Vector competence studies with hard ticks and *Borrelia burgdorferi* sensu lato spirochetes: A review

Lars Eisen

Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, 3156 Rampart Road, Fort Collins, CO 80521, United States

Abstract

Use of emerging technology allowing for identification of genetic material from pathogens and endosymbionts in ticks collected from humans, domestic animals, wildlife, or the environment has resulted in an avalanche of new data on tick-microorganism associations. This rapidly growing stream of new information is a tremendous resource but also presents challenges, including how detection of pathogen genetic material in ticks should best be interpreted. There is a tendency in the more recent published literature to incorrectly use the term "vector" based on detection of pathogen genetic material from tick species not experimentally confirmed to serve as vectors of the pathogen in question. To serve as a vector of a horizontally maintained pathogen, such as a *Borrelia burgdorferi* sensu lato (s.l.) Lyme borreliosis spirochete, the tick species in question must be capable of acquiring the pathogen while feeding in the larval or nymphal stage on an infectious host, maintaining it transstadially through the molt, and then transmitting the pathogen to a naïve host while feeding in the subsequent nymphal or adult stage. This review examines the experimental evidence for and against species of hard (ixodid) ticks from different genera to serve as vectors of *B. burgdorferi* s.l. spirochetes. Of the 18 *Ixodes* species ticks evaluated to date, 13 were experimentally confirmed as vectors of *B. burgdorferi* s.l. spirochetes. These studies focused primarily on the three major Lyme borreliosis agents: *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii*, and *Borrelia garinii*. In striking contrast, none of 8 tick species from other genera (1 *Amblyomma* species, 5 *Dermacentor* species, and 2 *Haemaphysalis* species) evaluated to date were unequivocally experimentally confirmed as vectors of *B. burgdorferi* s.l. spirochetes. The strength of the evidence for or against each tick species to serve as a vector of *B. burgdorferi* s.l. spirochetes is discussed together with key knowledge gaps and research challenges.

evp4@cdc.gov.

Disclaimer

The findings and conclusions of this study are by the author and do not necessarily represent the views of the Centers for Disease Control and Prevention.

1.   Rationale for tick vector competence studies with *Borrelia burgdorferi* sensu lato spirochetes

Epidemiological studies conducted in the United States in the late 1970s indicated a linkage between Lyme borreliosis (Lyme disease in North America) and bites by *Ixodes* ticks (Steere and Malawista, 1979). The etiological agent of this illness remained elusive until 1981, when a spirochete was discovered in host-seeking *Ixodes scapularis* ticks (including the junior synonym, *Ixodes dammini*) collected in New York state (Burgdorfer et al., 1982). Soon thereafter, this spirochete also was isolated from Lyme borreliosis patients (Benach et al., 1983; Steere et al., 1983). The new disease agent was named *Borrelia burgdorferi* (Johnson et al., 1984a). Examination of other tick species closely related to *I. scapularis* (other members of the *Ixodes ricinus*/*Ixodes persulcatus* species complex [Filippova et al., 1999; Keirans et al., 1999]) confirmed that *B. burgdorferi* was present in a suite of four notorious human-biting ticks with a collective distribution spanning a very large portion of the northern hemisphere: *I. scapularis* in eastern North America (Burgdorfer et al., 1982; Anderson et al., 1983; Bosler et al., 1983); *Ixodes pacificus* in far western North America (Burgdorfer et al., 1985); and *I. ricinus* and *I. persulcatus* in Eurasia (Barbour et al., 1983; Burgdorfer et al., 1983; Ackermann et al., 1984; Korenberg et al., 1987, 1988; Kryuchechnikov et al., 1988; Zhang, 1989; Ai et al., 1990; Miyamoto et al., 1991).

Another major development was the delineation in the early 1990s of *B. burgdorferi* into three species, associated with different clinical manifestations in afflicted humans, within the *Borrelia burgdorferi* sensu lato (s.l.) species complex: *Borrelia burgdorferi* sensu stricto (s.s.), *Borrelia afzelii,* and *Borrelia garinii* (Baranton et al., 1992; Canica et al., 1993). The description of new species within the *B. burgdorferi* s.l. complex is still ongoing with the most recent global count at 21 named species. In addition to the three major causative agents of human illness mentioned above, the *B. burgdorferi* s.l. complex also includes several species occasionally associated with human illness and other species with unknown pathogenicity to humans (Rudenko et al., 2011; Margos et al., 2016, 2017; Tables 1–2). The global burden of Lyme borreliosis is still poorly defined, but the United States alone now typically documents >30,000 annual reported cases (Rosenberg et al., 2018) and estimates of the true number of annual cases is tenfold higher (Hinckley et al., 2014; Nelson et al., 2015). Moreover, a recent study including 17 countries in western Europe produced an estimate of >200,000 Lyme borreliosis cases diagnosed annually (Sykes and Makiello, 2016).

The early descriptions of natural *B. burgdorferi* s.l. infection in *I. scapularis*, *I. pacificus*, *I. ricinus*, and *I. persulcatus* mentioned above resulted in an initial wave of experimental studies to formally demonstrate the vector competence of these four human-biting *I. ricinus*/*I. persulcatus* species complex ticks for *B. burgdorferi* s.l. (see references in Tables 3–6). Additional studies with *Ixodes* ticks have targeted other notable human-biting species as well as species that more rarely bite humans but may be involved in enzootic maintenance cycles. These include: *Ixodes affinis, Ixodes angustus, Ixodes cookei, Ixodes dentatus, Ixodes jellisoni, Ixodes minor, Ixodes muris,* and *Ixodes spinipalpis* [including the junior synonym, *Ixodes neotomae*] in North America (Table 7); *Ixodes arboricola, Ixodes frontalis,*

Ixodes hexagonus, *Ixodes ovatus*, and *Ixodes sinensis* in Eurasia (Table 8); and *Ixodes holocyclus* in Australia (Table 8).

In North America, another wave of vector competence studies was spurred by the description of *B. burgdorferi* s.l. spirochetes from naturally infected non-*Ixodes* human-biting ticks: *Amblyomma americanum*, *Dermacentor occidentalis*, and *Dermacentor variabilis* (Schulze et al., 1984; Anderson et al., 1985; Lane and Lavoie, 1988; Magnarelli and Anderson, 1988). The resulting vector competence evaluations included four major non-*Ixodes* human-biting ticks in the United States: *A. americanum* and *D. variabilis* in the east, *Dermacentor andersoni* in the Rocky Mountain region and *D. occidentalis* in the far west (Table 9). Later, two Eurasian human-biting *Dermacentor* species, *Dermacentor nuttalli* and *Dermacentor silvarum*, also were evaluated experimentally (Table 9). Finally, some of the most recent vector competence studies for *B. burgdorferi* s.l. spirochetes have focused on *Haemaphysalis* ticks (*Haemaphysalis concinna* and *Haemaphysalis longicornis*), spurred in part by the ongoing emergence in Lyme borreliosis-endemic areas of the United States of the invasive *H. longicornis* (Table 9).

The most recent review of tick vector competence studies with *B. burgdorferi* s.l. spirochetes was published nearly two decades ago (Eisen and Lane, 2002). Although relatively few studies have been published since then, the overall context has changed considerably due to the still ongoing description of new species within the *B. burgdorferi* s.l. complex. Consequently, as shown in Table 1, not even the most intensively studied tick species, *I. scapularis*, has been evaluated experimentally with all species within the *B. burgdorferi* s.l. complex known to occur within the tick's geographical range. Moreover, use of emerging technology allowing for detection of genetic material from pathogens and endosymbionts in ticks collected from humans, domestic animals, wildlife, or the environment has resulted in an avalanche of new data on tick-microorganism associations, including *B. burgdorferi* s.l. spirochetes (Kurilshikov et al., 2015; Cross et al., 2018; Greay et al., 2018; Zhuang et al., 2018; Thapa et al., 2019; Tokarz et al., 2019). This rapidly growing stream of new information is a tremendous resource but it also presents major challenges, including how detection of genetic material from *B. burgdorferi* s.l. in ticks should best be interpreted. There is a tendency in the more recent published literature to incorrectly use the term "vector" based on detection of pathogen genetic material from tick species that have not been experimentally confirmed to serve as vectors of the pathogen in question. This review both serves as a reminder of the concept of vector competence and provides an upto-date single resource for the state of our knowledge regarding tick vector competence for *B. burgdorferi* s.l. spirochetes.

Tables were compiled to (i) provide an overview of qualitative outcomes for tick species evaluated in vector competence studies with *B. burgdorferi* s.l. spirochetes (Tables 1–2) and (ii) present important details for individual studies (Tables 3–9). Within these tables and the specific descriptions of transmission studies by tick species (sections 4–8), *B. burgdorferi* s.l. refers to uncharacterized spirochetes and represent either wild strains from field-collected ticks or animals or named isolates which have yet to be assigned to a species in the published literature. The zoogeographic regions used to describe the broad geographical ranges of tick and *B. burgdorferi* s.l. species in the Tables follow those used in

the tick species descriptions in "The Hard Ticks of the World" by Gugliemone et al. (2014). In the text, I also sometimes use the more familiar terms of North America and Eurasia to broadly describe where different species of ticks and *B. burgdorferi* s.l. spirochetes occur. Published literature was queried by searching the Scopus database using combinations of the key words "vector", "tick", and "*Borrelia*" together with "transmission", "transmit", or "competence". The snowball technique, which identifies additional publications based on referenced materials, was then employed to identify additional publications of interest.

The issue of duration of tick attachment required for transmission of *B. burgdorferi* s.l. spirochetes is not addressed here because this topic was examined in a recent separate review (Eisen, 2018). All data presented in this review are based on ticks allowed to feed to repletion on the host; in some cases this includes subsets of data for ticks fed to repletion in studies that also examined spirochete acquisition or transmission during partial blood meals. Data were interpreted and broad conclusions drawn bearing in mind that the studies were conducted over more than 35 years and with substantial methodological variation, including the species and strain of experimental host, the route of initial host infection, and the methods used to detect spirochetes in ticks or hosts.

2. Conceptual underpinnings for tick vector competence studies with *Borrelia burgdorferi* sensu lato spirochetes

Enzootic maintenance of *B. burgdorferi* s.l. spirochetes was recognized early on to primarily involve horizontal transmission between vector ticks and reservoir hosts (Anderson et al., 1983; Bosler et al., 1983; Levine et al., 1985; Donahue et al., 1987). Consequently, experimental tick vector competence studies with *B. burgdorferi* s.l. spirochetes have included demonstration of three distinct processes: (i) acquisition of spirochetes by uninfected ticks feeding on infectious experimental hosts, (ii) maintenance of spirochetes through the molt to the next life stage (transstadial passage), and (iii) transmission of spirochetes to naïve hosts during a subsequent blood meal (Eisen and Lane, 2002; Kahl et al., 2002). A tick species should not be considered a vector of *B. burgdorferi* s.l. spirochetes unless all three processes have been experimentally demonstrated. In the case of field-derived data, detection of *B. burgdorferi* s.l. spirochetes in fed ticks (of any life stage) collected from wild animal hosts are only indicative of acquisition of spirochetes from an infected host, whereas detection of *B. burgdorferi* s.l. in host-seeking nymphal or adult ticks indicate that spirochetes were both acquired during the blood meal in the preceding life stage and passed transstadially through the molt (Eisen and Lane, 2002; Kahl et al., 2002). However, field-derived data alone can never satisfy the final criterion of vector competence: unequivocal demonstration of transmission of *B. burgdorferi* s.l. spirochetes by feeding ticks.

It also is worth noting another transmission scenario, which in light of new knowledge has become less relevant for *B. burgdorferi* s.l. spirochetes: demonstration of transmission by transovarially infected larval ticks where infection was passed from the female to her eggs and the resulting larvae. Although the importance of transovarial transmission of *B. burgdorferi* s.l. spirochetes in the four major *Ixodes* vectors has been hotly debated

(Randolph, 1994), a consensus is building that early records of field-collected infected unfed larvae (based on microscopy or immunofluorescence assays) may represent the more recently described relapsing fever group spirochete, *Borrelia miyamotoi* (Rollend et al., 2013). *Borrelia miyamotoi* occurs naturally in *Ixodes* ticks across the northern hemisphere (Wagemakers et al., 2015), and was demonstrated to be passed transovarially and transmitted by the resulting larvae for both *I. ricinus* and *I. scapularis* (Scoles et al., 2001; van Duijvendijk et al., 2016; Breuner et al., 2018). To the best of my knowledge, there is only a single study where *B. burgdorferi* s.l. spirochetes, characterized using methodology capable of distinguishing them from *B. miyamotoi*, were demonstrated to be transmitted by *Ixodes* larvae to an experimental host: van Duijvendijk et al. (2016) found that feeding of field-collected *I. ricinus* larvae on laboratory mice resulted in infection with *B. afzelii*. However, the use of field-collected larval ticks in such transmission experiments can be problematic unless it is clearly documented that the larvae had not previously taken a partial blood meal on another (potentially infectious) host that perished in the early stages of their blood meal, thus forcing them to seek another host to acquire the amount of blood required to complete their molt (Piesman, 1991). Previous studies demonstrated that *I. scapularis* and *I. persulcatus* ticks allowed to attach to an infectious host for 24–48 h and thereafter removed and placed on a new, naïve experimental host can effectively transmit *B. burgdorferi* s.s. while completing their blood meal (Shih and Spielman, 1993; Nakao and Sato, 1996).

Experimental animal hosts used in tick vector competence studies with *B. burgdorferi* s.l. spirochetes have, depending in part on the targeted tick species, typically included rabbits and various species of rodents, including natural tick hosts as well as laboratory animals (see Tables 3–9). Early experimental studies tended to use rabbit hosts (Burgdorfer et al., 1982, 1983; Burgdorfer, 1984; Kornblatt et al., 1984; Burgdorfer and Gage, 1986) but this soon gave way to use of rodents as these animals proved more effective sources to infect feeding immature ticks with *B. burgdorferi* s.l. spirochetes (Burgdorfer and Gage, 1987; Donahue et al., 1987; Piesman et al., 1987a, b; Piesman and Sinsky, 1988; Mather et al., 1990). Moreover, rodents develop long-lasting spirochete infections (Burgdorfer and Gage, 1987; Donahue et al., 1987; Piesman, 1988; Brown and Lane, 1992; Gern et al., 1994; Lindsay et al., 1997; Lane et al., 1999; States et al., 2017; Dolan et al., 2017a) readily detectable by culture of infected tissue, such as biopsies from ear or internal organs, including bladder, kidney, and spleen (Johnson et al., 1984b; Anderson et al., 1985, 1986; Piesman et al., 1987b; Schwan et al., 1988; Sinsky and Piesman, 1989). For tick species and life stages that feed willingly on mice, or can be compelled to take a blood meal from mice when confined to feeding capsules, outbred strains of the white mouse (*Mus musculus*) provide practical animal models for vector competence studies with rodent-associated *B. burgdorferi* s.l. spirochetes (Piesman, 1993). White mice infected with *B. burgdorferi* s.l. spirochetes via tick bite become highly infectious to feeding uninfected immature ticks within a predictable window of time post-infection (3–5 wk) and they also are excellent naïve hosts in the final transmission step with infected ticks because ear biopsies provide a reliable source of tissue for spirochete culture. Use of a natural tick host, such as the white-footed mouse (*Peromyscus leucopus*) or deer mouse (*Peromyscus maniculatus*) in North America, is another option that adds an element of realism to the experimental study.

For the initial step of spirochete acquisition via feeding by uninfected ticks, it is advisable to use experimental hosts that were infected via tick bite rather than needle inoculation in order to ensure that the spirochetes introduced into the host expressed surface proteins associated with tick transmission rather than artificial culturing (Gern et al., 1993; Piesman, 1993; Randolph and Nuttall, 1994). Moreover, for tick species suspected to be poor vectors of *B. burgdorferi* s.l. spirochetes it is critically important to examine both freshly fed ticks (to demonstrate spirochete acquisition) and molted resulting ticks of the next life stage (to document transstadial passage). There are several examples of studies where a non-*Ixodes* tick species effectively acquired spirochetes while feeding (infection prevalence >50 % in freshly fed ticks) but the infection was uniformly lost in the molt to the next life stage (Piesman and Happ, 1997; Sun and Xu, 2003; Sun et al., 2003a; Breuner et al., 2020). In cases where there is a high likelihood of efficient spirochete acquisition and transstadial passage, such as for *Ixodes* ticks, studies often bypass testing of freshly fed larvae to maximize the number of molted nymphs available for pathogen detection and demonstration of spirochete transmission by the nymphs (Tables 3–9). As part of providing evidence for transstadial passage of *B. burgdorferi* s.l., it is advisable to not rely solely on detection of spirochete DNA in the molted ticks but also demonstrate the presence of viable spirochetes via culture or feeds on naïve experimental hosts.

In the final transmission step, it is important to consider the purpose of the vector competence study. Because mass feeding of infected ticks can result in more effective transmission of *B. burgdorferi* s.l. spirochetes compared to use of a single or a few ticks (reviewed by Eisen, 2018), it may be useful to restrict the number of feeding ticks to match the typical number of that species and life stage found on a natural reservoir host (in the context of enzootic transmission) or to aim for a number projected to include only a single infected tick (in the context of transmission to humans). This final transmission step can be readily achieved in scenarios where a large proportion of the ticks both acquire and pass spirochetes transstadially but is extremely challenging when the prevalence of infection in the molted ticks is near zero. As an evaluation of transmission by infected ticks requires actual feeding by infected ticks it is critically important to test the fed ticks recovered from each host for presence of spirochetes in order to ascertain which individual experimental hosts were exposed to at least one infected tick versus only fed upon by uninfected ticks.

Seminal studies on experimental transmission of *B. burgdorferi* s.l. by *Ixodes* ticks used a model where field-collected adult ticks were fed upon New Zealand white rabbits (Burgdorfer et al., 1982, 1983; Burgdorfer, 1984). Following the recognition that the nymphal stage of *I. scapularis* is the principal vector of Lyme borreliosis spirochetes to humans in the northeastern United States (Spielman et al., 1985; Piesman et al., 1987c; Lane et al., 1991), there was a shift toward an experimental model with *B. burgdorferi* s.l. acquisition by larvae and the final transmission step involving infected nymphs. These developments produced a practical experimental system where laboratory-reared rodents infected via tick bite are used to infect feeding larval ticks with the *B. burgdorferi* s.l. species under study and the resulting molted nymphs then are fed on naïve animals to demonstrate transmission (Piesman, 1993). Variations of this experimental system may be needed for tick species or life stages that are unwilling to feed on rodents or *B. burgdorferi* s.l. spirochetes adapted to non-mammalian hosts, for example avian-associated ticks and *B. garinii* variants

(Kurtenbach et al., 2002; Heylen et al., 2014). Unequivocal demonstration of transmission of viable spirochetes from infected ticks to hosts should include either culture of spirochetes from host tissues or acquisition of spirochetes from the hosts by uninfected (xenodiagnostic) ticks. Serology can serve as a complement for demonstration of spirochete exposure but is not recommended as a stand-alone method in a transmission experiment.

There have been efforts to employ artificial means to infect ticks with *B. burgdorferi* s.l. spirochetes, including use of artificial feeders containing infected blood (Burkot et al., 2001), ingestion of culture containing spirochetes via capillary-feeding before or after a blood meal (Monin et al., 1989; Gern et al., 1991, 1993; Li and Lane, 1996; Fingerle et al., 2002), immersion of ticks into culture containing spirochetes (Policastro and Schwan, 2003; Fiserova et al., 2008), and microinjection of spirochetes into the tick hemocoel (Johns et al., 1998, 2000; Urbanova et al., 2017). Although such techniques can be useful complements to the traditional animal model, they also introduce biological deviations from the natural host-tick transmission chain which makes the results of transmission studies more difficult to interpret. One specific problem is the ingestion by ticks of spirochetes not exposed to a host's immune system and therefore not necessarily expressing the phenotype seen in a natural or experimental host-tick transmission chain.

3. Limitations to tick vector competence studies with *Borrelia burgdorferi* sensu lato spirochetes

When considering the results of tick vector competence studies, it is important to recognize their limitations. A tick species that experimentally proves incapable or very inefficient to serve as a vector for a given *B. burgdorferi* s.l. species will not play a major role as a vector in nature. However, it is equally important to recognize that experimental demonstration of vector competence does not necessarily imply that the tick species in question is important either as an enzootic vector (it may not feed commonly on *B. burgdorferi* s.l. reservoir hosts) or a vector to humans (its questing behavior may not be conducive to making contact with people). Field studies are critical to provide all the data needed for an accurate assessment of the potential (vector potential/vectorial capacity) of an experimentally confirmed vector tick species to contribute to infection of wildlife or human illness (Spielman et al., 1984; Kahl et al., 2002). In some cases there also may be interest in examining vector competence of notorious human-biting tick vectors for human-pathogenic *B. burgdorferi* s.l. spirochetes that are currently not present within the distribution range of the tick but conceivably could be introduced in the future. The demonstration of vector competence of the Nearctic *I. scapularis* for two Palearctic Lyme borreliosis spirochetes, *B. afzelii* and *B. garinii*, is perhaps the best example of such as study (Dolan et al., 1998).

As noted previously, the still ongoing description of new species within the *B. burgdorferi* s.l. complex has far outpaced the vector competence studies (Tables 1–2). For example, *I. scapularis* has been experimentally demonstrated to serve as a vector of *B. burgdorferi* s.s., *Borrelia mayonii*, and *Borrelia bissettiae* (Table 3) but has yet to be evaluated experimentally for several other *B. burgdorferi* s.l. complex spirochetes occurring naturally in the eastern United States: *Borrelia americana*, *Borrelia andersonii*, *Borrelia carolinensis*, and *Borrelia*

kurtenbachii. The same scenario, with vector competence studies conducted for only a subset of their associated *B. burgdorferi* s.l. complex spirochetes, holds true also for the other three major global vectors of Lyme borreliosis spirochetes to humans: *I. pacificus*, *I. ricinus* and *I. persulcatus* (Table 1). Because of the potential for further delineation of species within the *B. burgdorferi* s.l. complex, it is recommended to use well characterized *B. burgdorferi* s.l. isolates in vector competence studies. Should further delineation occur in the future, the *B. burgdorferi* s.l. species to which such isolates are re-assigned can most likely be elucidated. In contrast, vector competence studies using "wild type" *B. burgdorferi* s.l. spirochetes may be difficult to re-interpret and therefore less informative.

The ideal design for a vector competence study involves early generation ticks from a laboratory colony established using field-collected specimens from the same general geographical area as the *B. burgdorferi* s.l. species and strain/isolate under evaluation. Variation in genotype and phenotype (including infectivity for rodents and ticks) within a single *B. burgdorferi* s.l. species (Piesman, 1993; Lane et al., 1994; Nakao and Miyamoto, 1994; Peavey and Lane, 1996; Piesman and Happ, 1997; Piesman et al., 1999; Crippa et al., 2002; Eisen et al., 2003; Derdakova et al., 2004; Tonetti et al., 2015; States et al., 2017) can make this challenging, especially when also addressing a tick species with a large distribution range and geographical variation in genotype and phenotype across tick populations. For example, *I. scapularis* occurs widely across different ecological settings in the eastern United States and displays genetic variation as well as distinct behavioral differences to the north versus south (Van Zee et al., 2015; Eisen et al., 2016; Arsnoe et al., 2019). Within this broad geographical range, there also is considerable genetic and phenotypic variability within *B. burgdorferi* s.s. (Mathiesen et al., 1997; Qiu et al., 2002; Bunikis et al., 2004; Hoen et al., 2009; Brisson et al., 2010). To address this complex scenario, vector efficiency was assessed in a series of studies using northern and southern *I. scapularis* populations and *B. burgdorferi* s.s. isolates originating from different parts of the tick's geographical range (Oliver et al., 1993; Sanders and Oliver, 1995; Piesman and Happ, 1997; Jacobs et al., 2003; Goddard et al., 2015).

Experimental studies with tick species expected to be poor vectors of *B. burgdorferi* s.l. spirochetes are especially challenging. One logical end-point for such studies is to demonstrate that spirochetes acquired during a blood meal are not passed to the subsequent life stage (Piesman and Sinsky, 1988; Mather and Mather, 1990; Brown and Lane, 1992; Lane et al., 1994; Breuner et al., 2020), indicating that the tick species under evaluation is not a vector. This, however, requires a large sample size for a statistically robust evaluation. For example, as reported by Breuner et al. (2020), lack of *B. burgdorferi* s.s. infection in 520 examined *H. longicornis* nymphs fed as larvae on infectious hosts still resulted in an upper 95 % confidence limit estimate for infection prevalence of 0.7 %. Thus, the effort required to conclude that a given tick species is unlikely to be a vector is considerable. An even more challenging scenario arises when *B. burgdorferi* s.l. spirochetes are passed transstadially but only for a small percentage of ticks, as recorded in a small subset of experiments with *A. americanum* and *D. variabilis* (Ryder et al., 1992; Piesman and Happ, 1997; Soares et al., 2006). Based on the low infection prevalence in the remaining ticks, it can be challenging to subsequently succeed in exposing naïve hosts to infected, rather than only uninfected, feeding ticks. As a case in point, none of the three studies mentioned above succeeded in

unequivocally exposing a single animal to an infected tick. Moreover, as shown in Table 9, despite numerous attempts there is still a complete lack of documented exposures of experimental hosts to the feeding by a transstadially infected *Amblyomma*, *Dermacentor*, or *Haemaphysalis* tick.

A final emerging issue is the potential impact of the tick microbiome on vector competence studies (Narasimhan and Fikrig, 2015; Gall et al., 2016; de la Fuente et al., 2017; Kwan et al., 2017; Varela-Stokes et al., 2017; Couper et al., 2019). For example, our understanding of the impact of co-infection with multiple pathogens within a single tick on the transmission of *B. burgdorferi* s.l. spirochetes is very limited (Piesman et al., 1987a; Levin and Fish, 2000) despite common reports of co-infection in field-collected ticks (Diuk-Wasser et al., 2016; Moutailler et al., 2016; Eisen and Eisen, 2018). In addition, colony ticks considered "pathogen-free" and used in experimental studies may harbor endosymbionts and other microorganisms potentially impacting the outcome of a vector competence or vector efficiency study, positively or negatively. This is simultaneously very intriguing and quite frustrating as it may lead to a better understanding of pathogen transmission dynamics, and perhaps also to new ways of interrupting spirochete transmission, while also presenting new challenges for data interpretation. Future transmission studies may face the added challenge of having to incorporate broad metagenomics data on the microbiome of the individual ticks used in the experiments into the analysis model, potentially resulting in a need to increase the number of animals exposed in order to achieve adequate statistical power of the study design.

4. Vector competence studies with Nearctic/Neotropical *Ixodes* ticks

To date, 10 species of Nearctic/Neotropical *Ixodes* ticks have been examined in vector competence studies with *B. burgdorferi* s.l. spirochetes (Tables 3–4,7). Of these 10 tick species, 9 were experimentally confirmed as vectors of *B. burgdorferi* s.l. spirochetes. The strength of the evidence for or against vector competence is outlined for each tick species below.

4.1. Ixodes scapularis

Ixodes scapularis (a member of the *Ixodes ricinus*/*Ixodes persulcatus* species complex) has been confirmed as a vector of North American uncharacterized *B. burgdorferi* s.l. spirochetes (wild strains, 006 strain, MI129, and Valhalla), *B. burgdorferi* s.s. (JD1, B31, NC-2, SH2–82, SI-1, BL206, and B348), *B. mayonii* (MN14–1420 and MN17–4755) and *B. bissettiae* (MI-6) (Burgdorfer et al., 1982; Burgdorfer, 1984; Burgdorfer and Gage, 1986; Piesman et al., 1987a, 1987b, 1991, 1999; Piesman and Sinsky, 1988; Mather et al., 1990; Mukolwe et al., 1992; Ryder et al., 1992; Oliver et al., 1993; Lane et al., 1994; Levin et al., 1995; Sanders and Oliver, 1995; Richter et al., 1996; Dolan et al., 1997, 1998, 2016, 2017b; Piesman and Happ, 1997; Levin and Fish, 2000; des Vignes et al., 2001; Jacobs et al., 2003; Derdakova et al., 2004; Goddard et al., 2015; Eisen et al., 2017; Table 3). Moreover, *I. scapularis* was confirmed as a vector of Palearctic *B. afzelii* (Pgau.C3) and *B. garinii* (VS286) (Dolan et al., 1998).

The efficiency of spirochete acquisition by larval ticks and transstadial passage to the nymphal stage was highly variable (<10 to 98 %) across uncharacterized *B. burgdorferi* s.l. spirochetes (Burgdorfer, 1984; Burgdorfer and Gage, 1986; Telford and Spielman, 1989; Barker et al., 1993; Piesman, 1993; Maupin et al., 1994; Levin et al., 1995; Dolan et al., 1997; Piesman and Happ, 1997). For isolates of *B. burgdorferi* s.s., the efficiency of spirochete acquisition by larval ticks and transstadial passage to the nymphal stage was moderate to high (40–100 %), with most studies exceeding 80 % (Piesman et al., 1987b; Piesman and Sinsky, 1988; Mather and Mather, 1990; Mather et al., 1990; Piesman and Stone, 1991; Ryder et al., 1992; Piesman, 1993; Lane et al., 1994; Dolan et al., 1997, 1998; Piesman and Happ, 1997; Jacobs et al., 2003; Derdakova et al., 2004; States et al., 2017). The efficiency of spirochete acquisition by larval ticks and transstadial passage to the nymphal stage was lower (27 %) for *B. bissettiae* (Sanders and Oliver, 1995). For *B. mayonii*, the efficiency of spirochete acquisition by larval ticks and transstadial passage to the nymphal stage was low (12–13 %) for a human-derived isolate (MN14–1420) but higher (55 %) for a rodent-derived isolate (MN17–4755) (Dolan et al., 2016; Eisen et al., 2017; Parise et al., 2020; Table 3).

As shown in Table 3, some of the experiments demonstrating spirochete transmission to naïve hosts also include data for the number of animals known to have been exposed to infected ticks whereas in other cases these important data were not reported. Considering only those studies where the number of animals known to have been exposed to at least one infected tick was reported, the transmission rate (defined here as: number of hosts with evidence of spirochete exposure / number of hosts exposed to infected ticks) was 91 % (85/93) for uncharacterized *B. burgdorferi* s.l. spirochetes, 94 % (50/53) for *B. burgdorferi* s.s., and 69 % (49/71) for *B. mayonii* (Table 3). In all cases, this included subsets of naïve hosts exposed to a single infected tick. Including all studies presenting evidence for host infection following exposure to *I. scapularis* ticks, the total numbers of recorded instances of transmission to an individual host reach 95 for uncharacterized *B. burgdorferi* s.l. spirochetes (nymphal and female ticks), 93 for *B. burgdorferi* s.s. (nymphs), 7 for *B. bissettiae* (nymphs), 49 for *B. mayonii* (nymphs), 2 for *B. afzelii* (nymphs), and 1 for *B. garinii* (nymphs) (Table 3). Finally, as shown in Table 3, the evidence to date indicates that *I. scapularis* is a highly efficient vector for *B. burgdorferi* s.s. regardless of the geographical origins of the combination of tick population and spirochete isolate used in the transmission experiment.

4.2. Ixodes pacificus

Ixodes pacificus (a member of the *Ixodes ricinus*/*Ixodes persulcatus* species complex) has been confirmed as a vector of uncharacterized *B. burgdorferi* s.l. spirochetes (wild strain), *B. burgdorferi* s.s. (CA4 and JD1), and *B. bissettiae* (CA 592) (Lane et al., 1994; Peavey and Lane, 1995; Piesman et al., 1999; Eisen et al., 2003; Table 4). The efficiency of spirochete acquisition by larval ticks and transstadial passage to the nymphal stage was low to moderate (12–40 %) across uncharacterized *B. burgdorferi* s.l. spirochetes (Burgdorfer, 1984; Brown and Lane, 1992, 1996); highly variable (<5 to 60 %) for isolates of *B. burgdorferi* s.s. (Piesman, 1993; Lane et al., 1994; Peavey and Lane, 1995; Eisen et al., 2003); and highly variable (<5 to 75 %) for isolates of *B. bissettiae* (Eisen et al., 2003). Across the evaluated

isolates, known exposure of 27 naïve hosts to infected nymphs (n = 1–6 per host) resulted in documented spirochete transmission to 23 of the hosts (85 % transmission rate across exposed hosts): this included 2 recorded instances of transmission for uncharacterized *B. burgdorferi* s.l. spirochetes, 14 for *B. burgdorferi* s.s., and 7 for *B. bissettiae* (Table 4). An additional 6 instances of transmission of *B. bissettiae* were recorded in a study where it was not clear how many naïve hosts may have been exposed to infected ticks (Eisen et al., 2003).

4.3. Ixodes angustus

Ixodes angustus has been confirmed as a vector of *B. burgdorferi* s.s. (CA4) (Peavey et al., 2000; Table 7). The efficiency of spirochete acquisition by larval ticks and transstadial passage to the nymphal stage was low (8–12 %). A single instance of transmission to a host by feeding nymphs was recorded but it is not clear how many infected nymphs fed on the animal.

4.4. Ixodes dentatus

Ixodes dentatus has been confirmed as a vector of uncharacterized *B. burgdorferi* s.l. spirochetes (Telford and Spielman, 1989; Table 7). The efficiency of spirochete acquisition by larval ticks and transstadial passage to the nymphal stage was moderate (47 %). Known exposure of 1 naïve host to a single infected nymph resulted in transmission to the host.

4.5. Ixodes jellisoni

Ixodes jellisoni (a member of the *Ixodes ricinus*/*Ixodes persulcatus* species complex) has been confirmed as a vector of uncharacterized *B. burgdorferi* s.l. spirochetes (CA445) and *B. californiensis* (CA404, CA409, and CA446; Margos et al., 2016) (Lane et al., 1999; Table 7). Although the evidence is limited to a single study, the evaluation did include multiple *B. californiensis* isolates. The efficiency of spirochete acquisition by larval ticks and transstadial passage to the nymphal stage was low (25 %). Known exposure of 3 naïve hosts each to 1–7 nymphs infected with *B. californiensis* resulted in 2 instances of transmission to the host (67 % transmission rate across exposed hosts). The yet uncharacterized *B. burgdorferi* s.l. spirochete isolate (445) performed similarly in the study and one instance of transmission to a host was recorded (Table 7).

4.6. Ixodes muris

Ixodes muris has been confirmed as a vector of *B. burgdorferi* s.s. (B31) (Dolan et al., 2000; Table 7). The efficiency of spirochete acquisition by larval ticks and transstadial passage to the nymphal stage was moderate (38 %). Known exposure of 4 naïve hosts each to 1–3 infected nymphs resulted in a single instance of transmission to a host (25 % transmission rate across exposed hosts).

4.7. Ixodes spinipalpis

Ixodes spinipalpis has been confirmed as a vector of uncharacterized *B. burgdorferi* s.l. spirochetes (wild strains), *B. burgdorferi* s.s. (B31), and *B. bissettiae* (wild strain, N271, N501, and CA 592) (Brown and Lane, 1992; Dolan et al., 1997; Burkot et al., 2000; Eisen et al., 2003; Table 7). The efficiency of spirochete acquisition by larval ticks and transstadial

passage to the nymphal stage was highly variable (<5 to 80 %) across uncharacterized *B. burgdorferi* s.l. spirochetes (Brown and Lane, 1992; Dolan et al., 1997; Lane et al., 1999); highly variable (<10 to 70 %) for isolates of *B. burgdorferi* s.s. (Dolan et al., 1997; Peavey et al., 2000; Eisen et al., 2003); highly variable (<5 to 80 %) for isolates of *B. bissettiae* (Eisen et al., 2003); and low (25 %) for isolates of *B. californiensis* (Lane et al., 1999). Across the evaluated isolates, known exposure of 18 naïve hosts to infected nymphs (n = 1–12 per host) uniformly resulted in spirochete transmission to the hosts (100 % transmission rate across exposed hosts): this included 7 recorded instances of transmission for uncharacterized *B. burgdorferi* s.l. spirochetes, 2 for *B. burgdorferi* s.s., and 9 for *B. bissettiae* (Table 7). An additional 2 instances of transmission of *B. bissettiae* was recorded in a study where it was not clear how many naïve hosts may have been exposed to infected ticks (Burkot et al., 2000). The study (Lane et al., 1999) with spirochetes later characterized as *B. californiensis* (CA404, CA409, CA442, CA443 and CA446; Margos et al., 2016) did not proceed to evaluate transmission by infected *I. spinipalpis* ticks.

4.8. Ixodes affinis and Ixodes minor

Ixodes affinis (a member of the *Ixodes ricinus*/*Ixodes persulcatus* species complex) has reportedly been confirmed as a vector of *B. burgdorferi* s.s. (SI-1), and *Ixodes minor* as a vector of *B. burgdorferi* s.s. and *B. bissettiae* (Oliver, 1996; Oliver et al., 2003). However, as indicated in Table 7, the evidence for vector competence remains limited to references to unpublished data, and therefore must be classified as weak.

4.9. Ixodes cookei

The vector competence of *I. cookei* was evaluated for an uncharacterized *B. burgdorferi* s.l. spirochete (LI-231) and *B. burgdorferi* s.s. (SH2–82) in two studies (Ryder et al., 1992; Barker et al., 1993; Table 7). The efficiency of spirochete acquisition by larval ticks and transstadial passage to the nymphal stage was low (4–16 %) for the uncharacterized *B. burgdorferi* s.l. spirochete and zero for *B. burgdorferi* s.s. Unfortunately, in the last step to demonstrate spirochete transmission by feeding ticks there was no evidence of a single host having been exposed to an infected tick (Table 7). Consequently, the evidence against *I. cookei* as a vector of Lyme borreliosis spirochetes is weak and further studies are justified to clarify the vector competence status of this tick species.

5.   Vector competence studies with Palearctic/Oriental/Australian *Ixodes* ticks

To date, 8 species of Palearctic/Oriental/Australian *Ixodes* ticks have been examined in vector competence studies with *B. burgdorferi* s.l. spirochetes (Tables 5–6, 8). Of these 8 tick species, 4 were experimentally confirmed as vectors of *B. burgdorferi* s.l. spirochetes. The strength of the evidence for or against vector competence is outlined for each tick species below.

5.1. Ixodes ricinus

Ixodes ricinus (a member of the *Ixodes ricinus*/*Ixodes persulcatus* species complex) has been confirmed as a vector of uncharacterized *B. burgdorferi* s.l. spirochetes (wild strains), *B. burgdorferi* s.s. (wild strains, ZS7, and NE1849), *B. afzelii* (wild strains, Pgau.C3, NE496, NE2963, NE4053, NE5046, NE36, E61, P/sto, NE4054, NE4049, NE4051, and CB43), and *B. garinii* (wild strains and VS286) (Burgdorfer et al., 1983; Gern and Rais, 1996; Dolan et al., 1998; Kahl et al., 1998; Hu et al., 2001, 2003; Crippa et al., 2002; Tonetti et al., 2015; van Duijvendijk et al., 2016; Pospisilova et al., 2019; Table 5). The efficiency of spirochete acquisition by larval ticks and transstadial passage to the nymphal stage was low to moderate (16–50 %) across uncharacterized *B. burgdorferi* s.l. spirochetes (Burgdorfer, 1984; Gern et al., 1994), highly variable (<5 to 88 %) for isolates of *B. burgdorferi* s.s. (Gern et al., 1993; Gern et al., 1993; Dolan et al., 1998; Crippa et al., 2002), and highly variable (<5 to 90 %) for isolates of *B. afzelii* (Dolan et al., 1998; Crippa et al., 2002; Tonetti et al., 2015; Pospisilova et al., 2019). No similar robust data were presented for *B. garinii*. Very few studies on *I. ricinus* have presented data on the number of hosts known to have been exposed to infected ticks, but in both studies presenting such data (Burgdorfer et al., 1983; Gern and Rais, 1996) all hosts exposed to ticks infected with uncharacterized *B. burgdorferi* s.l. spirochetes (n = 7 hosts) or *B. burgdorferi* s.s. (n = 3 hosts) became infected. Including all studies presenting evidence for host infection following exposure to *I. ricinus* ticks, the total numbers of recorded instances of transmission to individual hosts reach 13 for uncharacterized *B. burgdorferi* s.l. spirochetes (nymphal and female ticks), 10 for *B. burgdorferi* s.s. (nymphs), 95 for *B. afzelii* (larval and nymphal ticks), and 17 for *B. garinii* (nymphs) (Table 5).

5.2. Ixodes persulcatus

Ixodes persulcatus (a member of the *Ixodes ricinus*/*Ixodes persulcatus* species complex) has been confirmed as a vector of *B. garinii* (wild strain and JEM 6) (Sato and Nakao, 1997; Sun et al., 2003a; Table 6). The efficiency of spirochete acquisition by larval ticks and transstadial passage to the nymphal stage was highly variable (21 to >90 %) across *B. garinii* isolates but in the 30–60 % range for most examined isolates (Nakao and Miyamoto, 1994; Sato and Nakao, 1997; Sun et al., 2003a). Overall, there were a minimum of 16 recorded instances of transmission to a naïve host by infected nymphal ticks, including 15 for the JEM6 isolate (Table 6). The transmission rate across animals exposed to infected ticks could not be calculated as there is no information in the relevant publications regarding the number of hosts known to have been exposed to infected ticks. I am not aware of any published experimental evaluation of the vector competence of *I. persulcatus* specifically for *B. afzelii*, but it seems a near certainty that an experimental study would confirm vector competence. *Ixodes persulcatus* is commonly naturally infected with *B. garinii* as well as *B. afzelii* spirochetes in Russia, East Asia, and Japan (Postic et al., 1997; Korenberg et al., 2002; Miyamoto and Masuzawa, 2002). It also should be noted that - in contrast to *I. ricinus*, *I. scapularis*, and *I. pacificus* for which the nymphal stage is considered the main vector of *B. burgdorferi* s.l. spirochetes to humans (Eisen and Lane, 2002) - the females of *I. persulcatus* are the main vectors of *B. burgdorferi* s.l. spirochetes to humans (Korenberg, 1994; Korenberg et al., 2002).

5.3.   Ixodes sinensis

Ixodes sinensis has been confirmed as a vector of *B. garinii* (wild strains) (Sun et al., 2003b; Table 8). The efficiency of spirochete acquisition by larval or nymphal ticks and transstadial passage to the resulting nymphal or adult stages was moderate (56–57 %). Exposure of naïve hosts to potentially infected ticks (nymphs or adults) resulted in 15 recorded instances of transmission to an individual host. The transmission rate could not be calculated as it is not clear how many hosts, in total, were exposed to infected ticks.

5.4.   Ixodes hexagonus

Ixodes hexagonus has been confirmed as a vector of *B. burgdorferi* s.s. (B31) (Gern et al., 1991; Table 8). Nymphal ticks infected via capillary feeding before taking a blood meal on an uninfected rabbit host were shown to maintain infection after molting to the adult stage, and the resulting female ticks transmitted spirochetes to all 4 hosts known to have been exposed to an infected female tick.

5.5.   Ixodes arboricola and Ixodes frontalis

The vector competence of the ornithophilic ticks *I. arboricola* and *I. frontalis* was evaluated using wild strains of *B. garinii* (both tick species), *B. spielmanii* (*I. frontalis* only), and *B. valaisiana* (*I. arboricola* only) (Heylen et al., 2014; Table 8). The efficiency of spirochete acquisition by larval ticks (from infected birds) and transstadial passage to the resulting nymphal stage was low (0.9–3.0 %) across combinations of tick and spirochete species. Subsequent attempts to feed infected nymphs on naïve avian hosts were unsuccessful as none of the ticks fed on the birds were found to be infected (Table 8). Consequently, as no single host has yet been unequivocally exposed to an infected tick, the evidence against *I. arboricola* and *I. frontalis* as vectors of Lyme borreliosis spirochetes is weak and additional studies are justified.

5.6.   Ixodes ovatus

The vector competence of *I. ovatus* was evaluated for *B. garinii* (JEM3, JEM4, JEM5, JEM6, JEM7, and JEM8) (Nakao and Miyamoto, 1994; Table 8). Larval ticks acquired spirochetes from infected hosts with variable efficiency (10–80 % of freshly fed larvae found to be infected across isolates, with an overall infection prevalence of 47 %) but there was no evidence of transstadial passage to the nymphal stage (none of 306 molted nymphs found to be infected). As there was no evidence of transstadial spirochete passage, there was no attempt to feed resulting nymphs on naïve hosts. Although the evidence against *I. ovatus* as a vector of the evaluated, human-derived *B. garinii* strains is robust, it nevertheless would be worthwhile to evaluate the vector competence of this tick species using rodent-associated isolates of *B. afzelii* and *B. garinii*.

5.7.   Ixodes holocyclus

The vector competence of *I. holocyclus* was evaluated for *B. burgdorferi* s.s. (JD1) (Piesman and Stone, 1991; Table 8). Larval ticks acquired spirochetes from infected hosts with low efficiency (17 % of freshly fed larvae found to be infected) but there was no evidence of transstadial passage to the nymphal stage (none of 84 molted nymphs found to be

infected). As there was no evidence of transstadial spirochete passage, there was no attempt to feed resulting nymphs on naïve hosts. Based on the limited number of ticks examined to assess transstadial spirochete passage and the use of a single isolate of a single species (*B. burgdorferi* s.s.), the evidence against *I. holocyclus* as a vector of Lyme borreliosis spirochetes is weak. Further studies are justified to clarify the vector competence status of this tick species for the species of Lyme borreliosis spirochetes most likely to occur in or be introduced to Australia.

6. *Ixodes* tick species likely to serve as vectors of *Borrelia burgdorferi* sensu lato spirochetes but still lacking formal experimental demonstration of vector competence

The seabird tick, *Ixodes uriae*, is perhaps the best example of a tick species which almost certainly is a vector of Lyme borreliosis spirochetes but where experimental demonstration of vector competence is still lacking (due to logistical challenges of conducting laboratory studies with this tick). There is very strong evidence from several field studies, including in settings lacking other tick species, to indicate that enzootic transmission cycles of *B. burgdorferi* s.l. spirochetes, particularly *B. garinii*, are maintained by *I. uriae* and seabirds (Olsen et al., 1993, 1995; Bunikis et al., 1996; Gylfe et al., 1999; Smith et al., 2006; Munro et al., 2017). Other examples of tick species where field evidence is strong enough to justify experimental transmission studies to formally demonstrate vector competence include the Palearctic ticks, *Ixodes trianguliceps* and *Ixodes pavlovskyi*, and the Palearctic/Oriental tick, *Ixodes granulatus* (Doby et al., 1990; Gorelova et al., 1996, 2001; Hubbard et al., 1998; Chao et al., 2009, 2012; Korenberg et al., 2010, 2015; Kovalevskii et al., 2013; Hou et al., 2015). *Ixodes trianguliceps* primarily infests small mammals, whereas *I. granulatus* and *I. pavlovskyi* have broader host ranges and occasionally bite humans. Based on the uniformly successful vector competence studies with *B. burgdorferi* s.l. spirochetes with the 6 members of the *I. ricinus*/*I. persulcatus* species complex evaluated to date (*I. affinis*, *I. jellisoni*, *I. pacificus*, *I. ricinus*, *I. persulcatus*, and *I. scapularis*), it seems likely that not only *I. pavlovskyi* but also the 7 additional remaining tick species within the complex (the Palearctic/Oriental *Ixodes gibbosus*, *Ixodes hyatti*, *Ixodes kashmiricus*, *Ixodes kazakstani*, *Ixodes nipponensis,* and *Ixodes nuttallianus*; and the Neotropical *Ixodes pararicinus*) would prove vectors of *B. burgdorferi* s.l. spirochetes if evaluated experimentally. A final tick species that deserves mention as a likely vector of *B. burgdorferi* s.l. spirochetes is the newly described *Ixodes inopinatus*, which occurs in southern Europe and northern Africa, and is closely related to *I. ricinus* (Estrada-Peña et al., 2014).

7. Vector competence studies with Nearctic/Neotropical *Amblyomma,* *Dermacentor* and *Haemaphysalis* ticks

To date, 5 species of Nearctic/Neotropical *Amblyomma*, *Dermacentor*, and *Haemaphysalis* ticks have been examined in vector competence studies with *B. burgdorferi* s.l. spirochetes (Table 9). None of these 5 tick species were experimentally confirmed as vectors of

any evaluated *B. burgdorferi* s.l. spirochete. The strength of the evidence against vector competence is outlined for each tick species below.

7.1. Amblyomma americanum

The vector competence of *A. americanum* was evaluated for *B. burgdorferi* s.s. (JD1, B31, NC-2, SI-1, and SH2–82), and *B. bissettiae* (MI-6) (Piesman and Sinsky, 1988; Mather and Mather, 1990; Mukolwe et al., 1992; Ryder et al., 1992; Oliver et al., 1993; Sanders and Oliver, 1995; Piesman and Happ, 1997; Soares et al., 2006; Table 9). The efficiency of spirochete acquisition by larval ticks was 0–19 % for isolates of *B. burgdorferi* s.s. (Piesman and Sinsky, 1988; Mather and Mather, 1990; Mukolwe et al., 1992: Ryder et al., 1992; Piesman and Happ, 1997). A single instance of transstadial spirochete transmission to a resulting nymph was recorded (Ryder et al., 1992), but the overall infection prevalence in resulting nymphs was very low: 0.3 % (1/361) for *B. burgdorferi* s.s., and 0 % (0/105) for *B. bissettiae* (Table 9). Five studies attempted to feed infected nymphs on naïve hosts but the uniformly negative outcomes (no hosts showing evidence of spirochete exposure) are difficult to interpret because none of the ticks fed on the animals were found to be infected in one study (Piesman and Happ, 1997) and the infection status of the fed nymphs was not examined in the other four studies (Mukolwe et al., 1992; Ryder et al., 1992; Oliver et al., 2003; Sanders and Oliver, 1995). Consequently, no single host has yet been unequivocally exposed to the feeding by an infected *A. americanum* tick in a vector competence study with Lyme borreliosis spirochetes.

7.2. Dermacentor andersoni

The vector competence of *D. andersoni* was evaluated for uncharacterized *B. burgdorferi* s.l. spirochetes (wild strain) and *B. burgdorferi* s.s. (B31) (Dolan et al., 1997; Table 9). Larval ticks acquired spirochetes from infected hosts with low efficiency (11–17 % of freshly fed larvae were infected) but there was no evidence of transstadial passage to the nymphal stage (none of 60 molted nymphs were infected). Subsequent attempts to feed infected nymphs on naïve hosts were unsuccessful as none of the ticks fed on the animals were found to be infected (Table 9). Consequently, no single host has yet been exposed to the feeding by an infected *D. andersoni* tick in a vector competence study with Lyme borreliosis spirochetes.

7.3. Dermacentor occidentalis

The vector competence of *D. occidentalis* was evaluated for uncharacterized *B. burgdorferi* s.l. spirochetes (wild strains) and *B. burgdorferi* s.s. (CA5) (Brown and Lane, 1992; Lane et al., 1994; Table 9). Larval ticks acquired spirochetes from infected hosts with low efficiency (10 % of freshly fed larvae were infected) but there was no evidence of transstadial passage to the nymphal stage (none of 84 molted nymphs were infected). As there was no evidence of transstadial spirochete passage, no attempt was made to feed resulting nymphs on naïve hosts.

7.4. Dermacentor variabilis

The vector competence of *D. variabilis* was evaluated for uncharacterized *B. burgdorferi* s.l. spirochetes (MI-119, MI-128, and MI-129), *B. burgdorferi* s.s. (JD1, B31, NC-2, and SI-1),

and *B. bissettiae* (MI-6) (Piesman and Sinsky, 1988; Mather and Mather, 1990; Mukolwe et al., 1992; Sanders and Oliver, 1995; Piesman and Happ, 1997; Soares et al., 2006; Table 9). The efficiency of spirochete acquisition by larval ticks varied from 10 to 61% across uncharacterized *B. burgdorferi* s.l. spirochetes (Piesman and Happ, 1997) and 0–58 % for isolates of *B. burgdorferi* s.s. (Piesman and Sinsky, 1988; Mather and Mather, 1990; Mukolwe et al., 1992; Piesman and Happ, 1997). A few instances of transstadial spirochete transmission to the resulting nymphs were recorded (Piesman and Happ, 1997; Soares et al., 2006), but the overall infection prevalence in resulting nymphs was very low: 3.3 % (2/61) for uncharacterized *B. burgdorferi* s.l. spirochetes, 0.3 % (1/306) for *B. burgdorferi* s.s., and 0 % (0/105) for *B. bissettiae* (Table 9). Four studies attempted to feed infected nymphs on naïve hosts but the outcomes are difficult to interpret because none of the ticks fed on the animals were found to be infected in one study (Piesman and Happ, 1997) and the infection status of the fed nymphs was not examined in the other three studies (Mukolwe et al., 1992; Sanders and Oliver, 1995; Soares et al., 2006). Of the 51 hosts included in these transmission experiments, 50 showed no evidence of spirochete exposure following tick feeding. The remaining host, a rabbit, reportedly seroconverted after exposure to *D. variabilis* nymphs, but there was no evidence presented for infection in the nymphal ticks fed on the rabbit and no spirochetes could be isolated from rabbit tissues (liver, spleen, heart, kidneys, and urinary bladder) taken at necropsy (Mukolwe et al., 1992). In contrast, rabbits exposed to *I. scapularis* ticks infected with the same spirochete isolate in the study uniformly were both seroreactive and yielded tissues from which spirochetes were cultured (Mukolwe et al., 1992). Consequently, no single host has yet been unequivocally exposed to the feeding by an infected *D. variabilis* tick in a vector competence study with Lyme borreliosis spirochetes.

7.5. Haemaphysalis longicornis (North American population)

The vector competence of a North American population of *H. longicornis* was evaluated for *B. burgdorferi* s.s. (B31) (Breuner et al., 2020; Table 9). Larval ticks efficiently acquired spirochetes from infected hosts (56 % of fed ticks harvested within 1 d after repletion were infected) but there was no evidence of transstadial spirochete passage as all 520 examined resulting nymphs tested negative for *B. burgdorferi* s.s. As there was no evidence of transstadial spirochete passage, no attempt was made to feed resulting nymphs on naïve hosts. Similar results for an Asian population of *H. longicornis* are described in section 8.4.

8. Vector competence studies with Palearctic/Oriental *Dermacentor* and *Haemaphysalis* ticks

To date, 4 species of Palearctic/Oriental *Dermacentor* and *Haemaphysalis* ticks have been examined in vector competence studies with *B. burgdorferi* s.l. spirochetes (Table 9). None of these 4 tick species were experimentally confirmed as vectors of any evaluated *B. burgdorferi* s.l. spirochete. The strength of the evidence against vector competence is outlined for each tick species below.

8.1. Dermacentor nuttalli

The vector competence of *D. nuttalli* was evaluated for *B. garinii* (wild strains) (Sun et al., 2003a; Table 9). Larval or nymphal ticks efficiently acquired spirochetes from infected hosts (50–60 % of fed ticks harvested 1 d after repletion were infected) but spirochetes could no longer be detected from fed larval ticks harvested 8 d after repletion or nymphal ticks harvested 12 d after repletion (Sun et al., 2003a). Moreover, there was no evidence of transstadial passage to the resulting nymphs or adults examined (no data presented for numbers of ticks tested). The subsequent attempts to feed infected ticks on naïve hosts had uniformly negative outcomes (no hosts showing evidence of spirochete exposure) but are difficult to interpret because the infection status of the fed ticks was not examined (Table 9). Consequently, no single host has yet been unequivocally exposed to the feeding by an infected *D. nuttalli* tick in a vector competence study with Lyme borreliosis spirochetes.

8.2. Dermacentor silvarum

The vector competence of *D. silvarum* was evaluated for *B. garinii* (wild strains) (Sun and Xu, 2003; Table 9). Larval or nymphal ticks efficiently acquired spirochetes from infected hosts (40–50 % of fed ticks harvested 1 d after repletion were infected) but spirochetes could no longer be detected from fed larval ticks harvested 8 d after repletion or nymphal ticks harvested 15 d after repletion (Sun and Xu, 2003). Moreover, there was no evidence of transstadial passage to the resulting nymphs or adults examined (no data presented for numbers of ticks tested). The subsequent attempts to feed infected ticks on naïve hosts had uniformly negative outcomes (no hosts showing evidence of spirochete exposure) but are difficult to interpret because the infection status of the fed ticks was not examined (Table 9). Consequently, no single host has yet been unequivocally exposed to the feeding by an infected *D. silvarum* tick in a vector competence study with Lyme borreliosis spirochetes.

8.3. Haemaphysalis concinna

The vector competence of *H. concinna* was evaluated for *B. garinii* (wild strains) (Sun and Xu, 2003; Table 9). Larval or nymphal ticks efficiently acquired spirochetes from infected hosts (70–80 % of fed ticks harvested 1 d after repletion were infected) but the prevalence of infection fell to <10 % for fed larval ticks harvested 8 d after repletion or fed nymphal ticks harvested 15 d after repletion (Sun and Xu, 2003). Moreover, there was no evidence of transstadial passage to the resulting nymphs or adults examined (no data presented for numbers of ticks tested). The subsequent attempts to feed infected ticks on naïve hosts had uniformly negative outcomes (no hosts showing evidence of spirochete exposure) but are difficult to interpret because the infection status of the fed ticks was not examined (Table 9). Consequently, no single host has yet been unequivocally exposed to the feeding by an infected *H. concinna* tick in a vector competence study with Lyme borreliosis spirochetes.

8.4. Haemaphysalis longicornis (Asian population)

The vector competence of an Asian population of *H. longicornis* was evaluated for *B. garinii* (wild strains) (Sun et al., 2003a; Table 9). Larval or nymphal ticks efficiently acquired spirochetes from infected hosts (60–70 % of fed ticks harvested 1 d after repletion were infected) but spirochetes could no longer be detected from fed larval ticks harvested 8 d

after repletion or nymphal ticks harvested 20 d after repletion (Sun et al., 2003a). Moreover, there was no evidence of transstadial passage to the resulting nymphs or adults examined (no data presented for numbers of ticks tested). The subsequent attempts to feed infected ticks on naïve hosts had uniformly negative outcomes (no hosts showing evidence of spirochete exposure) but are difficult to interpret because the infection status of the fed ticks was not examined (Table 9). Consequently, no single host has yet been unequivocally exposed to the feeding by an infected *H. longicornis* tick in a vector competence study with Lyme borreliosis spirochetes. Similar results for a North American population of *H. longicornis* are described in section 7.5.

9. Conclusions and future directions

A reasonable goal for each zoogeographic region is to conduct experimental vector competence studies for combinations of the most commonly human-biting *Ixodes* ticks and geographically relevant potentially human-pathogenic *B. burgdorferi* s.l. species. In North America, both *I. scapularis* and *I. pacificus* were confirmed as vectors of the major Lyme borreliosis spirochete, *B. burgdorferi* s.s., and *I. scapularis* also was shown to be a vector of the recently recognized, human-pathogenic *B. mayonii* (Table 1). Both *I. scapularis* and *I. pacificus* also were confirmed as vectors of the potentially human-pathogenic *B. bissettiae*, but neither species has yet been evaluated for the more recently recognized, potentially human-pathogenic *B. kurtenbachii* (Table 1). The Eurasian tick *I. ricinus* has been experimentally confirmed as a vector of the three major Eurasian Lyme borreliosis spirochetes (*B. burgdorferi* s.s., *B. afzelii*, and *B. garinii*) but not for other geographically relevant *B. burgdorferi* s.l. species occasionally associated with human disease (*Borrelia bavariensis*, *B. bissettiae*, *B. kurtenbachii*, *Borrelia lusitaniae*, *Borrelia spielmanii*, and *Borrelia valaisiana*) (Table 1). The other major human-biting Eurasian tick, *I. persulcatus*, has to date been experimentally confirmed as a vector for *B. garinii* but not for either *B. afzelii* or *B. burgdorferi* s.s. (Table 1). Although demonstrations of vector competence are worth pursuing to complete the evidence base, it is nevertheless important to keep in mind that the four above-mentioned major human-biting species within the *Ixodes ricinus/Ixodes persulcatus* complex uniformly have proven to serve as vectors for every *B. burgdorferi* s.l. species evaluated experimentally to date.

Efforts have been more sporadic for other *Ixodes* species, with vector competence for *B. burgdorferi* s.l. spirochetes experimentally confirmed for an additional 9 species (*I. affinis*, *I. angustus*, *I. dentatus*, *I. hexagonus*, *I. jellisoni*, *I. minor*, *I. muris*, *I. sinensis*, and *I. spinipalpis*). The evidence is weakest for *I. affinis* and *I. minor* (based on unpublished data), and renewed studies to confirm the vector competence of these ticks for *B. burgdorferi* s.s. are merited. As outlined in sections 4–5 above, arguments can be made for renewed studies on all 5 species of *Ixodes* ticks (*I. arboricola*, *I. cookei*, *I. frontalis*, *I. holocyclus*, and *I. ovatus*) where previous studies failed to demonstrate vector competence for *B. burgdorferi* s.l. spirochetes. Although none of these tick species are likely to be highly efficient vectors, which greatly increases the effort required to demonstrate their vector competence, it is overstated to label them incapable of serving as vectors of Lyme borreliosis spirochetes without the benefit of additional studies. As noted in section 6, several additional *Ixodes* species merit experimental evaluation as vectors of geographically relevant Lyme borreliosis

spirochetes: among others these include *I. granulatus*, *I. pavlovskyi*, *I. trianguliceps* and *I. uriae*.

Despite shortcomings of individual studies on the vector competence of different species of *Amblyomma*, *Dermacentor*, and *Haemaphysalis* ticks for *B. burgdorferi* s.l. spirochetes, the collective evidence indicates that ticks of these genera are unlikely to contribute more than minimally, if at all, to transmission of Lyme borreliosis spirochetes. Overall, extensive studies involving more than 1600 ticks have produced only a handful (n = 4) of records of spirochete acquisition followed by transstadial spirochete passage in individual ticks, including 3 records for *D. variabilis* and 1 record for *A. americanum* (Table 9). Moreover, there is still no single documented instance of an infected *Amblyomma*, *Dermacentor*, or *Haemaphysalis* tick having fed on a naïve host in a transmission experiment. This again underscores the difficulty of experimental evaluations involving tick species expected to at best be inefficient vectors of *B. burgdorferi* s.l. spirochetes. To produce even a few infected ticks for the final step of demonstrating transmission from infected ticks to naïve hosts, extreme effort may be needed in the initial step of feeding uninfected ticks of the preceding life stage on infected hosts. Moreover, the importance of documenting the infection status of the ticks having fed on naïve hosts in the final step cannot be overemphasized: if all ticks that fed on the hosts were free of spirochetes nothing can be concluded regarding the capacity of infected ticks to transmit spirochetes while feeding.

Several factors probably contribute to *Amblyomma*, *Dermacentor*, and *Haemaphysalis* ticks being poor vectors for *B. burgdorferi* s.l. spirochetes. Soares et al. (2006) demonstrated that spirochete acquisition by larvae fed on mice infected with *B. burgdorferi* s.s. (B31) was highly effective for *I. scapularis*, moderately effective for *D. variabilis*, and ineffective for *A. americanum*. Moreover, *B. burgdorferi* s.l. spirochetes acquired during a larval blood meal are effectively passed to the resulting nymphs for *I. scapularis* (similar prevalence of infection from fed larvae to molted nymphs) but ineffectively for *D. variabilis* and *A. americanum* (decreasing prevalence of infection from fed larvae to molted nymphs) (Piesman and Sinsky, 1988; Mather and Mather, 1990; Ryder et al., 1992; Piesman and Happ, 1997). Another contributing factor to consider is the presence in *Amblyomma*, *Dermacentor*, and *Haemaphysalis* ticks of antimicrobial peptides (defensins) that are lytic to *B. burgdorferi* s.l. spirochetes (Johns et al., 2000, 2001a, 2001b; Sonenshine et al., 2005; Todd et al., 2007; Chrudimska et al., 2014; Sun et al., 2017). Future research to better understand the mechanisms resulting in some ticks being permissive and others refractory to infection with Lyme borreliosis spirochetes is warranted.

Acknowledgments

References

Ackermann R, Kabatzki J, Boisten HP, Steere AC, Grodzicki RL, Hartung S, Runne U, 1984. *Ixodes ricinus* spirochete and European erythema chronicum migrans disease. Yale J. Biol. Med 57, 573–580. [PubMed: 6393609]

Ai C, Hu R, Hyland KE, Wen Y, Zhang Y, Qiu Q, Li D, Liu X, Shi Z, Zhao J, Cheng D, 1990. Epidemiological and aetiological evidence for transmission of Lyme disease by adult *Ixodes persulcatus* in an endemic area in China. Int. J. Epidemiol 19, 1061–1065. [PubMed: 2083990]

Anderson JF, Magnarelli LA, Burgdorfer W, Barbour AG, 1983. Spirochetes in *Ixodes dammini* and mammals from Connecticut. Am. J. Trop. Med. Hyg 32, 818–824. [PubMed: 6881431]

Anderson JF, Johnson RC, Magnarelli LA, Hyde FW, 1985. Identification of endemic foci of Lyme disease: isolation of *Borrelia burgdorferi* from feral rodents and ticks (Dermacentor variabilis). J. Clin. Microbiol 22, 36–38. [PubMed: 3926816]

Anderson JF, Johnson RC, Magnarelli LA, Hyde FW, 1986. Culturing *Borrelia burgdorferi* from spleen and kidney tissues of wild-caught white-footed mice, *Peromyscus leucopus*. Zentralbl. Bakteriol. Mikrobiol. Hyg. B 263, 34–39.

Arsnoe I, Tsao JI, Hickling GJ, 2019. Nymphal *Ixodes scapularis* questing behavior explains geographic variation in Lyme borreliosis risk in the eastern United States. Ticks Tick. Dis 10, 553–563.

Baranton G, Postic D, Saint Girons I, Boerlin P, Piffaretti JC, Assous M, Grimont PAD, 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov. and Group VS461 sssociated with Lyme borreliosis. Int. J. Syst. Bacteriol 42, 378–383. [PubMed: 1380285]

Barbour AG, Burgdorfer W, Hayes SF, Péter O, Aeschlimann A, 1983. Isolation of a cultivable spirochete from *Ixodes ricinus* ticks of Switzerland. Curr. Microbiol 8, 123–126.

Barker IK, Lindsay LR, Campbell GD, Surgeoner GA, McEwen SA, 1993. The groundhog tick Ixodes cookei (Acari: Ixodidae): a poor potential vector of Lyme borreliosis. J. Wildl. Dis 29, 416–422. [PubMed: 8355343]

Beard CB, Occi J, Bonilla DL, Egizi AM, Fonseca DM, Mertins JW, Backenson BP, Bajwa WI, Barbarin AM, Bertone MA, Brown J, Connally NP, Connell ND, Eisen RJ, Falco RC, James AM, Krell RK, Lahmers K, Lewis N, Little SE, Neault M, Pérez de León AA, Randall AR, Ruder MG, Saleh MN, Schappach BL, Schroeder BA, Seraphin LL, Wehtje M, Wormser GP, Yabsley MJ, Halperin W, 2018. Multistate infestation with the exotic disease-vector tick *Haemaphysalis longicornis*— United States, August 2017–September 2018. Morb. Mortal. Rep. Surveill. Summ 67, 1310–1313.

Benach JL, Bosler EM, Hanrahan JP, Coleman JL, Habicht GS, Bast TF, Cameron DJ, Ziegler JL, Barbour AG, Burgdorfer W, Edelman R, Kaslow RA, 1983. Spirochetes isolated from the blood of two patients with Lyme disease. N. Engl. J. Med 308, 740–742. [PubMed: 6828119]

Bosler EM, Coleman JL, Benach JL, Massey DA, Hanrahan JP, Burgdorfer W, Barbour AG, 1983. Natural distribution of the *Ixodes dammini* spirochete. Science 220, 321–322. [PubMed: 6836274]

Breuner NE, Hojgaard A, Replogle AJ, Boegler KA, Eisen L, 2018. Transmission of the relapsing fever spirochete, *Borrelia miyamotoi*, by single transovarially-infected larval *Ixodes scapularis* ticks. Ticks Tick. Dis 9, 1464–1467.

Breuner NE, Ford SL, Hojgaard A, Osikowicz LM, Parise CM, Rosales Rizzo MF, Bai Y, Levin ML, Eisen RJ, Eisen L, 2020. Failure of the Asian longhorned tick, Haemaphysalis longicornis, to serve as an experimental vector of the Lyme disease spirochete, Borrelia burgdorferi sensu stricto. Ticks Tick. Dis 11, 101311.

Brisson D, Vandermause MF, Meece JK, Reed KD, Dykhuizen DE, 2010. Evolution of Northeastern and Midwestern *Borrelia burgdorferi*, United States. Emerg. Infect. Dis 16, 911–917. [PubMed: 20507740]

Brown RN, Lane RS, 1992. Lyme disease in California: a novel enzootic transmission cycle of *Borrelia burgdorferi*. Science 256, 1439–1442. [PubMed: 1604318]

Brown RN, Lane RS, 1996. Reservoir competence of four chaparral-dwelling rodents for *Borrelia burgdorferi* in California. Am. J. Trop. Med. Hyg 54, 84–91. [PubMed: 8651378]

Bunikis J, Olsen B, Fingerle V, Bonnedahl J, Wilske B, Bergström S, 1996. Molecular polymorphism of the Lyme disease agent *Borrelia garinii* in Northern Europe is influenced by a novel enzootic *Borrelia* focus in the North Atlantic. J. Clin. Microbiol 34, 364–368. [PubMed: 8789017]

Bunikis J, Garpmo U, Tsao J, Berglund J, Fish D, Barbour AG, 2004. Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. Microbiology 150, 1741–1755. [PubMed: 15184561]

Burgdorfer W, 1984. The New Zeland white rabbit: an experimental host for infecting ticks with Lyme disease spirochetes. Yale J. Biol. Med 57, 609–612. [PubMed: 6393612]

Burgdorfer W, Gage KL, 1986. Susceptibility of the black-legged tick, *Ixodes scapularis*, to the Lyme disease spirochete *Borrelia burgdorferi*. Zentralbl. Bakteriol. Mikrobiol. Hyg. B 263, 15–20.

Burgdorfer W, Gage KL, 1987. Susceptibility of the hispid cotton rat (Sigmodon hispidus) to the Lyme disease spirochete (Borrelia burgdorferi). Am. J. Trop. Med. Hyg 37, 624–628. [PubMed: 3688316]

Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP, 1982. Lyme disease - a tick-borne spirochetosis? Science 216, 1317–1319. [PubMed: 7043737]

Burgdorfer W, Barbour AG, Hayes SF, Péter O, Aeschlimann A, 1983. Erythema chronicum migrans - a tick-borne spirochetosis. Acta Trop. 40, 79–84. [PubMed: 6134457]

Burgdorfer W, Lane RS, Barbour AG, Gresbrink RA, Anderson JR, 1985. The western black-legged tick, *Ixodes pacificus*: a vector of *Borrelia burgdorferi*. Am. J. Trop. Med. Hyg 34, 925–930. [PubMed: 3898886]

Burkot TR, Schneider BS, Pieniazek NJ, Happ CM, Rutherford JS, Slemenda SB, Hoffmeister E, Maupin GO, Zeidner NS, 2000. *Babesia microti* and *Borrelia bissettii* transmission by *Ixodes spinipalpis* ticks among prairie voles, *Microtus* ochrogaster, in Colorado. Parasitology 121, 595–599. [PubMed: 11155930]

Burkot TR, Happ CM, Dolan MC, Maupin GO, 2001. Infection of *Ixodes scapularis* (Acari: Ixodidae) with *Borrelia burgdorferi* using a new artificial feeding technique. J. Med. Entomol. 38, 167–171. [PubMed: 11296818]

Canica MM, Nato F, Du Merle L, Mazie JC, Baranton G, Postic D, 1993. Monoclonal antibodies for identification of *Borrelia afzelii* sp. nov. associated with late cutaneous manifestations of Lyme borreliosis. Scand. J. Infect. Dis 25, 441–448.

Chao L-L, Wu W-J, Shih C-M, 2009. First detection and molecular identification of *Borrelia burgdorferi*-like spirochetes in *Ixodes granulatus* ticks collected on Kinmen Island of Taiwan. Am. J. Trop. Med. Hyg 80, 389–394. [PubMed: 19270287]

Chao L-L, Wu W-J, Shih C-M, 2012. Prevalence and molecular identification of *Borrelia* spirochetes in *Ixodes granulatus* ticks collected from *Rattus losea* on Kinmen Island of Taiwan. Parasit. Vectors 5, 167. [PubMed: 22882955]

Chrudimska T, Cerovsky V, Slaninova J, Rego ROM, Grubhoffer L, 2014. Defensin from the ornate sheep tick *Dermacentor marginatus* and its effect on Lyme borreliosis spirochetes. Dev. Comp. Immunol 46, 165–170. [PubMed: 24747431]

Couper LI, Kwan JY, Ma J, Swei A, 2019. Drivers and patterns of microbial community assembly in a Lyme disease vector. Ecol. Evol 9, 7768–7779. [PubMed: 31346439]

Crippa M, Rais O, Gern L, 2002. Investigations on the mode and dynamics of transmission and infectivity of *Borrelia burgdorferi* sensu stricto and *Borrelia afzelii* in *Ixodes ricinus* ticks. Vector Borne Zoonotic Dis. 2, 3–9. [PubMed: 12656125]

Cross ST, Kapuscinski ML, Perino J, Maertens BL, Weger-Lucarelli J, Ebel GD, Stenglein MD, 2018. Co-infection patterns in individual *Ixodes scapularis* ticks reveal associations between viral, eukaryotic and bacterial microorganisms. Viruses 10, 388.

de la Fuente J, Antunes S, Bonnet S, Cabezas-Cruz A, Domingos AG, Estrada-Peña A, Johnson N, Kocan KM, Mansfield KL, Nijhof AM, Papa A, Rudenko N, Villar M, Alberdi P, Torina A, Ayllón N, Vancova M, Golovchenko M, Grubhoffer L, Caracappa S, Fooks AR, Gortazar C, Rego ROM, 2017. Tickpathogen interactions and vector competence: identification of molecular drivers for tick-borne diseases. Front. Cell. Infect. Microbiol 7, 114. [PubMed: 28439499]

Derdakova M, Dudioak V, Brei B, Brownstein JS, Schwartz I, Fish D, 2004. Interaction and transmission of two *Borrelia burgdorferi* sensu stricto strains in a tickrodent maintenance system. Appl. Environ. Microbiol 70, 6783–6788. [PubMed: 15528545]

Des Vignes F, Piesman J, Heffernan R, Schulze TL, Stafford Stafford III, Fish D, 2001. Effect of tick removal on transmission of *Borrelia burgdorferi* and *Ehrlichia phagocytophila* by *Ixodes scapularis* nymphs. J. Infect. Dis 183, 773–778. [PubMed: 11181154]

Diuk-Wasser MA, Vannier E, Krause PJ, 2016. Coinfection by *Ixodes* tick-borne pathogens: ecological, epidemiological, and clinical consequences. Trends Parasitol. 32, 30–42. [PubMed: 26613664]

Doby JM, Bigaignon G, Launay H, Costil C, Lambert MC, Lorvellec O, Rolland C, 1990. Presence de Borrelia burgdorferi, agent de spirochetoses a tiques, chez Ixodes (Exopalpiger) trianguliceps Birula, 1895 et Ixodes (Ixodes) acuminatus Neumann 1901 (Acariens Ixodidae) et chez Ctenophthalmus baeticus arvernus Jordan, 1931 et Megabothris turbidus (Rothschild, 1909) (Insectes Siphonaptera), ectoparasites de micromammiferes de forets dans l'ouest de la France. Bull. Soc. Franc. Parasitol 8, 311–322 [in French].

Dolan MC, Maupin GO, Panella NA, Golde WT, Piesman J, 1997. Vector competence of Ixodes scapularis, I. spinipalpis, and Dermacentor andersoni (Acari: Ixodidae) in transmitting Borrelia burgdorferi, the etiologic agent of Lyme disease. J. Med. Entomol 34, 128–135. [PubMed: 9103755]

Dolan MC, Piesman J, Mbow ML, Maupin GO, Péter O, Brossard M, Golde WT, 1998. Vector competence of Ixodes scapularis and Ixodes ricinus (Acari: Ixodidae) for three genospecies of Borrelia burgdorfer*i*. J. Med. Entomol 35, 465–470. [PubMed: 9701928]

Dolan MC, Lacombe EH, Piesman J, 2000. Vector competence of Ixodes muris (Acari: Ixodidae) for Borrelia bur*gdorferi*. J. Med. Entomol 37, 766–768. [PubMed: 11004792]

Dolan MC, Hojgaard A, Hoxmeier JC, Replogle AJ, Respicio-Kingry LB, Sexton C, Williams MA, Pritt BS, Schriefer ME, Eisen L, 2016. Vector competence of the blacklegged tick, *Ixodes scapularis*, for the recently recognized Lyme borreliosis spirochete Candidatus *Borrelia mayonii*. Ticks Tick. Dis 7, 665–669.

Dolan MC, Breuner NE, Hojgaard A, Hoxmeier JC, Pilgard MA, Replogle AJ, Eisen L, 2017a. Duration of *Borrelia mayonii* infectivity in an experimental mouse model for feeding *Ixodes scapularis* larvae. Ticks Tick. Dis. 8, 196–200.

Dolan MC, Breuner NE, Hojgaard A, Boegler KA, Hoxmeier JC, Replogle AJ, Eisen L, 2017b. Transmission of the Lyme disease spirochete *Borrelia mayonii* in relation to duration of attachment by nymphal Ixodes scapularis (Acari: Ixodidae). J. Med. Entomol 54, 1360–1364. [PubMed: 28874016]

Donahue JG, Piesman J, Spielman A, 1987. Reservoir competence of white-footed mice for Lyme disease spirochetes. Am. J. Trop. Med. Hyg 36, 92–96. [PubMed: 3812887]

Eisen L, 2018. Pathogen transmission in relation to duration of attachment by *Ixodes scapularis* ticks. Ticks Tick. Dis 9, 535–542.

Eisen L, Lane RS, 2002. Vectors of *Borrelia burgdorferi* sensu lato. In: Gray JS, Kahl O, Lane RS, Stanek G (Eds.), Lyme Borreliosis Biology, Epidemiology and Control. CABI Publishing, New York, pp. 91–115.

Eisen L, Dolan MC, Piesman J, Lane RS, 2003. Vector competence of Ixodes pacificus and I. spinipalpis (Acari: Ixodidae), and reservoir competence of the dusky-footed woodrat (Neotoma fuscipes) and the deer mouse (Peromyscus maniculatus), for *Borrelia bissettii*. J. Med. Entomol. 40, 311–320. [PubMed: 12943110]

Eisen L, Breuner NE, Hojgaard A, Hoxmeier JC, Pilgard MA, Replogle AJ, Biggerstaff BJ, Dolan MC, 2017. Comparison of vector efficiency of Ixodes scapularis (Acari: Ixodidae) from the Northeast and Upper Midwest of the United States for the Lyme disease spirochete *Borrelia mayonii* J. Med. Entomol 54, 239–242.

Eisen RJ, Eisen L, 2018. The blacklegged tick, *Ixodes scapularis*: an increasing health concern. Trends Parasitol. 34, 295–309. [PubMed: 29336985]

Eisen RJ, Eisen L, Beard CB, 2016. County-scale distribution of Ixodes scapularis and Ixodes pacificus (Acari: Ixodidae) in the continental United States. J. Med. Entomol 53, 349–386. [PubMed: 26783367]

Estrada-Peña A, Nava S, Petney T, 2014. Description of all the stages of Ixodes inopinatus n. sp. (Acari: Ixodidae). Ticks Tick. Dis 5, 734–743.

Filippova NA, 1999. Systematic relationships of the *Ixodes ricinus* complex in the Palearctic faunal region. In: Needham GR, Mitchell R, Horn DJ, Welbourn WC (Eds.), Acarology IX, Volume 2. Ohio Biological Survey, Columbus, Ohio, USA, pp. 355–361.

Fingerle V, Rauser S, Hammer B, Kahl O, Heimerl C, Schulte-Spechtel U, Gern L, Wilske B, 2002. Dynamics of dissemination and outer surface protein expression of different European *Borrelia burgdorferi* sensu lato strains in artificially infected *Ixodes ricinus* nymphs. J. Clin. Microbiol 40, 1456–1463. [PubMed: 11923373]

Fiserova L, Cerna K, Horka H, Kopecky J, 2008. Two ways of experimental infection of Ixodes ricinus ticks (Acari: Ixodidae) with spirochetes of Borrelia burgdorferi sensu lato complex. Folia Parasitol. 55, 150–154.

Gall CA, Reif KE, Scoles GA, Mason KL, Mousel M, Noh SM, Brayton KA, 2016. The bacterial microbiome of *Dermacentor andersoni* ticks influences pathogen susceptibility. ISME J. 10, 1846–1855. [PubMed: 26882265]

Gern L, Rais O, 1996. Efficient transmission of *Borrelia* burgdorferi between cofeeding Ixodes ricinus ticks (Acari: Ixodidae). J. Med. Entomol. 33, 189–192. [PubMed: 8906929]

Gern L, Toutoungi LN, Hu CM, Aeschilimann A, 1991. Ixodes (Pholeoixodes) hexagonus, an efficient vector of Borrelia burgdorferi in the laboratory. Med. Vet. Entomol 5, 431–436. [PubMed: 1773121]

Gern L, Schaible UE, Simon MM, 1993. Mode of inoculation of the Lyme disease agent *Borrelia burgdorferi* influences infection and immune responses in inbred strains of mice. J. Infect. Dis 167, 971–975. [PubMed: 8450262]

Gern L, Siegenthaler M, Hu CM, Leuba-Garcia S, Humair PF, Moret J, 1994. Borrelia burgdorferi in rodents (Apodemus flavicollis and A. sylvaticus): duration and enhancement of infectivity for Ixodes ricinus ticks. Eur. J. Epidemiol 10, 75–80. [PubMed: 7957795]

Goddard J, Embers M, Hojgaard A, Piesman J, 2015. Comparison of tick feeding success and vector competence for Borrelia burgdorferi among immature Ixodes scapularis (Ixodida: Ixodidae) of both southern and northern clades. J. Med. Entomol 52, 81–85. [PubMed: 26336283]

Gorelova NB, Korenberg EI, Kovalevskii, Yu V, Postic D, Baranton G, 1996. Isolation of Borrelia from Ixodes trianguliceps (Ixodidae) ticks and possible significance of this species in epizootiology of ixodid tick-borne borrelioses. Parazitologiya 30, 13–18 [in Russian].

Gorelova NB, Korenberg EI, Filippova NA, Postic D, 2001. First isolation of *Borrelia* pathogenic for humans from ticks *Ixodes pavlovskyi* Pom. Dokl. Ross. Akad. Nauk 378, 558–559 [in Russian].

Greay TL, Gofton AW, Paparini A, Ryan UM, Oskam CL, Irwin PJ, 2018. Recent insights into the tick microbiome gained through next-generation sequencing. Parasit. Vectors 11, 12.

Guglielmone AA, Robbins RG, Apanaskevich DA, Petney TA, Estrada-Peña A, Horak IG, 2014. The Hard Ticks of the World (Acari: Ixodida: Ixodidae). Springer, Heidelberg, Germany.

Gylfe Å, Olsen B, Strasevicius D, Ras NM, Weihe P, Noppa L, Östberg Y, Baranton G, Bergström S, 1999. Isolation of Lyme disease Borrelia from puffins (Fratercula arctica) and seabird ticks (Ixodes uriae) on the Faeroe Islands. J. Clin. Microbiol 37, 890–896. [PubMed: 10074497]

Heylen D, Sprong H, van Oers K, Fonville M, Leirs H, Matthysen E, 2014. Are the specialized bird ticks, *Ixodes arboricola* and *I. frontalis*, competent vectors for *Borrelia burgdorferi* sensu lato? Environ. Microbiol 16, 1081–1089. [PubMed: 24237635]

Hinckley AF, Connally NP, Meek JI, Johnson BJ, Kemperman MM, Feldman KA, White JL, Mead PS, 2014. Lyme disease testing by large commercial laboratories in the United States. Clin. Infect. Dis 59, 676–681. [PubMed: 24879782]

Hoen AG, Margos G, Bent SJ, Diuk-Wasser MA, Barbour A, Kurtenbach K, Fish D, 2009. Phylogeography of *Borrelia burgdorferi* in the eastern United States reflects multiple independent Lyme disease emergence events. Proc. Natl. Acad. Sci. U.S.A 106, 15013–15018. [PubMed: 19706476]

Hou J, Ling F, Chai C, Lu Y, Yu X, Lin J, Sun J, Chang Y, Ye X, Gu S, Pang W, Wang C, Zheng X, Jiang J, Chen Z, Gong Z, 2015. Prevalence of *Borrelia burgdorferi* sensu lato in ticks from eastern China. Am. J. Trop. Med. Hyg 92, 262–266. [PubMed: 25548382]

Hu CM, Wilske B, Fingerle V, Lobet Y, Gern L, 2001. Transmission of *Borrelia garinii* OspA serotype 4 to BALB/c mice by *Ixodes ricinus* ticks collected in the field. J. Clin. Microbiol 39, 1169–1171. [PubMed: 11230451]

Hu CM, Cheminade Y, Perret JL, Weynants V, Lobet Y, Gern L, 2003. Early detection of *Borrelia burgdorferi* sensu lato infection in Balb/c mice by co-feeding *Ixodes ricinus* ticks. Zentralbl. Bakteriol. 293, 421–426.

Hubbard MJ, Baker AS, Cann KJ, 1998. Distribution of Borrelia burgdorferi s.l. spirochaete DNA in British ticks (Argasidae and Ixodidae) since the 19th Century, assessed by PCR. Med. Vet. Entomol. 12, 89–97. [PubMed: 9513944]

Jacobs MB, Purcell JE, Philipp MT, 2003. Ixodes scapularis ticks (Acari: Ixodidae) from Louisiana are competent to transmit Borrelia burgdorferi, the agent of Lyme borreliosis. J. Med. Entomol 40, 964–967. [PubMed: 14765677]

Johns R, Sonenshine DE, Hynes WL, 1998. Control of bacterial infections in the hard tick Dermacentor variabilis (Acari: Ixodidae): evidence for the existence of antimicrobial proteins in the tick hemolymph. J. Med. Entomol 35, 458–464. [PubMed: 9701927]

Johns R, Sonenshine DE, Hynes WL, 2000. Response of the tick Dermacentor variabilis (Acari: Ixodidae) to hemocoelic inoculation of Borrelia burgdorferi (Spirochetales). J. Med. Entomol 37, 265–270. [PubMed: 10730498]

Johns R, Sonenshine DE, Hynes WL, 2001a. Identification of a defensin from the hemolymph of the American dog tick, *Dermacentor variabilis*. Insect Biochem. Mol. Biol 31, 857–865. [PubMed: 11439245]

Johns R, Ohnishi J, Broadwater AH, Sonenshine DE, de Silva AM, Hynes WL, 2001b. Contrasts in tick innate immune responses to *Borrelia burgdorferi* challenge: Immunotolerance in *Ixodes scapularis* versus immunocompetence in Dermacentor variabilis (Acari: Ixodidae). J. Med. Entomol 38, 99–107. [PubMed: 11268698]

Johnson RC, Schmid GP, Hyde FW, Steigerwalt AG, Brenner DJ, 1984a. *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. Int. J. Syst. Bacteriol 34, 496–497.

Johnson RC, Marek N, Kodner C, 1984b. Infection of Syrian hamsters with Lyme disease spirochetes. J. Clin, Bacteriol 20, 1099–1101.

Kahl O, Janetzki-Mittman C, Gray JS, Jonas R, Stein J, de Boer R, 1998. Risk of infection with *Borrelia burgdorferi* sensu lato for a host in relation to the duration of nymphal *Ixodes ricinus* feeding and the method of tick removal. Zentralbl. Bakteriol 287, 41–52. [PubMed: 9532263]

Kahl O, Gern L, Eisen L, Lane RS, 2002. Ecological research on Borrelia burgdorferi sensu lato: terminology and some methodological pitfalls. In: Gray JS, Kahl O, Lane RS, Stanek G (Eds.), Lyme Borreliosis Biology, Epidemiology and Control. CABI Publishing, New York, pp. 29–46.

Keirans JE, Needham GR, Oliver JH Jr., 1999. The *Ixodes ricinus* complex worldwide: diagnosis of the species in the complex, hosts and distribution. In: Needham GR, Mitchell R, Horn DJ, Welbourn WC (Eds.), Acarology IX, Volume 2. Ohio Biological Survey, Columbus, Ohio, USA, pp. 341–347.

Korenberg EI, 1994. Comparative ecology and epidemiology of Lyme disease and tick-borne encephalitis in the former Soviet Union. Parasitol. Today 10, 157–160. [PubMed: 15275486]

Korenberg EI, Kryuchechnikov VN, Kovalevsky YV, Shcherbakov SV, Kuznetsova RI, Levin ML, 1987. The tick *Ixodes persulcatus* Schulze as a new vector of *Borrelia burgdorferi*. Dokl. Akad. Nauk 297, 1268–1270 [In Russian].

Korenberg EI, Shcherbakov SV, Kovalevsky YV, Kryuchechnikov VN, Nikitina OV, 1988. Transmission of *Borrelia burgdorferi* from nymphs to adults in the tick *Ixodes persulcatus* Schulze. Dokl. Akad. Nauk 302, 759–760 [In Russian].

Korenberg EI, Gorelova NB, Kovalevskii YV, 2002. Ecology of Borrelia burgdorferi sensu lato in Russia. In: Gray JS, Kahl O, Lane RS, Stanek G (Eds.), Lyme Borreliosis Biology, Epidemiology and Control. CABI Publishing, New York, pp. 175–200.

Korenberg EI, Nefedova VV, Romanenko VN, Gorelova NB, 2010. The tick *Ixodes pavlovskyi* as a host of spirochetes pathogenic for humans and its possible role in the epizootiology and epidemiology of borrelioses. Vector-Borne Zoonotic Dis. 10, 453–458. [PubMed: 19929222]

Korenberg EI, Kovalevskii YV, Gorelova NB, Nefedova VV, 2015. Comparative analysis of the roles of *Ixodes persulcatus* and *I. trianguliceps* ticks in natural foci of ixodid tick-borne borrelioses in the Middle Urals, Russia. Ticks Tick. Dis 6, 316–321.

Kornblatt AN, Steere AC, Brownstein DG, 1984. Experimental Lyme disease in rabbits: spirochetes found in erythema migrans and blood. Infect. Immun 46, 220–223. [PubMed: 6480108]

Kovalevskii YV, Korenberg EI, Gorelova NB, Nefedova VV, 2013. The ecology of *Ixodes trianguliceps* ticks and their role in the natural foci of ixodid tick-borne borrelioses in the Middle Urals. Entomol. Rev 93, 1073–1083.

Kryuchechnikov VN, Korenberg EI, Shcherbakov SV, Kovalevsky YV, Levin ML, 1988. Identification of *Borrelia* isolated in the USSR from *Ixodes persulcatus* Schulze ticks. Zh. Mikrobiol. Epidemiol. Immunobiol 1988 (12), 41–44 [In Russian].

Kurilshikov A, Livanova NN, Fomenko NV, Tupikin AE, Rar VA, Kabilov MR, Livanov SG, Tikunova NV, 2015. Comparative metagenomic profiling of symbiotic bacterial communities associated with *Ixodes persulcatus*, *Ixodes pavlovskyi* and *Dermacentor reticulatus* ticks. PLoS One 10, e0131413. [PubMed: 26154300]

Kurtenbach K, Schäfer SM, Sewell H-S, Peacey M, Hoodless AN, Nuttall PA, Randolph SE, 2002. Differential survival of Lyme borreliosis spirochetes in ticks that feed on birds. Infect. Immun 70, 5893–5895. [PubMed: 12228325]

Kwan JY, Griggs R, Chicana B, Miller C, Swei A, 2017. Vertical vs. horizontal transmission of the microbiome in a key disease vector, *Ixodes pacificus*. Mol. Ecol 26, 6578–6589. [PubMed: 29178531]

Lane RS, Lavoie PE, 1988. Lyme borreliosis in California USA: acarological clinical and epidemiological studies. Ann. N.Y. Acad. Sci 539, 192–203. [PubMed: 3263826]

Lane RS, Piesman J, Burgdorfer W, 1991. Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. Annu. Rev. Entomol 36, 587–609. [PubMed: 2006870]

Lane RS, Brown RN, Piesman J, Peavey CA, 1994. Vector competence of Ixodes pacificus and Dermacentor occidentalis (Acari: Ixodidae) for various isolates of Lyme disease spirochetes. J. Med. Entomol 31, 417–424. [PubMed: 8057316]

Lane RS, Peavey CA, Padgett KA, Hendson M, 1999. Life history of Ixodes (Ixodes) jellisoni (Acari: Ixodidae) and its vector competence for Borrelia burgdorferi sensu lato. J. Med. Entomol 36, 329–340. [PubMed: 10337104]

Levin ML, Fish D, 2000. Acquisition of coinfection and simultaneous transmission of *Borrelia burgdorferi* and *Ehrlichia phagocytophila* by *Ixodes scapularis* ticks. Infect. Immun. 68, 2183–2186. [PubMed: 10722618]

Levin ML, Levine JF, Apperson CS, Norris DE, Howard PB, 1995. Reservoir competence of the rice rat (Rodentia: Cricetidae) for Borrelia burgdorferi. J. Med. Entomol 32, 138–142. [PubMed: 7608919]

Levine JF, Wilson ML, Spielman A, 1985. Mice as reservoirs of the Lyme disease spirochete. Am. J. Trop. Med. Hyg 34, 355–360. [PubMed: 3985277]

Li X, Lane RS, 1996. Vector competence of ixodid ticks (Acari) for Borrelia burgdorferi as determined with a capillary-feeding technique. J. Spirochetal Tick-borne Dis 3, 116–123.

Lindsay LR, Barker IK, Surgeoner GA, McEwen SA, Campbell GD, 1997. Duration of *Borrelia burgdorferi* infectivity in white-footed mice for the tick vector *Ixodes scapularis* under laboratory and field conditions in Ontario. J. Wildl. Dis 33, 766–775. [PubMed: 9391960]

Magnarelli LA, Anderson JF, 1988. Ticks and biting insects infected with the etiologic agent of Lyme disease, *Borrelia burgdorferi*. J. Clin. Microbiol 26, 1482–1486. [PubMed: 3170711]

Margos G, Lane RS, Fedorova N, Koloczek J, Piesman J, Hojgaard A, Sing A, Fingerle V, 2016. *Borrelia bissettiae* sp. nov. and *Borrelia californiensis* sp. nov. prevail in diverse enzootic transmission cycles. Int. J. Syst. Evol. Microbiol 66, 1447–1452. [PubMed: 26813420]

Margos G, Fedorova N, Kleinjan JE, Hartberger C, Schwan TG, Sing A, Fingerle V, 2017. *Borrelia lanei* sp. nov. extends the diversity of *Borrelia* species in California. Int. J. Syst. Evol. Microbiol 67, 3872–3876. [PubMed: 28884668]

Mather TN, Mather ME, 1990. Intrinsic competence of three ixodid ticks (Acari) as vectors of the Lyme disease spirochete. J. Med. Entomol 27, 646–650. [PubMed: 2388239]

Mather TN, Telford SR III, Moore SI, Spielman A, 1990. *Borrelia burgdorferi* and *Babesia* microti: efficiency of transmission from reservoirs to vector ticks (Ixodes dammini). Exp. Parasitol 70, 55–61. [PubMed: 2295326]

Mathiesen DA, Oliver JH Jr., Kolbert CP, Tullson ED, Johnson BJB, Campbell GL, Mitchell PD, Reed KD, Telford III SR, Anderson JF, Lane RS, Persing DH, 1997. Genetic heterogeneity of *Borrelia burgdorferi* in the United States. J. Infect. Dis 175, 98–107. [PubMed: 8985202]

Maupin GO, Gage KL, Piesman J, Montenieri J, Sviat SL, VanderZanden L, Happ CM, Dolan M, Johnson BJB, 1994. Discovery of an enzootic cycle of *Borrelia burgdorferi* in *Neotoma mexicana* and *Ixodes spinipalpis* from northern Colorado, an area where Lyme disease is nonendemic. J. Infect. Dis 170, 636–643. [PubMed: 8077722]

Miyamoto K, Masuzawa T, 2002. Ecology of Borrelia burgdorferi sensu lato in Japan and East Asia. In: Gray JS, Kahl O, Lane RS, Stanek G (Eds.), Lyme Borreliosis Biology, Epidemiology and Control. CABI Publishing, New York, pp. 201–222.

Miyamoto K, Nakao M, Sato N, Mori M, 1991. Isolation of Lyme disease spirochetes from an ixodid tick in Hokkaido, Japan. Acta Trop. 49, 65–68. [PubMed: 1678577]

Monin R, Gern L, Aeschlimann A, 1989. A study of the different modes of transmission of *Borrelia burgdorferi* by *Ixodes ricinus*. Zentralbl. Bakteriol. Hyg 263, 14–20.

Moutailler S, Moro CV, Vaumourin E, Michelet L, Tran FH, Devillers E, Cosson J-F, Gasqui P, Van VT, Mavingui P, Vourc'h G, Vayssier-Taussat M, 2016. Coinfection of ticks: the rule rather than the exception. PLoS Negl. Trop. Dis 10, e0004539. [PubMed: 26986203]

Mukolwe SW, Kocan AA, Barker RW, Kocan KM, Murphy GL, 1992. Attempted transmission of Borrelia burgdorferi (Spirochaetales: Spirochaetaceae) (JD1 strain) by Ixodes scapularis (Acari: Ixodidae), Dermacentor *variabilis*, and *Amblyomma americanum*. J. Med. Entomol 29, 673–677. [PubMed: 1495078]

Munro HJ, Ogden NH, Lindsay LR, Robertson GJ, Whitney H, Lang AS, 2017. Evidence for *Borrelia bavariensis* infections of *Ixodes uriae* within seabird colonies of the North Atlantic Ocean. Appl. Environ. Microbiol 83, e01087–17. [PubMed: 28802273]

Nakao M, Miyamoto K, 1994. Susceptibility of Ixodes persulcatus and I. ovatus (Acari: Ixodidae) to Lyme disease spirochetes isolated from humans in Japan. J. Med. Entomol 31, 467–473. [PubMed: 8057322]

Nakao M, Sato Y, 1996. Refeeding activity of immature ticks of *Ixodes persulcatus* and transmission of Lyme disease spirochete by partially fed larvae. J. Parasitol 82, 669–672. [PubMed: 8691387]

Narasimhan S, Fikrig E, 2015. Tick microbiome: the force within. Trends Parasitol. 31, 315–323. [PubMed: 25936226]

Nelson CA, Saha S, Kugeler KJ, Delorey MJ, Shankar MB, Hinckley AF, Mead PS, 2015. Incidence of clinician-diagnosed Lyme disease, United States, 2005–2010. Emerg. Infect. Dis 21, 1625–1631. [PubMed: 26291194]

Oliver J, 1996. Lyme borreliosis in the southern United States: a review. J. Parasitol 82, 926–935. [PubMed: 8973401]

Oliver JH Jr., Chandler FW Jr., Luttrell MP, James AM, Stallknecht DE, McGuire BS, Hutcheson HJ, Cummins GA, Lane RS, 1993. Isolation and transmission of the Lyme disease spirochete from the southeastern United States. Proc. Natl. Acad. Sci. U.S.A 90, 7371–7375. [PubMed: 8346258]

Oliver JH Jr., Lin T, Gao L, Clark KL, Banks CW, Durden LA, James AM, Chandler FW Jr., 2003. An enzootic transmission cycle of Lyme borreliosis spirochetes in the southeastern United States. Proc. Natl. Acad. Sci. U.S.A 100, 11642–11645. [PubMed: 14500917]

Olsen B, Jaenson TGT, Noppa L, Bunikis J, Bergström S, 1993. A Lyme borreliosis cycle in seabirds and *Ixodes uriae* ticks. Nature 362, 340–342. [PubMed: 8455718]

Olsen B, Duffy DC, Jaenson TGT, Gylfe Å, Bonnedahl J, Bergström S, 1995. Transhemispheric exchange of Lyme disease spirochetes by seabirds. J. Clin. Microbiol 33, 3270–3274. [PubMed: 8586715]

Parise CM, Breuner NE, Hojgaard A, Osikowicz LM, Replogle AJ, Eisen RJ, Eisen L, 2020. Experimental demonstration of reservoir competence of the white-footed mouse (Peromyscus leucopus) for the Lyme disease spirochete, Borrelia mayonii. J. Med. Entomol 57. 10.1093/jme/tjz242.

Peavey CA, Lane RS, 1995. Transmission of Borrelia burgdorferi by Ixodes pacificus nymphs and reservoir competence of deer mice (Peromyscus maniculatus) infected by tick- bite. J. Parasitol 81, 175–178. [PubMed: 7707191]

Peavey CA, Lane RS, 1996. Comparison of infectivities of six tick-derived isolates of *Borrelia burgdorferi* for rodents and ticks. J. Clin. Microbiol 34, 71–75. [PubMed: 8748276]

Peavey CA, Lane RS, Damrow T, 2000. Vector competence of Ixodes angustus (Acari: Ixodidae) for Borrelia *burgdorferi* sensu stricto. Exp. Appl. Acarol 24, 77–84. [PubMed: 10823359]

Piesman J, 1988. Intensity and duration of *Borrelia burgdorferi* and *Babesia microti* infectivity in rodent hosts. Int. J. Parasitol 18, 687–690. [PubMed: 3170076]

Piesman J, 1991. Experimental acquisition of the Lyme disease spirochete, Borrelia burgdorferi, by larval Ixodes dammini (Acari: Ixodidae) during partial blood meals. J. Med. Entomol 28, 259–262. [PubMed: 2056507]

Piesman J, 1993. Standard system for infecting ticks (Acari: Ixodidae) with the Lyme disease spirochete, *Borrelia burgdorferi*. J. Med. Entomol 30, 199–203. [PubMed: 8433326]

Piesman J, Happ CM, 1997. Ability of the Lyme disease spirochete (Borrelia burgdorferi) to infect rodents and three species of human-biting ticks (blacklegged tick, American dog tick, lone star tick) (Acari: Ixodidae). J. Med. Entomol 34, 451–456. [PubMed: 9220680]

Piesman J, Sinsky RJ, 1988. Ability of Ixodes scapularis, Dermacentor variabilis and Amblyomma americanum (Acari: Ixodidae) to acquire, maintain, and transmit Lyme disease spirochetes (Borrelia burgdorferi). J. Med. Entomol 25, 336–339. [PubMed: 3193425]

Piesman J, Stone BF, 1991. Vector competence of the Australian paralysis tick, *Ixodes holocyclus*, for the Lyme disease spirochete *Borrelia burgdorferi*. Int. J. Parasitol 21, 109–112. [PubMed: 2040556]

Piesman J, Hicks TC, Sinsky RJ, Obiri G, 1987a. Simultaneous transmission of *Borrelia burgdorferi* and *Babesia microti* by individual nymphal *Ixodes dammini* ticks. J. Clin. Microbiol 25, 2012–2013. [PubMed: 3667924]

Piesman J, Mather TN, Sinsky RJ, Spielman A, 1987b. Duration of tick attachment and *Borrelia burgdorferi* transmission. J. Clin. Microbiol 25, 557–558. [PubMed: 3571459]

Piesman J, Mather TN, Dammin GJ, Telford III SR, Lastavica CC, Spielman A, 1987c. Seasonal variation of transmission risk of Lyme dsease and human babesiosis. Am. J. Epidemiol 126, 1187–1189. [PubMed: 3687924]

Piesman J, Maupin GO, Campos EG, Happ CM, 1991. Duration of adult female *Ixodes dammini* attachment and transmission of *Borrelia burgdorferi* with description of a needle aspiration isolation method. J. Infect. Dis 163, 895–897. [PubMed: 2010643]

Piesman J, Clark KL, Dolan MC, Happ CM, Burkot TR, 1999. Geographic survey of vector ticks (Ixodes scapularis and Ixodes pacificus) for infection with the Lyme disease spirochete, Borrelia burgdorferi. J. Vector Ecol 24, 91–98. [PubMed: 10436883]

Policastro PF, Schwan TG, 2003. Experimental infection of Ixodes scapularis larvae (Acari: Ixodidae) by immersion in low passage cultures of *Borrelia burgdorferi*. J. Med. Entomol 40, 364–370. [PubMed: 12943118]

Pospisilova T, Urbanova V, Hes O, Kopacek P, Hajdusek O, Sima R, 2019. Tracking of *Borrelia afzelii* transmission from infected *Ixodes ricinus* nymphs to mice. Infect. Immun 87, e00896–18. [PubMed: 30910791]

Postic D, Korenberg E, Gorelova N, Kovalevski YV, Bellenger E, Baranton G, 1997. *Borrelia burgdorferi* sensu lato in Russia and neighbouring countries: high incidence of mixed isolates. Res. Microbiol 148, 691–702. [PubMed: 9765854]

Qiu WG, Dykhuizen DE, Acosta MS, Luft BJ, 2002. Geographic uniformity of the Lyme disease spirochete (Borrelia burgdorferi) and its shared history with tick vector (Ixodes scapularis) in the northeastern United States. Genetics 160, 833–849. [PubMed: 11901105]

Randolph SE, 1994. The relative contributions of transovarial and transstadial transmission to the maintenance of tick-borne diseases. In: Axford JS, Rees DHE (Eds.), Lyme Borreliosis. Plenum Press, New York, USA, pp. 131–134.

Randolph SE, Nuttall PA, 1994. Nearly right or precisely wrong? Natural versus laboratory studies of vector-borne diseases. Parasitol. Today 10, 458–462. [PubMed: 15275510]

Richter PJ Jr., Kimsey RB, Madigan JE, Brooks DL, 1996. Compatibility of two species of *Ixodes* ticks with murid hosts and its effect on transmission of Lyme disease spirochaetes. Med. Vet. Entomol 10, 291–294. [PubMed: 8887343]

Rollend L, Fish D, Childs JE, 2013. Transovarial transmission of *Borrelia* spirochetes by *Ixodes scapularis*: a summary of the literature and recent observations. Ticks Tick. Dis 4, 46–51.

Rosenberg R, Lindsey NP, Fischer M, Gregory CJ, Hinckley AF, Mead PS, Paz-Bailey G, Waterman SH, Drexler NA, Kersh GJ, Hooks H, Partridge SK, Visser SN, Beard CB, Petersen LR, 2018. Vital signs: trends in reported vector-borne disease cases — United States and Territories, 2004–2016. Morb. Mortal. Rep. Surveill. Summ 67, 496–501.

Rudenko N, Golovchenko M, Grubhoffer L, Oliver JH Jr., 2011. Updates on *Borrelia burgdorferi* sensu lato complex with respect to public health. Ticks Tick. Dis 2, 123–128.

Ryder JW, Pinger RR, Glancy T, 1992. Inability of Ixodes cookei and Amblyomma americanum nymphs (Acari: Ixodidae) to transmit Borrelia burgdorferi. J. Med. Entomol 29, 525–530. [PubMed: 1625302]

Sanders FH, Oliver JH Jr., 1995. Evaluation of Ixodes scapularis, Amblyomma americanum, and Dermacentor variabilis (Acari: Ixodidae) from Georgia as vectors of a Florida strain of the Lyme disease spirochete, *Borrelia burgdorferi*. J. Med. Entomol 32, 402–406. [PubMed: 7650697]

Sato Y, Nakao M, 1997. Transmission of the Lyme disease spirochete, *Borrelia garinii*, between infected and uninfected immature *Ixodes persulcatus* during cofeeding on mice. J. Parasitol 83, 547–550. [PubMed: 9194849]

Scoles GA, Papero M, Beati L, Fish D, 2001. A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. Vector Borne Zoonotic Dis. 1, 21–34. [PubMed: 12653133]

Schulze TL, Bowen GS, Bosler EM, Lakat MF, Parkin WE, Altman R, Ormiston BG, Shisler JK, 1984. *Amblyomma americanum*: a potential vector of Lyme disease in New Jersey. Science 224, 601–603. [PubMed: 6710158]

Schwan TG, Burgdorfer W, Schrumpf ME, Karstens RH, 1988. The urinary bladder, a consistent source of *Borrelia burgdorferi* in experimentally infected white-footed mice (Peromyscus leucopus). J. Clin. Microbiol. 26, 893–895. [PubMed: 3290239]

Shih CM, Spielman A, 1993. Accelerated transmission of Lyme disease spirochetes by partially fed vector ticks. J. Clin. Microbiol 31, 2878–2881. [PubMed: 8263171]

Sinsky RJ, Piesman J, 1989. Ear punch biopsy method for detection and isolation of *Borrelia burgdorferi* from rodents. J. Clin. Microbiol 27, 1723–1727. [PubMed: 2768461]

Smith RP Jr., Muzaffar SB, Lavers J, Lacombe EH, Cahill BK, Lubelczyk CB, Kinsler A, Mathers AJ, Rand PW, 2006. Borrelia garinii in seabird ticks (Ixodes uriae), Atlantic Coast, North America. Emerg. Infect. Dis 12, 1909–1912. [PubMed: 17326943]

Soares CAG, Zeidner NS, Beard CB, Dolan MC, Dietrich G, Piesman J, 2006. Kinetics of *Borrelia burgdorferi* infection in larvae of refractory and competent tick vectors. J. Med. Entomol 43, 61–67. [PubMed: 16506448]

Sonenshine DE, Hynes WE, Ceraul SM, Mitchell R, Benzine T, 2005. Host blood proteins and peptides in the midgut of the tick *Dermacentor variabilis* contribute to bacterial control. Exp. Appl. Acarol 36, 207–223. [PubMed: 16132735]

Spielman A, Levine JF, Wilson ML, 1984. Vectorial capacity of North American *Ixodes* ticks. Yale J. Biol. Med 67, 507–513.

Spielman A, Levine JF, Wilson ML, Piesman J, 1985. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. Annu. Rev. Entomol 30, 439–460. [PubMed: 3882050]

States SL, Huang CI, Davis S, Tufts DM, Diuk-Wasser MA, 2017. Co-feeding transmission facilitates strain coexistence in *Borrelia burgdorferi*, the Lyme disease agent. Epidemics 19, 33–42. [PubMed: 28089780]

Steere AC, Malawista SE, 1979. Cases of Lyme disease in the United States: locations correlated with distribution of *Ixodes dammini*. Ann. Intern. Med 91, 730–733. [PubMed: 496106]

Steere AC, Grodzicki RL, Kornblatt AN, Craft JE, Barbour AG, Burgdorfer W, Schmid GP, Johnson E, Malawista SE, 1983. The spirochetal etiology of Lyme disease. N. Engl. J. Med 308, 733–740. [PubMed: 6828118]

Sun Y, Xu R, 2003. Ability of *Ixodes persulcatus*, *Haemaphysalis concinna* and *Dermacentor silvarum* ticks to acquire and transstadially transmit *Borrelia garinii*. Exp. Appl. Acarol 31, 151–160. [PubMed: 14756409]

Sun Y, Xu R, Tianyu G, Panhe Z, Wuchun C, 2003a. Incapability of *Haemaphysalis longicornis* and *Dermacentor nuttalli* to acquire and trans-stadial transmit the Lyme spirochetes *Borrelia garinii*. Acta Parasitol. Med. Entomol. Sin. 10, 174–180.

Sun Y, Xu R, Cao W, 2003b. *Ixodes sinensis*: competence as a vector to transmit the Lyme disease spirochete *Borrelia garinii*. Vector-borne Zoonotic Dis 3, 39–44. [PubMed: 12804379]

Sun T, Pan W, Song Y, Zhang J, Wang J, Dai J, 2017. Functional characterization of two defensins, HlDFS1 and HlDFS2, from the hard tick *Haemaphysalis longicornis*. Parasit. Vectors 10, 455. [PubMed: 28969703]

Sykes RA, Makiello P, 2016. An estimate of Lyme borreliosis incidence in Western Europe. J. Publ. Health 39, 74–81.

Telford III SR, Spielman A, 1989. Competence of a rabbit-feeding Ixodes (Acari: Ixodidae) as a vector of the Lyme disease spirochete. J. Med. Entomol 26, 118–121. [PubMed: 2709387]

Thapa S, Zhang Y, Allen MS, 2019. Bacterial microbiomes of *Ixodes scapularis* ticks collected from Massachusetts and Texas, USA. BMC Microbiol. 19, 138. [PubMed: 31234774]

Todd SM, Sonenshine DE, Hynes WE, 2007. Tissue and life-stage distribution of a defensin gene in the lone star tick, *Amblyomma americanum*. Med. Vet. Entomol 21, 141–147. [PubMed: 17550433]

Tokarz R, Tagliafierro T, Sameroff S, Cucura DM, Oleynik A, Che X, Jain K, Lipkin WI, 2019. Microbiome analysis of *Ixodes scapularis* ticks from New York and Connecticut. Ticks Tick. Dis 10, 894–900.

Tonetti N, Voordouw MJ, Durand J, Monnier S, Gern L, 2015. Genetic variation in transmission success of the Lyme borreliosis pathogen *Borrelia afzelii*. Ticks Tick. Dis 6, 334–343.

Urbanova V, Hajdusek O, Mondekova HH, Sima R, Kopacek P, 2017. Tick thioester-containing proteins and phagocytosis do not affect transmission of *Borrelia afzelii* from the competent vector *Ixodes ricinus*. Front. Cell. Infect. Microbiol 7, 73. [PubMed: 28361038]

van Duijvendijk G, Coipan C, Wagemakers A, Fonville M, Ersöz J, Oei A, Földvári G, Hovius J, Takken W, Sprong H, 2016. Larvae of *Ixodes ricinus* transmit *Borrelia afzelii* and *B. miyamotoi* to vertebrate hosts. Parasit. Vectors 9, 97. [PubMed: 26896940]

Van Zee J, Piesman JF, Hojgaard A, Black IVWC, 2015. Nuclear markers reveal predominantly north to south gene flow in Ixodes scapularis, the tick vector of the Lyme disease spirochete. PLoS One 10, e0139630. [PubMed: 26536360]

Varela-Stokes AS, Park SH, Kim SA, Ricke SC, 2017. Microbial communities in North American ixodid ticks of veterinary and medical importance. Front. Vet. Sci 4, 179. [PubMed: 29104867]

Wagemakers A, Staarink PJ, Sprong H, Hovius JWR, 2015. *Borrelia miyamotoi:* a widespread tick-borne relapsing fever spirochete. Trends Parasitol. 31, 260–269. [PubMed: 25892254]

Zhang ZF, 1989. Investigation of Lyme disease in northeast of China. Chin. J. Epidemiol 10, 261–264 [In Chinese].

Zhuang L, Du J, Cui X-M, Li H, Tang F, Zhang P-H, Hu J-G, Tong Y-G, Feng Z-C, Liu W, 2018. Identification of tick-borne pathogen diversity by metagenomic analysis in *Haemaphysalis longicornis* from Xinyang, China. Infect. Dis. Poverty 7, 45. [PubMed: 29730989]

Table 1

Overview of outcomes for *Ixodes* tick species evaluated in vector competence studies with *Borrelia burgdorferi* sensu lato spirochetes.

Borrelia burgdorferi sensu lato complex species	Geographic range [b]	Experimental evidence for vector competence of *Ixodes* ticks [c]																	
		affinis	*angustus*	*arboricola*	*cookei*	*dentatus*	*frontalis*	*hexagonus*	*holocyclus*	*jellisoni*	*minor*	*muris*	*ovatus*	*pacificus*	*persulcatus*	*ricinus*	*scapularis*	*sinensis*	*spinipalpis*
Geographic range [b]		NA/NT	NA/PA	OR/PA	NA/NT	NA	PA	PA	AU	NA	NA/NT	NA	OR/PA	NA	OR/PA	PA	NA/NT	OR	NA/NT
Major cause of human illness																			
Borrelia burgdorferi sensu stricto	**NA/PA/OR**	Yes	Yes		No			Yes	No		Yes	Yes		Yes		Yes	Yes		Yes
Borrelia afzelii	**PA/OR**															Yes	Yes		
Borrelia garinii	**PA/OR**			No			No						No		Yes	Yes	Yes	Yes	
Minor cause of human illness																			
Borrelia bavariensis	**PA**																		
Borrelia bissettiae	**NA/PA**										Yes			Yes			Yes		Yes
Borrelia kurtenbachii	**NA/PA**																		
Borrelia lusitaniae	**PA**																		
Borrelia mayonii	**NA**																Yes		
Borrelia spielmanii	**PA**						No												
Borrelia valaisiana	**PA/OR**			No															
Unknown human pathogenicity																			
Borrelia americana	**NA**																		
Borrelia andersonii	**NA**																		
Borrelia californiensis	**NA**									Yes									
Borrelia carolinensis	**NA**																		
Borrelia chilensis	**NT**																		
Borrelia japonica	**PA**																		
Borrelia lanei	**NA**																		
Borrelia sinica	**PA/OR**																		
Borrelia tanukii	**PA**																		
Borrelia turdi	**PA**																		
Borrelia yangtzensis	**PA/OR**																		

Experimental evidence for vector competence of *Ixodes* ticks[c]

	affinis	*angustus*	*arboricola*	*cookei*	*dentatus*	*frontalis*	*hexagonus*	*holocyclus*	*jellisoni*	*minor*	*muris*	*ovatus*	*pacificus*	*persulcatus*	*ricinus*	*scapularis*	*sinensis*	*spinipalpis*
Wild type[a]				No	Yes				Yes				Yes		Yes	Yes		Yes

[a]Uncharacterized *Borrelia burgdorferi* sensu lato spirochetes.

[b]NA, Nearctic; PA, Palearctic; AT, Afrotropical; OR, Oriental; AU, Australian. Based on zoogeographic regions of the world and tick distributions as defined in Guglielmone et al. (2014), and distributions of *B. burgdorferi* s.l. spirochetes from various sources.

[c]Yes, vector competence was confirmed experimentally; No, vector competence was evaluated experimentally but could not be confirmed; Blank space, tick species not yet evaluated for this *B. burgdorferi* s.l. species.

Table 2

Overview of outcomes for *Amblyomma* (*A.*), *Dermacentor* (*D.*), and *Haemaphysalis* (*H.*) tick species evaluated in vector competence studies with *Borrelia burgdorferi* sensu lato spirochetes.

	Geographic range [b]	Experimental evidence for vector competence [c]							
		A. americanum	*D. andersoni*	*D. nuttalli*	*D. occidentalis*	*D. silvarum*	*D. variabilis*	*H. concinna*	*H. longicornis* [d]
Borrelia burgdorferi sensu lato complex species		NA	NA	PA	NA	PA	NA/NT	OR/PA	AU/NA/OR/PA
Major cause of human illness									
Borrelia burgdorferi sensu stricto	NA/PA/OR	No	No		No		No		No
Borrelia afzelii	PA/OR								
Borrelia garinii	PA/OR			No		No		No	No
Minor cause of human illness									
Borrelia bavariensis	PA								
Borrelia bissettiae	NA/PA	No					No		
Borrelia kurtenbachii	NA/PA								
Borrelia lusitaniae	PA								
Borrelia mayonii	NA								
Borrelia spielmanii	PA								
Borrelia valaisiana	PA/OR								
Unknown human pathogenicity									
Borrelia americana	NA								
Borrelia andersonii	NA								
Borrelia californiensis	NA								
Borrelia carolinensis	NA								
Borrelia chilensis	NT								
Borrelia japonica	PA								
Borrelia lanei	NA								
Borrelia sinica	PA/OR								
Borrelia tanukii	PA								
Borrelia turdi	PA								
Borrelia yangtzensis	PA/OR								

	Experimental evidence for vector competence[c]							
	A. americanum	*D. andersoni*	*D. nuttalli*	*D. occidentalis*	*D. silvarum*	*D. variabilis*	*H. concinna*	*H. longicornis*
Wild type [a]		No		No		No		

[a]Uncharacterized *Borrelia burgdorferi* sensu lato spirochetes.

[b]NA, Nearctic; NT, Neotropical; PA, Palearctic; OR, Oriental; AT, Afrotropical; AU, Australian. Based on zoogeographic regions of the world and tick distributions as defined in Gugliemone et al. (2014), and distributions of *B. burgdorferi* s.l. spirochetes from various sources.

[c]Yes, vector competence was confirmed experimentally; No, vector competence was evaluated experimentally but could not be confirmed; Blank space, tick species not yet evaluated for this *B. burgdorferi* s.l. species.

[d]Recently established in the Nearctic (United States) (Beard et al., 2018).

Table 3

Detailed results for studies to evaluate the vector competence of the Nearctic/Neotropical tick *Ixodes scapularis* for *Borrelia burgdorferi* sensu lato spirochetes.

Spirochete species and source/ isolate	Hosts used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection				Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Geographic origin of ticks[f]	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[k]	No. hosts known to have been exposed to at least 1 infected tick[k]	No. hosts for which infection was confirmed after tick feeding	
Uncharacterized *Borrelia burgdorferi* sensu lato															
Wild strain	–	–	NY	–	–	–	–	–	–	Female[j]	White rabbit	2	8	8	Burgdorfer et al., 1982
Wild strain	–	–	NY	–	–	–	–	–	–	Female[j]	White rabbit	10	1	1	Burgdorfer, 1984
Wild strain	White rabbit	Needle[c]	NY	Larva	No data	No data	Nymph	90	8[i]	–	–	–	–	–	Burgdorfer, 1984
Wild strain	White rabbit	Needle[c]	OK	Larva	No data	No data	Nymph	316	22[i]	Nymph	White rabbit	6	1	1	Burgdorfer and Gage, 1986
Wild strain	White rabbit	Tick bite[d]	OK	Larva	No data	No data	Nymph	198	31[i]	–	–	–	–	–	Burgdorfer and Gage, 1986
Wild strain	White rabbit	Tick bite[d]	MA	Larva	No data	No data	Nymph	34	71	–	–	–	–	–	Telford and Spielman, 1989
Wild strain	–	–	NY	–	–	–	–	–	–	Female[j]	White rabbit	3–8	4	4	Piesman et al., 1991
Wild strain	White mouse	Needle[e]	MA	Larva	No data	No data	Nymph	30	40	–	–	–	–	–	Maupin et al., 1994
Wild strain	White mouse	Needle[e]	MA	Larva	60	65[g]	Nymph	20	75	Nymph	White mouse	7	1	1	Dolan et al., 1997
Wild strain	–	–	CT/MD/NJ/ NY	–	–	–	–	–	–	Female[j]	White rabbit	3–28	14	14	Piesman et al, 1999
Wild strain	–	–	MI/MN/WI	–	–	–	–	–	–	Female[j]	White rabbit	4–14	3	3	Piesman et al, 1999

Spirochete species and source/ isolate	Hosts used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection				Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Geographic origin of ticks[f]	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[k]	No. hosts known to have been exposed to at least 1 infected tick[k]	No. hosts for which infection was confirmed after tick feeding	
Wild strain	–	–	FL/GA/MS/ SC	–	–	–	–	–	–	Female[j]	White rabbit	0	0	0 (out of 12)[l]	Piesman et al, 1999
Wild strain	–	–	CT/NJ/NY	–	–	–	–	–	–	Nymph[j]	White mouse	1	16	15	des Vignes et al., 2001
WI210	Hamster	Tick bite[d]	MA/NY	Larva	No data	No data	Nymph	90	98	–	–	–	–	–	Piesman, 1993
LI-231	Hamster	Needle[e]	–	Larva	82	28[h]	Nymph	271	45	–	–	–	–	–	Barker et al., 1993
LI-231	Rat	Needle[e]	–	Larva	No data	No data	Nymph	70	36	–	–	–	–	–	Barker et al., 1993
LI-231	M. monax[a]	Needle[e]	–	Larva	No data	No data	Nymph	57	23	–	–	–	–	–	Barker et al., 1993
006 strain	White mouse	Tick bite[d]	MA	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	4	Richter et al, 1996
006 strain	White mouse	Tick bite[d]	MA	Larva	No data	No data	Nymph	No data	No data	Nymph	P. leucopus[a]	No data	No data	4	Richter et al, 1996
MI-119	White mouse	Needle[e]	NJ/NY	Larva	40	75[g]	Nymph	30	90	–	–	–	–	–	Piesman and Happ, 1997
MI-128	White mouse	Needle[e]	NJ/NY	Larva	20	10[g]	Nymph	10	90	–	–	–	–	–	Piesman and Happ, 1997
MI-129	White mouse	Needle[e]	NJ/NY	Larva	40	78[g]	Nymph	30	80	Nymph	White mouse	No data	4	4	Piesman and Happ, 1997
Valhalla	Hamster	Tick bite[d]	–	–	–	–	–	–	–	Nymph	Hamster	No data	3	3	Levin et al, 1995

Spirochete species and source/isolate	Hosts used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection				Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Geographic origin of ticks [f]	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host [k]	No. hosts known to have been exposed to at least 1 infected tick [k]	No. hosts for which infection was confirmed after tick feeding	
Valhalla	O. palustris [a]	Tick bite [d]	–	Larva	No data	No data	Nymph	694	75	Nymph	O. palustris [a]	No data	No data	2	Levin et al, 1995
Valhalla	P. leucopus [a]	Tick bite [d]	–	Larva	No data	No data	Nymph	No data	No data	Nymph	P. leucopus [a]	1	38	31	Levin and Fish, 2000
Borrelia burgdorferi sensu stricto															
JD1	P. leucopus [a]	Tick bite [d]	MA	Larva	No data	No data	Nymph	No data	>90	Nymph	Hamster	1–3	6	5	Piesman et al, 1987b
JD1	P. leucopus [a]	Tick bite [d]	MA	Larva	No data	No data	Nymph	No data	>90	Nymph	P. leucopus [a]	1–3	7	7	Piesman et al, 1987b
JD1	Hamster	Tick bite [d]	MA	Larva	No data	No data	Nymph	No data	No data	Nymph	Hamster	1	No data	6	Piesman et al, 1987a
JD1	P. leucopus [a]	Tick bite [d]	–	Larva	No data	No data	Nymph	215	92	Nymph	P. leucopus [a]	1–3	10	10	Mather et al, 1990
JD1	Hamster	Tick bite [d]	MA	Larva	18	61 [g]	Nymph	24	75	–	–	–	–	–	Piesman and Stone, 1991
JD1	White rabbit	Needle [e]	OK	Larva	200	20 [h]	Nymph	No data	No data	Nymph	White rabbit	No data	No data	6	Mukolwe et al., 1992
JD1	Hamster	Tick bite [d]	MA/NY	Larva	No data	No data	Nymph	180	99	–	–	–	–	–	Piesman, 1993
JD1	Hamster	Tick bite [d]	MA	Larva	132	44 [h]	Nymph	73	88	Nymph	Hamster	1–14	4	4	Piesman and Sinsky, 1988

Spirochete species and source/ isolate	Hosts used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection				Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Geographic origin of ticks[f]	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[k]	No. hosts known to have been exposed to at least 1 infected tick[k]	No. hosts for which infection was confirmed after tick feeding	
JD1	P. leucopus [a]	Tick bite[d]	–	Larva	75	53[h]	Nymph	161	89	–	–	–	–	–	Mather and Mather, 1990
JD1	White mouse	Needle[e]	LA	Larva	34	79[g]	Nymph	10	90	Nymph	White mouse	9	1	1	Jacobs et al., 2003
B31	Hamster	Tick bite[d]	MA/NY	Larva	No data	No data	Nymph	90	84	–	–	–	–	–	Piesman, 1993
B31	White mouse	Needle[e]	MA	Larva	60	77[g]	Nymph	20	85	Nymph	White mouse	9	1	1	Dolan et al., 1997
B31	White mouse	Needle[e]	NY	Larva	20	85[g]	Nymph	20	90	Nymph	White mouse	No data	No data	2	Dolan et al., 1998
B31	White mouse	Needle[e]	LA	Larva	51	90[g]	Nymph	10	90	Nymph	White mouse	3–4	2	2	Jacobs et al., 2003
B31	White mouse	Tick bite[d]	CT	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	1–4	8	7	Goddard et al., 2015
B31	White mouse	Tick bite[d]	LA	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	1–4	6	5	Goddard et al., 2015
B31	White mouse	Tick bite[d]	MS	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	1–4	4	4	Goddard et al., 2015
NC-2	White mouse	Needle[e]	NJ/NY	Larva	30	100[g]	Nymph	40	100	Nymph	White mouse	No data	4	4	Piesman and Happ, 1997
SH2-82	Hamster	Needle[e]	GA	Larva	36	89[h]	Nymph	107	88	Nymph	Hamster	No data	No data	3	Ryder et al., 1992
SH2-82	Hamster	Needle[e]	GA	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	5	Sanders and Oliver, 1995
SH2-82	Hamster	Needle[e]	GA	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	1	Oliver et al., 1993

Spirochete species and source/isolate	Hosts used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection				Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Geographic origin of ticks[f]	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[k]	No. hosts known to have been exposed to at least 1 infected tick[k]	No. hosts for which infection was confirmed after tick feeding	
SI-1	White mouse	Needle[e]	NJ/NY	Larva	30	73[g]	Nymph	20	75	–	–	–	–	–	Piesman and Happ, 1997
SI-1	Hamster	Needle[e]	GA	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	2	Oliver et al., 1993
SI-1	Hamster	Needle[e]	MA	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	4	Oliver et al, 1993
CA4	Hamster	Tick bite[d]	MA/NY	Larva	No data	No data	Nymph	90	58	–	–	–	–	–	Piesman, 1993
CA5	Hamster	Tick bite[d]	MA/NY	Larva	No data	No data	Nymph	30	70	–	–	–	–	–	Piesman, 1993
CA5	Hamster	Tick bite[d]	–	Larva	20	25[g]	Nymph	43	40	Nymph	Hamster	No data	No data	2	Lane et al., 1994
BL206	White mouse	Needle[e]	CT	Larva	No data	No data	Nymph	No data	82	Nymph	P. leucopus[a]	No data	No data	6	Derdakova et al., 2004
B348	White mouse[b]	Needle[e]	CT	Larva	No data	No data	Nymph	No data	97	Nymph	P. leucopus[a]	No data	No data	6	Derdakova et al., 2004
B348	P. leucopus[a]	Tick bite[d]	–	Larva	No data	No data	Nymph	No data	>90	–	–	–	–	–	States et al., 2017
BBC13	P. leucopus[a]	Tick bite[d]	–	Larva	No data	No data	Nymph	No data	>95	–	–	–	–	–	States et al., 2017
Borrelia bissettiae															
MI-6	Hamster	Needle[e]	GA	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	5	Sanders and Oliver, 1995

Spirochete species and source/isolate	Hosts used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection				Transtadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Geographic origin of ticks[f]	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[k]	No. hosts known to have been exposed to at least 1 infected tick[k]	No. hosts for which infection was confirmed after tick feeding	
MI-6	Hamster	Needle[e]	GA	Larva	No data	No data	Nymph	52	27	Nymph	S. hispidus[a]	No data	No data	2	Sanders and Oliver, 1995
Borrelia mayonii															
MN14-1420	White mouse	Needle[e]	CT	Larva	No data	No data	Nymph	241	13	Nymph	White mouse	1–3	13	9	Dolan et al. 2016
MN14-1420	White mouse	Tick bite[d]	CT/MN	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	1–2	20	13	Dolan et al., 2017b
MN14-1420	White mouse	Tick bite[d]	CT	Larva	No data	No data	Nymph	301	13	Nymph	White mouse	1	9	4	Eisen et al., 2017
MN14-1420	White mouse	Tick bite[d]	MN	Larva	No data	No data	Nymph	268	12	Nymph	White mouse	1	6	2	Eisen et al., 2017
MN-17-4755	White mouse	Tick bite[d]	–	Larva	No data	No data	Nymph	20	55	Nymph	P. leucopus[a]	1–10	23	21	Parise et al., 2020
Borrelia afzelii															
Pgau.C3	White mouse	Needle[e]	NY	Larva	20	90[g]	Nymph	20	85	Nymph	White mouse	No data	No data	2	Dolan et al. 1998
Borrelia garinii															
VS286	White mouse	Needle[e]	NY	Larva	20	10[g]	Nymph	20	0	Nymph	White mouse	No data	No data	1	Dolan et al. 1998
VSBP	White mouse	Needle[e]	NY	Larva	20	5[g]	Nymph	20	0	Nymph	White mouse	No data	No data	0 (out of 4)[l]	Dolan et al. 1998

[a] Laboratory colony of ground hogs (*Marmota monax*), marsh rice rats (*Oryzomys palustris*), white-footed mice (*Peromyscus leucopus*), or cotton rats (*Sigmodon hispidus*).

[b] Immunodeficient white mouse strain.

[c] Inoculated with a suspension containing material from field-collected *I. scapularis* ticks.

[d] Bite by infected *I. scapularis* ticks.

[e] Inoculated with a suspension containing cultured spirochetes.

[f] Northeast and Mid-Atlantic region: CT, MA, MD, NJ, NY; Midwestern region: MI, MN, OK, WI; Southeast region: FL, GA, LA, MS, SC.

[g] Larvae harvested within the first 2 wk after completing their blood meal.

[h] Larvae harvested within the first 2 d after completing their blood meal.

[i] Including only nymphs resulting from larvae fed 14–30 d post-infection.

[j] Field-collected infected ticks.

[k] By examination of either fed ticks or the resulting unfed ticks of the next life stage; or elucidated via transmission to uninfected ticks in a co-feeding experiment. Listed as no data when the number was not clearly stated in the publication.

[l] The study presented no evidence that any of these naïve hosts were exposed to an infected tick.

Table 4

Detailed results for studies to evaluate the vector competence of the Nearctic tick *Ixodes pacificus* for *Borrelia burgdorferi* sensu lato spirochetes.

Spirochete species and source/isolate	Hosts used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[j]	No. hosts known to have been exposed to at least 1 infected tick[j]	No. hosts for which infection was confirmed after tick feeding	
Uncharacterized *Borrelia burgdorferi* sensu lato														
Wild strain	White rabbit	Tick bite[c]	Larva	No data	No data	Nymph	120	12[h]	–	–	–	–	–	Burgdorfer, 1984
Wild strain	Wild rodents[a]	Tick bite[a]	Larva	No data	No data	Nymph	80	24	–	–	–	–	–	Brown and Lane, 1992
Wild strain	Wild rodents[a]	Tick bite[a]	Larva	No data	No data	Nymph	418	40	–	–	–	–	–	Brown and Lane, 1996
Wild strain	–	–	–	–	–	–	–	–	Female[i]	White rabbit	1	2	2	Piesman et al., 1999
006 strain	White mouse	Tick bite[c]	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	0 (out of 14)[k]	Richter et al., 1996
006 strain	White mouse	Tick bite[c]	Larva	No data	No data	Nymph	No data	No data	Nymph	*P. leucopus*[b]	No data	No data	0 (out of 5)[k]	Richter et al., 1996
Borrelia burgdorferi sensu stricto														
CA4	Hamster	Tick bite[c]	Larva	No data	No data	Nymph	70	13	–	–	–	–	–	Piesman, 1993
CA4	White rabbit	Needle[d]	Larva	259	3[g]	Nymph	140	6	Nymph	*P. maniculatus*[b]	1–2	6	5[l]	Lane et al., 1994
CA4	*P. maniculatus*[b]	Needle[d]	Larva	No data	No data	Nymph	107	0	–	–	–	–	–	Lane et al., 1994

Spirochete species and source/isolate	Hosts used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[i]	No. hosts known to have been exposed to at least 1 infected tick[j]	No. hosts for which infection was confirmed after tick feeding	
CA4	White rabbit	Needle[d]	Larva	No data	No data	Nymph	No data	20–60	Nymph	P. maniculatus[b]	1–2	10	8	Peavey and Lane, 1995
CA4	P. maniculatus[b]	Tick bite[e]	Larva	No data	No data	Nymph	103	34	–	–	–	–	–	Peavey and Lane, 1995
CA4	P. maniculatus[b]	Needle[d]	Larva	No data	No data	Nymph	182	10	–	–	–	–	–	Eisen et al., 2003
CA4	White mouse	Needle[d]	Larva	No data	No data	Nymph	200	19	–	–	–	–	–	Eisen et al., 2003
CA5	Hamster	Tick bite[c]	Larva	No data	No data	Nymph	30	27	–	–	–	–	–	Piesman, 1993
CA5	Hamster	Tick bite[c]	Larva	20	25[g]	Nymph	18	33	–	–	–	–	–	Lane et al., 1994
CA7	Hamster	Needle[d]	Larva	No data	No data	Nymph	60	2	–	–	–	–	–	Lane et al., 1994
CA10	P. maniculatus[b]	Needle[d]	Larva	No data	No data	Nymph	300	<1	–	–	–	–	–	Eisen et al., 2003
CA11	P. maniculatus[b]	Needle[d]	Larva	No data	No data	Nymph	32	3	–	–	–	–	–	Lane et al., 1994
JD1	Hamster	Tick bite[c]	Larva	No data	No data	Nymph	No data	No data	Nymph	Hamster	2	1	1	Lane et al., 1994
JD1	Hamster	Tick bite[c]	Larva	No data	No data	Nymph	No data	No data	Nymph	P. maniculatus[b]	1	1	0	Lane et al., 1994

Borrelia bissettiae

Spirochete species and source/isolate	Hosts used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[j]	No. hosts known to have been exposed to at least 1 infected tick[j]	No. hosts for which infection was confirmed after tick feeding	
CA389	P. maniculatus [b]	Needle [d]	Larva	No data	No data	Nymph	42	0	–	–	–	–	–	Eisen et al., 2003
CA389	White mouse	Needle [d]	Larva	No data	No data	Nymph	350	<1	–	–	–	–	–	Eisen et al., 2003
CA559	White mouse	Needle [d]	Larva	No data	No data	Nymph	240	0	–	–	–	–	–	Eisen et al., 2003
CA589	P. maniculatus [b]	Tick bite [f]	Larva	No data	No data	Nymph	12	42	–	–	–	–	–	Eisen et al., 2003
CA591	P. maniculatus [b]	Tick bite [f]	Larva	No data	No data	Nymph	32	43	–	–	–	–	–	Eisen et al., 2003
CA592	P. maniculatus [b]	Tick bite [f]	Larva	No data	No data	Nymph	12	75	Nymph	P. maniculatus [b]	1	3	3	Eisen et al., 2003
CA589/591/592	P. maniculatus [b]	Tick bite [f]	Larva	No data	No data	Nymph	No data	No data	Nymph	P. maniculatus [b]	1–6	4	4	Eisen et al., 2003
CA589/591/592	P. maniculatus [b]	Tick bite [f]	Larva	No data	No data	Nymph	No data	No data	Nymph	P. maniculatus [b]	No data	No data	6	Eisen et al., 2003

[a] Field-collected and naturally tick-bite infected dusky-footed woodrats (*Neotoma fuscipes*) or California kangaroo rats (*Dipodomys californicus*).

[b] Laboratory colonies of deer mice (*Peromyscus maniculatus*) or white-footed mice (*Peromyscus leucopus*).

[c] Bite by infected *I. scapularis* ticks.

[d] Inoculated with a suspension containing cultured spirochetes.

[e]Bite by infected *I. pacificus* ticks.

[f]Bite by infected *I. spinipalpis* ticks.

[g]Larvae harvested within the first 2 wk after completing their blood meal.

[h]Including only nymphs resulting from larvae fed 14–30 d post-infection.

[i]Field-collected infected ticks.

[j]By examination of either fed ticks or the resulting unfed ticks of the next life stage; or elucidated via transmission to uninfected ticks in a co-feeding experiment. Listed as no data when the number was not clearly stated in the publication.

[k]The study presented no evidence that any of these naïve hosts were exposed to an infected tick.

[l]Five mice had ear biopsies positive for spirochetes by culture and were seroreactive. A sixth mouse had ear biopsy negative for spirochetes by culture but was seroreactive; this mouse was not included in the number for which infection was confirmed.

Table 5

Detailed results for studies to evaluate the vector competence of the Palearctic tick *Ixodes ricinus* for *Borrelia burgdorferi* sensu lato spirochetes.

Spirochete species and source/ isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naive hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[i]	No. hosts known to be exposed to at least 1 infected tick[i]	No. hosts for which infection was confirmed after tick feeding	
Uncharacterized *Borrelia burgdorferi* sensu lato														
Wild	–	–	–	–	–	–	–	–	Female[h]	White rabbit	1–19	7	7	Burgdorfer et al., 1983
Wild	White rabbit	Needle[b]	Larva	No data	No data	Nymph	185	16[g]	–	–	–	–	–	Burgdorfer, 1984
Wild	Wild rodents[a]	Tick bite[a]	Larva	No data	No data	Nymph	1254	50	–	–	–	–	–	Gern et al., 1994
Wild (including *B. afzelii*)	Gerbil	Tick bite[c]	Larva	No data	No data	Nymph	162	88	Nymph	Gerbil	No data	No data	6	Kahl et al., 1998; Olaf Kahl (personal communication)
Borrelia burgdorferi sensu stricto														
Wild	–	–	–	–	–	–	–	–	Nymph[h]	White mouse	No data	No data	2	Hu et al., 2001
Wild	–	–	–	–	–	–	–	–	Nymph[h]	White mouse	No data	No data	3	Hu et al., 2003
ZS7	White mouse	Tick bite[d]	Larva	No data	No data	Nymph	No data	42–88	–	–	–	–	–	Gern et al., 1993
ZS7	White mouse	Tick bite[d]	Larva	No data	No data	Nymph	No data	70	Nymph	White mouse	No data	3	3	Gern and Rais, 1996
B31	White mouse	Needle[e]	Larva	20	10[f]	Nymph	20	0	–	–	–	–	–	Dolan et al., 1998
ZS7, NE1849	White mouse	Needle[e]	Larva	No data	No data	Nymph	No data	60–70	Nymph	White mouse	No data	No data	2	Crippa et al., 2002
Borrelia afzelii														

Spirochete species and source/isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[i]	No. hosts known to be exposed to at least 1 infected tick[i]	No. hosts for which infection was confirmed after tick feeding	
Wild	–	–	–	–	–	–	–	–	Larva[h]	White mouse	No data	No data	2	van Duijvendijk et al., 2016
Wild	–	–	–	–	–	–	–	–	Nymph[h]	White mouse	No data	No data	9	Hu et al., 2001
Wild	–	–	–	–	–	–	–	–	Nymph[h]	White mouse	No data	No data	31	Hu et al., 2003
Pgau.C3	White mouse	Needle[e]	Larva	20	85[f]	Nymph	20	90	Nymph	White mouse	No data	No data	3	Dolan et al., 1998
NE496, NE2963	White mouse	Needle[e]	Larva	No data	No data	Nymph	No data	60–80	Nymph	White mouse	No data	No data	2	Crippa et al., 2002
NE4053	White mouse	Needle[e]	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	2	Tonetti et al., 2015
NE4053	White mouse	Tick bite[e]	Larva	No data	No data	Nymph	26	4	–	–	–	–	–	Tonetti et al., 2015
NE5046	White mouse	Needle[e]	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	4	Tonetti et al., 2015
NE5046	White mouse	Tick bite[c]	Larva	No data	No data	Nymph	52	92	–	–	–	–	–	Tonetti et al., 2015
NE36	White mouse	Needle[e]	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	4	Tonetti et al., 2015
NE36	White mouse	Tick bite[e]	Larva	No data	No data	Nymph	52	67	–	–	–	–	–	Tonetti et al., 2015
E61	White mouse	Needle[e]	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	3	Tonetti et al., 2015
E61	White mouse	Tick bite[c]	Larva	No data	No data	Nymph	39	54	–	–	–	–	–	Tonetti et al., 2015
P/sto	White mouse	Needle[e]	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	4	Tonetti et al., 2015
P/sto	White mouse	Tick bite[c]	Larva	No data	No data	Nymph	52	15	–	–	–	–	–	Tonetti et al., 2015

Spirochete species and source/isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[i]	No. hosts known to be exposed to at least 1 infected tick[i]	No. hosts for which infection was confirmed after tick feeding	
NE4054	White mouse	Needle[e]	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	4	Tonetti et al., 2015
NE4054	White mouse	Tick bite[c]	Larva	No data	No data	Nymph	39	31	–	–	–	–	–	Tonetti et al., 2015
NE4049	White mouse	Needle[e]	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	8	Tonetti et al., 2015
NE4049	White mouse	Tick bite[c]	Larva	No data	No data	Nymph	117	85	–	–	–	–	–	Tonetti et al., 2015
NE4051	White mouse	Needle[e]	Larva	No data	No data	Nymph	No data	No data	Nymph	White mouse	No data	No data	9	Tonetti et al., 2015
NE4051	White mouse	Tick bite[c]	Larva	No data	No data	Nymph	115	70	–	–	–	–	–	Tonetti et al., 2015
CB43	White mouse	Tick bite[c]	Larva	No data	No data	Nymph	No data	90	Nymph	White mouse	No data	No data	10	Pospisilova et al., 2019
Borrelia garinii														
Wild	–	–	–	–	–	–	–	–	Nymph[h]	White mouse	No data	No data	7	Hu et al., 2001
Wild	–	–	–	–	–	–	–	–	Nymph[h]	White mouse	No data	No data	7	Hu et al., 2003
VS286	White mouse	Needle[e]	Larva	20	5[f]	Nymph	20	0	Nymph	White mouse	No data	No data	3	Dolan et al., 1998
VSBP	White mouse	Needle[e]	Larva	20	10[f]	Nymph	20	0	Nymph	White mouse	No data	No data	0 (out of 3)[j]	Dolan et al., 1998

[a] Field-collected and naturally tick-bite infected yellow-necked mice (*Apodemus flavicollis*) or wood mice (*Apodemus sylvaticus*).

[b] Inoculated with a suspension containing material from field-collected *I. scapularis* ticks.

[c] Bite by infected *I. ricinus* ticks.

[d] Nymphal ticks infected via capillary feeding before taking their blood meal.

[e]Inoculated with a suspension containing cultured spirochetes.

[f]Larvae harvested within the first 2 wk after completing their blood meal.

[g]Including only nymphs resulting from larvae fed 14–30 d post-infection.

[h]Field-collected infected ticks.

[i]By examination of either fed ticks or the resulting unfed ticks of the next life stage; or elucidated via transmission to uninfected ticks in a co-feeding experiment. Listed as no data when the number was not clearly stated in the publication.

[j]The study presented no evidence that any of these naïve hosts were exposed to an infected tick.

Table 6

Detailed results for studies to evaluate the vector competence of the Palearctic/Oriental tick *Ixodes persulcatus* for *Borrelia burgdorferi* sensu lato spirochetes.

Spirochete species and source/isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[f]	No. hosts known to be exposed to at least 1 infected tick[f]	No. hosts for which infection was confirmed after tick feeding	
Uncharacterized *Borrelia burgdorferi* sensu lato														
Wild strain	Wild host[a]	Tick bite[a]	Nymph	No data	No data	Adult	200	4[e]	–	–	–	–	–	Korenberg et al., 1988
Borrelia garinii														
Wild strain	White mouse	Needle[b]	Larva	No data	>90[d]	Nymph	No data	40–50	Nymph	White mouse	No data	No data	1[g]	Sun et al., 2003a
JEM3	Jird	Needle[b]	Larva	20	15[d]	Nymph	68	38	–	–	–	–	–	Nakao and Miyamoto, 1994
JEM4	Jird	Needle[b]	Larva	20	0[d]	Nymph	68	21	–	–	–	–	–	Nakao and Miyamoto, 1994
JEM5	Jird	Needle[b]	Larva	30	30[d]	Nymph	102	56	–	–	–	–	–	Nakao and Miyamoto, 1994
JEM6	Jird	Needle[b]	Larva	40	67[d]	Nymph	136	74	–	–	–	–	–	Nakao and Miyamoto, 1994
JEM7	Jird	Needle[b]	Larva	30	17[d]	Nymph	102	39	–	–	–	–	–	Nakao and Miyamoto, 1994
JME8	Jird	Needle[b]	Larva	30	43[d]	Nymph	102	37	–	–	–	–	–	Nakao and Miyamoto, 1994
JEM6	Jird	Tick bite[c]	Larva	No data	No data	Nymph	No data	>90	Nymph	Jird	No data	No data	15	Sato and Nakao, 1997

[a]Unknown naturally infected reservoir host.

[b]Inoculated with a suspension containing cultured spirochetes.

[c]Bite by infected *I. persulcatus* ticks.

[d]Larvae harvested within the first 2 d after completing their blood meal.

[e]Demonstration of transstadial passage from naturally infected nymphs to adults following nymphal feeding on naïve hosts.

[f]By examination of either fed ticks or the resulting unfed ticks of the next life stage; or elucidated *via* transmission to uninfected ticks in a co-feeding experiment. Listed as no data when the number was not clearly stated in the publication.

[g]The study noted that transmission occurred but it was not clear how many animals had evidence of infection.

Table 7

Detailed results for studies with *Borrelia burgdorferi* sensu lato spirochetes to evaluate the vector competence of *Ixodes* ticks (excluding *Ixodes scapularis* and *Ixodes pacificus*) occurring in the Nearctic (NA) Zoogeographic region and in some cases with ranges extending also to the Neotropic (NT) or Palearctic (PA) Zoogeographic regions.

Spirochete species and source/isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[i]	No. hosts known to have been exposed to at least 1 infected tick[i]	No. hosts for which infection was confirmed after tick feeding	
***Ixodes affinis* (NA/NT)**														
B. burgdorferi s.s. (SI-1)	–	–	–	–	–	–	–	–	No data	No data	No data	No data	No data[j]	Oliver et al., 2003
***Ixodes angustus* (NA/PA)**														
B. burgdorferi s.s. (CA4)	White mouse	Needle[d]	Larva	No data	No data	Nymph	50	12	Nymph	*P. maniculatus*[b]	No data	No data	1	Peavey et al., 2000
B. burgdorferi s.s. (CA4)	*P. maniculatus*[b]	Needle[d]	Larva	No data	No data	Nymph	98	8	Nymph	*P. maniculatus*[b]	0	0	0 (out of 9)[k]	Peavey et al., 2000
***Ixodes cookei* (NA/NT)**														
B. burgdorferi s.l. (LI-231)[a]	Hamster	Needle[d]	Larva	59	5[f]	Nymph	92	5	–	–	–	–	–	Barker et al., 1993
B. burgdorferi s.l. (LI-231)[a]	Rat	Needle[d]	Larva	No data	No data	Nymph	50	16	–	–	–	–	–	Barker et al., 1993
B. burgdorferi s.l. (LI-231)[a]	*M. monax*[b]	Needle[d]	Larva	No data	No data	Nymph	100	4	Nymph	*M. monax*[b]	0	0	0 (out of 1)[k]	Barker et al., 1993
B. burgdorferi s.s. (SH2-82)	Hamster	Needle[d]	Larva	36	14[f]	Nymph	30	0	Nymph	Hamster	No data	No data	0 (out of 3)[k]	Ryder et al., 1992

Spirochete species and source/isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[i]	No. hosts known to have been exposed to at least 1 infected tick[i]	No. hosts for which infection was confirmed after tick feeding	
Ixodes dentatus (NA)														
B. burgdorferi s.l. (Wild)[a]	White rabbit	Tick bite[e]	Larva	No data	No data	Nymph	45	47	Nymph	White rabbit	1	1	1	Telford and Spielman, 1989
Ixodes jellisoni (NA)														
B. burgdorferi s.l. (CA444)[a]	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	21	14	–	–	–	–	–	Lane et al., 1999
B. burgdorferi s.l. (CA445)[a]	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	28	25	Nymph	*D. californicus*[b]	4	1	1	Lane et al., 1999
B. burgdorferi s.l. (CA447)[a]	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	43	2	–	–	–	–	–	Lane et al., 1999
B. californiensis (CA404)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	3	0	Nymph	*D. californicus*[b]	1	1	1	Lane et al., 1999
B. californiensis (CA409)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	30	23	Nymph	*D. californicus*[b]	7	1	1	Lane et al., 1999
B. californiensis (CA442)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	32	3	–	–	–	–	–	Lane et al., 1999
B. californiensis (CA443)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	7	0	–	–	–	–	–	Lane et al., 1999
B. californiensis (CA446)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	13	15	Nymph	*D. californicus*[b]	1	1	0	Lane et al., 1999

Spirochete species and source/isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[i]	No. hosts known to have been exposed to at least 1 infected tick[i]	No. hosts for which infection was confirmed after tick feeding	
Ixodes minor (NA/NT)														
B. burgdorferi s.s. (unknown)	–	–	–	–	–	–	–	–	No data	No data	No data	No data	No data [l]	Oliver et al., 2003
B. bissettiae (unknown)	–	–	–	–	–	–	–	–	No data	No data	No data	No data	No data [l]	Oliver et al., 2003
Ixodes muris (NA)														
B. burgdorferi s.s. (B31)	White mouse	Tick bite [e]	Larva	12	67[g]	Nymph	No data	38	Nymph	White mouse	1-3	4	1	Dolan et al., 2000
Ixodes spinipalpis (NA/NT)														
B. burgdorferi s.l. (Wild)[a]	Wild rodents[c]	Tick bite [c]	Larva	No data	No data	Nymph	76	33	Nymph	2 hamsters, 1 P. truei[b], and 1 N. fuscipes[b]	1	4	4	Brown and Lane, 1992
B. burgdorferi s.l. (Wild)[a]	White mouse	Needle[d]	Larva	60	70[g]	Nymph	20	80	Nymph	White mouse	1-6	3	3	Dolan et al., 1997
B. burgdorferi s.l. (CA444)[a]	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	21	10	–	–	–	–	–	Lane et al., 1999
B. burgdorferi s.l. (CA445)[a]	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	32	3	–	–	–	–	–	Lane et al., 1999
B. burgdorferi s.l. (CA447)[a]	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	28	4	–	–	–	–	–	Lane et al., 1999
B. burgdorferi s.s. (B31)	White mouse	Needle[d]	Larva	60	58[g]	Nymph	20	70	Nymph	White mouse	1-6	2	2	Dolan et al., 1997

Spirochete species and source/isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[i]	No. hosts known to have been exposed to at least 1 infected tick[i]	No. hosts for which infection was confirmed after tick feeding	
B. burgdorferi s.s. (CA4)	*P. maniculatus*[b]	Needle[d]	Larva	No data	No data	Nymph	141	12	–	–	–	–	–	Peavey et al., 2000
B. burgdorferi s.s. (CA4)	White mouse	Needle[d]	Larva	No data	No data	Nymph	12	8	–	–	–	–	–	Eisen et al., 2003
B. bissettiae (Wild)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	No data	No data	Nymph[h]	*M. ochrogaster*[b]	No data	No data	2	Burkot et al., 2000
B. bissettiae (CA389)	White mouse	Needle[d]	Larva	No data	No data	Nymph	19	0	–	–	–	–	–	Eisen et al., 2003
B. bissettiae (N271)	White mouse	Needle[d]	Larva	No data	No data	Nymph	62	2	Nymph	White mouse	1	1	1	Eisen et al., 2003
B. bissettiae (N501)	White mouse	Needle[d]	Larva	No data	No data	Nymph	56	4	Nymph	White mouse	2	1	1	Eisen et al., 2003
B. bissettiae (CA589)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	10	80	Nymph	*P. maniculatus*[b]	2	1	1	Eisen et al., 2003
B. bissettiae (CA590)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	4	75	–	–	–	–	–	Eisen et al., 2003
B. bissettiae (CA591)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	20	35	Nymph	*P. maniculatus*[b]	2–12	4	4	Eisen et al., 2003
B. bissettiae (CA592)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	8	38	Nymph	*P. maniculatus*[b]	4–5	2	2	Eisen et al., 2003
B. californiensis (CA404)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	33	6	–	–	–	–	–	Lane et al., 1999

Spirochete species and source/isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[i]	No. hosts known to have been exposed to at least 1 infected tick[i]	No. hosts for which infection was confirmed after tick feeding	
B. californiensis (CA409)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	43	5	–	–	–	–	–	Lane et al., 1999
B. californiensis (CA442)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	28	0	–	–	–	–	–	Lane et al., 1999
B. californiensis (CA443)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	11	0	–	–	–	–	–	Lane et al., 1999
B. californiensis (CA446)	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	20	25	–	–	–	–	–	Lane et al., 1999

[a] Uncharacterized *Borrelia burgdorferi* sensu lato spirochetes.

[b] Laboratory colonies of deer mice (*Peromyscus maniculatus*), ground hogs (*Marmota monax*), California kangaroo rats (*Dipodomys californicus*), piñon mice (*Peromyscus true*), dusky-footed woodrats (*Neotoma fuscipes*), or prairie voles (*Microtus ochrogaster*).

[c] Field-collected and naturally tick-bite infected dusky-footed woodrats (*Neotoma fuscipes*) or California kangaroo rats (*Dipodomys californicus*).

[d] Inoculated with a suspension containing cultured spirochetes.

[e] Bite by infected *I. scapularis* ticks.

[f] Larvae harvested within 2 d after completing their blood meal.

[g] Larvae harvested within the first 2 wk after completing their blood meal.

[h] Naturally infected nymphs recovered as fed larvae from field-collected prairie voles.

[i] By examination of either fed ticks or the resulting unfed ticks of the next life stage; or elucidated via transmission to uninfected ticks in a co-feeding experiment. Listed as no data when the number was not clearly stated in the publication.

[j] Evidence for vector competence limited to the following statement: "The closely related but usually non-human biting *I. affinis* also experimentally transmitted the *B. burgdorferi* s.s. isolate SI-1 (J.H.O., A.M.J., and C.W.B., unpublished data).".

[k]The study presented no evidence that any naïve host was exposed to an infected tick.

[l]Evidence for vector competence limited to the following statement: "Although *I. minor* is not currently considered a member of the *Ixodes ricinus* species complex, as are *I. scapularis* and *I. affinis*, it is an efficient vector of *B. bissettii* and *B. burgdorferi* s.s. (J.H.O., J. B. Phillips, C.W.B., L.G., T.L., and A.M.J., unpublished data)." .

Table 8

Detailed results for studies with *Borrelia burgdorferi* sensu lato spirochetes to evaluate the vector competence of *Ixodes* ticks (excluding *Ixodes ricinus* and *Ixodes persulcatus*) occurring in the Palearctic (PA), Oriental (OR), or Australian (AU) zoogeographic regions.

Spirochete species and source/ isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[h]	No. hosts known to have been exposed to at least 1 infected tick[h]	No. hosts for which infection was confirmed after tick feeding	
***Ixodes arboricola* (OR/PA)**														
B. garinii (Wild)	Bird[a]	Tick bite[b]	Larva	No data	No data	Nymph	164	3	Nymph	Bird[a]	0	0	0 (out of 4)[i]	Heylen et al., 2014
B. valaisiana (Wild)	Bird[a]	Tick bite[b]	Larva	No data	No data	Nymph	164	3	Nymph	Bird[a]	0	0	0 (out of 4)[i]	Heylen et al., 2014
***Ixodes frontalis* (PA)**														
B. garinii (Wild)	Bird[a]	Tick bite[b]	Larva	No data	No data	Nymph	113	2	Nymph	Bird[a]	0	0	0 (out of 4)[i]	Heylen et al., 2014
B. spielmanii (Wild)	Bird[a]	Tick bite[b]	Larva	No data	No data	Nymph	113	<1	Nymph	Bird[a]	0	0	0 (out of 4)[i]	Heylen et al., 2014
***Ixodes hexagonus* (PA)**														
B. burgdorferi s.s. (B31)	–	–	Nymph[e]	6	67[f]	Adult	47	70	Female	White mouse	1	4	4	Gem et al., 1991
***Ixodes holocyclus* (AU)**														
B. burgdorferi s.s. (JD1)	Hamster	Tick bite[c]	Larva	36	17[f]	Nymph	84	0	–	–	–	–	–	Piesman and Stone, 1991
***Ixodes ovatus* (OR/PA)**														
B. garinii (JEM3)	Jird	Needle[d]	Larva	20	80[g]	Nymph	68	0	–	–	–	–	–	Nakao and Miyamoto, 1994
B. garinii (JEM4)	Jird	Needle[d]	Larva	10	20[g]	Nymph	34	0	–	–	–	–	–	Nakao and Miyamoto, 1994

Spirochete species and source/isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[h]	No. hosts known to have been exposed to at least 1 infected tick[h]	No. hosts for which infection was confirmed after tick feeding	
B. garinii (JEM5)	Jird	Needle[d]	Larva	10	10[g]	Nymph	34	0	–	–	–	–	–	Nakao and Miyamoto, 1994
B. garinii (JEM6)	Jird	Needle[d]	Larva	20	70[g]	Nymph	68	0	–	–	–	–	–	Nakao and Miyamoto, 1994
B. garinii (JEM7)	Jird	Needle[d]	Larva	20	25[g]	Nymph	68	0	–	–	–	–	–	Nakao and Miyamoto, 1994
B. garinii (JME8)	Jird	Needle[d]	Larva	10	40[g]	Nymph	34	0	–	–	–	–	–	Nakao and Miyamoto, 1994
Ixodes sinensis (OR)														
B. garinii (Wild)	White mouse	Needle[d]	Larva	50	96[g]	Nymph	50	56	Nymph	White mouse	No data	No data	9	Sun et al., 2003b
B. garinii (Wild)	White mouse	Needle[d]	Nymph	100	98[g]	Adult	56	57	Adult	White mouse	No data	No data	6	Sun et al., 2003b

[a] Bird (*Parus major*).

[b] Bite by infected *I. ricinus* ticks.

[c] Bite by infected *I. scapularis* ticks.

[d] Inoculated with a suspension containing cultured spirochetes.

[e] Nymphal ticks infected via capillary feeding before taking a blood meal on an uninfected rabbit host.

[f] Larvae or nymphs harvested within the first 2 wk after completing their blood meal.

[g] Larvae or nymphs harvested within the first 2 d after completing their blood meal.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

[h]By examination of either fed ticks or the resulting unfed ticks of the next life stage; or elucidated via transmission to uninfected ticks in a co-feeding experiment. Listed as no data when the number was not clearly stated in the publication.

[i]The study presented no evidence that any of these naïve hosts were exposed to an infected tick.

Table 9

Detailed results for studies with *Borrelia burgdorferi* sensu lato spirochetes to evaluate the vector competence of *Amblyomma*, *Dermacentor*, or *Haemaphysalis* ticks occurring in the Nearctic (NA), Palearctic (PA), Oriental (OR), or Australian (AU) zoogeographic regions.

Spirochete species and source/isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[h]	No. hosts known to have been exposed to at least 1 infected tick[h]	No. hosts for which infection was confirmed after tick feeding	
Amblyomma americanum (NA)														
B. burgdorferi s.s. (JD1)	Hamster	Tick bite[d]	Larva	118	4[f]	Nymph	218	0	–	–	–	–	–	Piesman and Sinsky, 1988
B. burgdorferi s.s. (JD1)	*P. leucopus*[b]	Tick bite[d]	Larva	16	19[f]	Nymph	33	0	–	–	–	–	–	Mather and Mather, 1990
B. burgdorferi s.s. (JD1)	White rabbit	Needle[e]	Larva	200	0[f]	Nymph	No data	No data	Nymph	White rabbit	No data	No data	0 (out of 6)[i]	Mukolwe et al., 1992
B. burgdorferi s.s. (B31)	White mouse	Tick bite[d]	Larva	No data	0[g]	Nymph	No data	0	–	–	–	–	–	Soares et al., 2006
B. burgdorferi s.s. (NC-2)	White mouse	Needle[e]	Larva	30	0[g]	Nymph	40	0	Nymph	White mouse	0	0	0 (out of 2)[i]	Piesman and Happ, 1997
B. burgdorferi s.s. (SI-1)	Hamster	Needle[e]	Larva	No data	No data	Nymph	No data	No data	Nymph	Hamster	No data	No data	0 (out of 15)[i]	Oliver et al., 1993
B. burgdorferi s.s. (SI-1)	White mouse	Needle[e]	Larva	20	0[g]	Nymph	10	0	–	–	–	–	–	Piesman and Happ, 1997
B. burgdorferi s.s. (SH2-82)	Hamster	Needle[e]	Larva	36	19[f]	Nymph	60	2	Nymph	Hamster	No data	No data	0 (out of 3)[i]	Ryder et al., 1992
B. bissettiae (MI-6)	Hamster	Needle[e]	Larva	No data	No data	Nymph	105	0	Nymph	White mouse	No data	No data	0 (out of 23)[i]	Sanders and Oliver, 1995

Spirochete species and source/isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[h]	No. hosts known to have been exposed to at least 1 infected tick[h]	No. hosts for which infection was confirmed after tick feeding	
Dermacentor andersoni (NA)														
B. burgdorferi s.l. (Wild)[a]	White mouse	Needle[e]	Larva	90	17[g]	Nymph	30	0	Nymph	White mouse	0	0	0 (out of 2)[i]	Dolan et al., 1997
B. burgdorferi s.s. (B31)	White mouse	Needle[e]	Larva	90	11[g]	Nymph	30	0	Nymph	White mouse	0	0	0 (out of 2)[i]	Dolan et al., 1997
Dermacentor mittalli (PA)														
B. garinii (Wild)	White mouse	Needle[e]	Larva	No data	50[f]	Nymph	No data	0	Nymph	White mouse	No data	No data	0[j]	Sun et al., 2003a
B. garinii (Wild)	White mouse	Needle[e]	Nymph	No data	60[f]	Adult	No data	0	Adult	White mouse	No data	No data	0[j]	Sun et al., 2003a
Dermacentor occidentalis (NA)														
B. burgdorferi s.l. (Wild)[a]	Wild rodents[c]	Tick bite[c]	Larva	No data	No data	Nymph	44	0	–	–	–	–	–	Brown and Lane, 1992
B. burgdorferi s.s. (CA5)	Hamster	Tick bite[d]	Larva	20	10[g]	Nymph	40	0	–	–	–	–	–	Lane et al., 1994
Dermacentor silvarum (PA)														
B. garinii (Wild)	White mouse	Needle[e]	Larva	No data	40[f]	Nymph	No data	0	Nymph	White mouse	No data	No data	0[j]	Sun and Xu, 2003
B. garinii (Wild)	White mouse	Needle[e]	Nymph	No data	50[f]	Adult	No data	0	Adult	White mouse	No data	No data	0[j]	Sun and Xu, 2003
Dermacentor variabilis (NA/NT)														
B. burgdorferi s.l. (MI-119)[a]	White mouse	Needle[e]	Larva	23	61[g]	Nymph	21	10	–	–	–	–	–	Piesman and Happ, 1997

Spirochete species and source/isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[h]	No. hosts known to have been exposed to at least 1 infected tick[h]	No. hosts for which infection was confirmed after tick feeding	
B. burgdorferi s.l. (MI-128)[a]	White mouse	Needle[e]	Larva	20	10[g]	Nymph	10	0	—	—	—	—	—	Piesman and Happ, 1997
B. burgdorferi s.l. (MI-129)[a]	White mouse	Needle[e]	Larva	30	37[g]	Nymph	30	0	Nymph	White mouse	0	0	0 (out of 18)[j]	Piesman and Happ, 1997
B. burgdorferi s.s. (JD1)	Hamster	Tick bite[d]	Larva	49	14[f]	Nymph	77	0	—	—	—	—	—	Piesman and Sinsky, 1988
B. burgdorferi s.s. (JD1)	P. leucopus[b]	Tick bite[d]	Larva	75	28[f]	Nymph	150	0	—	—	—	—	—	Mather and Mather, 1990
B. burgdorferi s.s. (JD1)	White rabbit	Needle[e]	Larva	200	0[f]	Nymph	No data	No data	Nymph	White rabbit	No data	No data	0 (out of 6)[i,k]	Mukolwe et al., 1992
B. burgdorferi s.s. (B31)	White mouse	Tick bite[d]	Larva	No data	>0[g]	Nymph	No data	>0	Nymph	White mouse	No data	No data	0[j]	Soares et al., 2006
B. burgdorferi s.s. (NC-2)	White mouse	Needle[e]	Larva	47	28[g]	Nymph	49	2	Nymph	White mouse	0	0	0 (out of 6)[i]	Piesman and Happ, 1997
B. burgdorferi s.s. (SI-1)	White mouse	Needle[e]	Larva	48	58[g]	Nymph	30	0	—	—	—	—	—	Piesman and Happ, 1997
B. bissettiae (MI-6)	Hamster	Needle[e]	Larva	No data	No data	Nymph	105	0	Nymph	White mouse	No data	No data	0 (out of 21)[j]	Sanders and Oliver, 1995
Haemaphysalis concinna (OR/PA)														
B. garinii (Wild)	White mouse	Needle[e]	Larva	No data	70–80[f]	Nymph	No data	0	Nymph	White mouse	No data	No data	0[j]	Sun and Xu, 2003
B. garinii (Wild)	White mouse	Needle[e]	Nymph	No data	70[f]	Adult	No data	0	Adult	White mouse	No data	No data	0[j]	Sun and Xu, 2003

Spirochete species and source/isolate	Host used as source of infection for feeding ticks		Spirochete acquisition by ticks fed on hosts with active infection			Transstadial passage of spirochetes to molted ticks of the next life stage			Spirochete transmission by ticks fed on naïve hosts					Reference
	Species	Route of spirochete infection	Life stage	No. examined	% infected	Life stage	No. examined	% infected	Life stage	Host	No. infected ticks known to have fed per host[h]	No. hosts known to have been exposed to at least 1 infected tick[h]	No. hosts for which infection was confirmed after tick feeding	
Haemaphysalis longicornis (AU/NA/OR/PA)														
B. garinii (Wild)	White mouse	Needle[e]	Larva	No data	60–70[f]	Nymph	No data	0	Nymph	White mouse	No data	No data	0[j]	Sun et al., 2003a
B. garinii (Wild)	White mouse	Needle[e]	Nymph	No data	60–70[f]	Adult	No data	0	Adult	White mouse	No data	No data	0[j]	Sun et al., 2003a
B. burgdorferi s.s. (B31)	White mouse	Tick bite[d]	Larva	32	56[f]	Nymph	520	0	–	–	–	–	–	Breuner et al., 2020

[a] Uncharacterized *Borrelia burgdorferi* sensu lato spirochetes.

[b] Laboratory colony of white-footed mice (*Peromyscus leucopus*).

[c] Field-collected and naturally tick-bite infected dusky-footed woodrats (*Neotoma fuscipes*) or California kangaroo rats (*Dipodomys californicus*).

[d] Bite by infected *I. scapularis* ticks.

[e] Inoculated with a suspension containing cultured spirochetes.

[f] Larvae or nymphs harvested within 2 d after completing their blood meal.

[g] Larvae or nymphs harvested within the first 2 wk after completing their blood meal.

[h] By examination of either fed ticks or the resulting unfed ticks of the next life stage; or elucidated via transmission to uninfected ticks in a co-feeding experiment. Listed as no data when the number was not clearly stated in the publication.

[i] The study presented no evidence that any naïve host was exposed to an infected tick.

[j] The study presented no evidence that any naïve host was exposed to an infected tick, and the number of hosts used was not given.

[k] One of these rabbits was seroreactive but no spirochetes were isolated from tissues (liver, spleen, heart, kidneys, and urinary bladder) taken at necropsy; the evidence for this rabbit was therefore not considered sufficient to conclude that infection was confirmed. In contrast, rabbits exposed to *I. scapularis* ticks infected with the same spirochete isolate in this study uniformly were both seroreactive and yielded tissues from which spirochetes were cultured.