LYME BORRELIOSIS: OF TICKS AND SPIROCHETES

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ABSTRACT

Lyme disease is now recognized as the most prevalent tick-borne spirochetosis in North America, Europe and in many Asien countries, especially Japan. Its global occurrence coincides with the geographic distribution of ticks belonging to the *Ixodes ricinus/persulcatus* complex. In this complex, four species, *Ixodes ricinus*, *Ixodes persulcatus*, *Ixodes scapularis*, and *Ixodes pacificus* are recognized as efficient vectors of the Lyme disease spirochete, *Borrelia burgdorferi*. At least ten additional species of ixodid ticks have been found occasionally infected, but of these only three, namely *Ixodes ovatus* from Japan, *Ixodes holocyclus* from Australia and *Amblyomma americanum* from the U.S.A., appear to be associated with Lyme disease in humans; the remaining seven ticks usually do not attack humans, but are important in maintaining natural foci in which Lyme disease spirochetes persist. Special attention is given to *Ixodes uriae*, a bird tick that maintains *Borrelia burgdorferi* among seabirds in Sweden.

On the basis of DNA homology, rRNA gene restriction patterns, and immunological reactivities, *Borrelia burgdorferi* has been classified into several gene species. Their distribution and possible relationship to clincial manifestations of Lyme disease will be discussed.

It is speculated that ongoing and future tick/spirochete surveys will discover additional distinct gene types of *Borrelia burgdorferi* as well as hitherto undescribed spirochetes.

KEY WORDS

Lyme disease, tick vectors, spirochetes, taxonomy

Erythema (chronicum) migrans (EM) was initially described as a rare skin disease in the Scandinavian countries (1). Today, it is recognized in various parts of the world, particularly in Europe, North America, and Asia as the most prevalent tick-borne spirochetosis. It is a complex illness that may affect not only the skin but also the skeleton, muscles, heart, eye, and nervous system of children and adults alike (2).

Ever since Afzelius described EM in 1910 (3), ticks had been suspected as vectors of a then unknown causal agent. Once the spirochetal etiology was established in late 1981 (4), intensive tick/spirochete surveys led to the realization that this disease - now known as Lyme disease (LD) or Lyme Borreliosis (LB) - occurs around the globe where it coincides with the geographic distribution of ixodid ticks of the *Ixodes ricinus/persulcatus* complex

- a group of hard ticks whose distribution is limited to the temperate zone, i.e., to regions between the 33rd and 65th degrees latitude (see Fig. 1) (5).

In addition to the availability of suitable hosts these ticks require a constant relative humidity of at least 80% and above - a condition found in deciduous forests with damp soil and rich undergrowth and brush. Of the 7 species listed in the *ricinus/persulcatus* complex (see Table 1), 4 are recognized as efficient vectors of LD spirochetes to humans;

(1) the European castor bean or sheep tick, *Ixodes ricinus*, - the most common tick of western and central Europe,

(2) the Taiga tick, *Ixodes persulcatus*, the main vector in Russia, China, and Japan,

(3) the black-legged deer tick, *Ixodes scapularis* that occurs in eastern, southeastern, and midwestern parts of the United States,

(4) the black-legged western deer tick, *Ixodes pacificus* found abundantly in California, the north western states, and in western Canada.

The eastern deer tick, *Ixodes dammini*, is no longer recognized as a separate species. Extensive studies have shown that it is morphologically and genetically indistinguishable from *Ixodes scapularis* and therefore, should be considered a synonym of the latter (6).

All three parasitic stages of these ticks - larvae, nymphs, and adults, have a wide range of vertebrate hosts. The immature larvae and nymphs attack predominantly rodents, lizards, birds, and humans, whereas the adults parasitize larger animals, such as deer, dogs, cattle, and humans.

Intensive surveys around the globe have identified several additional tick/spirochete associations that are less significant from a public health point of view, because the ticks involved do not, or only rarely, come into contact with humans. Yet such ticks play a significant role in maintaining and distributing terrestrial and maritime enzootic foci of LD spirochetes.

In Europe for instance, the hedgehog tick, *Ixodes hexagonus*, is said to be a competent vector of *Borrelia burgdorferi* (*Bb*) among its hosts, primarily hedgehogs, foxes, martens, dogs and cats (7,8).

Certain terrestrial species of birds have long been suspected of playing an important role in the ecology of LD spirochetes; they not only are the means by which ticks are spread over large geographic regions, but also are sources for infecting ticks (9). In a recent study in northern Sweden, a marine enzootic cycle of LD was discovered (10). Its major components are said to be seabirds (Razorbills and Guillemots) and their tick parasite, *Ixodes uriae*. This tick is found abundantly on islands of the Baltic Sea where terrestrial mammals are absent. It is associated with seabird colonies in both the northern and southern hemispheres. Spirochetes similar to those isolated from *Ixodes uriae* in Sweden were recovered from the same species of ticks collected off seabirds in New Zealand, Crozet Islands, Falkland Islands, Alaska, Labrador, Iceland, Faeroer, and France. The study confirms the role of birds, not only in the dispersal of infected ticks, but also in the multiplication and survival of *Borrelia* spirochetes where rodent reservoirs are absent (11).

Involvement of ticks in enzootic cycles of LD spirochetes has been reported also from California where the woodrat tick, *Ixodes neotomae*, maintains a primary cycle among its hosts (woodrats). They serve as sources of spirochetal infections for the simultaneously parasitizing *Ixodes pacificus* - the primary vector to humans in California (12).

Similarly, the rabbit tick, *Ixodes dentatus* has been found to maintain spirochetal infections among its host, the cottontail rabbit (*Sylvilagus floridanus*). Isolations of spirochetes from this tick, as well as rabbits within New York City, New York, suggested the presence of enzootic foci that could become the source of infection for other ticks, such as the human-biting *Ixodes scapularis* (13). Molecular and genetic analyses of isolates from *Ixodes dentatus* and cottontail rabbits indicated a previously undescribed spirochete, for which the name *Borrelia andersonii* sp. nov. was proposed in honor of Dr. J.E. Anderson who first isolated and characterized this species (14).

Other species of rabbit ticks that do not attack humans, but are important in maintaining natural foci of spirochetes belong to the genus *Haemaphysalis*. They have been found infected with spirochetes in the United States (15), China (16), Japan (17), and Australia (18).

Early molecular investigations of North American and European isolates of spirochetes from patients and ticks have indicated those from North America are more homogenous than those from Europe. Subsequently on the basis of DNA relatedness, *Bb* was classified into three genospecies: *Bb* sensu stricto (s.s.), *B. garinii*, and *B. afzelii* (19).

Bb s.s. was found on the North American, European, and Asian continents, whereas *B. garinii* and *B. afzelii* have been reported so far only from Europe

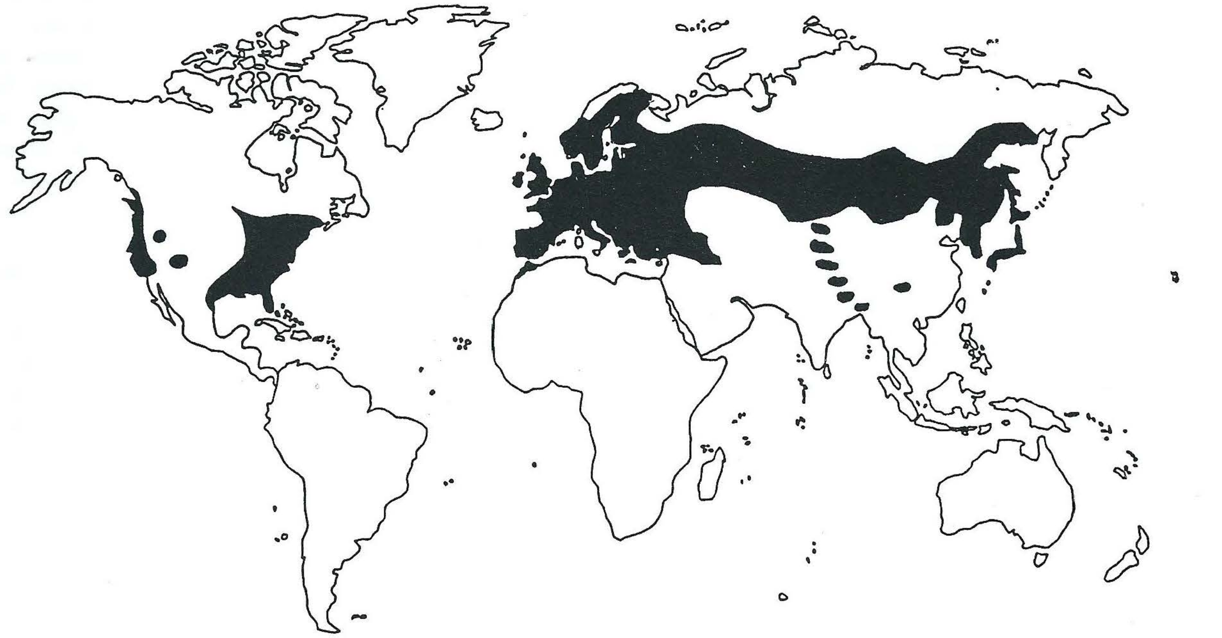

Fig. 1. Global distribution of Ixodes ticks able to transmit Lyme disease spirochetes. Modified from Filippova NA, editor: Taiga tick, Ixodes persulcatus Schulze (Acarina, Ixodidae). Leningrad, 1985, Nauka Publishers.

and Asia. Unidentified isolates are referred to as *Bb* sensu lato.

Several studies conducted in Europe have shown that the specific genospecies are associated with distinct clinical manifestations of LD (20). Thus, *Bb* s.s. tends to lead to arthritic symptoms, whereas *B. garinii* and *B. afzelii* cause neurological complications and cutaneous manifestations, respectively.

Of particular interest is a recent report from Belgium on the simultaneous presence of different *Bb* genotypes in the same patient (21). Whereas 10 patients were infected with a single genospecies, 8 were infected with more than one. Such multi-infections may be due to consecutive infections by separate individual ticks, or by a single tick infected with more than one genospecies. Obviously multi-infections are likely to occur in small geographic regions where more than one genospecies is found.

This has been demonstrated in a recent tick/spirochete survey in the southern part of Switzerland. Of 51 isolates from *Ixodes ricinus*, 26 were typed as *Bb* s.s., 20 as *B. garinii*, 3 as *B. afzelii*, and 2 (referred to as VS 116) as a hitherto unclassified spirochete (22).

Intensive studies have been conducted also in Japan where the first human case with characteristic EM was diagnosed and serologically confirmed in 1986 (23). The ticks, *Ixodes persulcatus* and *Ixodes ovatus*, are considered the main vectors, although no human case has ever been attributed to spirochetes from *Ixodes ovatus*, even though this tick is said to attack humans more frequently than does the recognized main vector, *Ixodes persulcatus* (24). It has been speculated that the spirochete in *Ixodes ovatus* represents a low virulent or even a nonpathogenic variant of *Bb*. Geno- and phenotypic analyses (restriction fragment length polymorphism ribotyping) indicated that the strains from *Ixodes persulcatus* represent the genospecies *B. garinii* and *B. afzelii*, whereas those from *Ixodes ovatus* differed from all available genospecies, and therefore were named *Borrelia japonica* sp. nov. (25). The genospecies, *Bb* s.s., does not occur in Japan. Recent genomic analyses of spirochetes from the main vector, *Ixodes persulcatus* also showed that certain isolates were unrelated to any of the known genospecies of *Bb*; the name *Borrelia miyamotoi* sp. nov. was proposed for these isolates (26).

LD is supposed to occur also on the Australian continent, where several cases of EM, arthritis, and radiculopathy have been diagnosed since 1986 (27). The paralysis tick, *Ixodes holocyclus*, was incriminated as the most logical vector, although none of the patients claimed having been bitten by this tick which in the laboratory has been shown to be an incompetent vector of LD spirochetes (28). Nevertheless, large percentages (up to 42%) of field-collected *Ixodes holocyclus* and *Haemaphysalis* spp., were found to harbor spirochetes that could not be isolated and established in BSK II medium because of fast-growing bacterial contamination (29). In a few instances, sufficient quantities of spirochetes could be used for limited molecular and immunochemical characterization. The results of these tests (PAGE, monoclonal antibody reactions, PCR), along with clinical case reports, suggested the existence of a genuine, indigenous form of LB in Australia (30).

In spite of these findings, the occurrence of LB in Australia is being questioned, at least until spirochetes are isolated from either ticks or patients (31). The fact that LD has been contracted in a region where *Ixodes holocyclus* does not occur, suggests that other species of ticks may be involved.

The behavior of the Australian spirochete in *Ixodes holocyclus* is reminiscent of the behavior of spirochetes in the lone star tick, *Amblyomma americanum*, from the Southeastern United States (32,33). Also, this spirochete could not be recovered either from ticks or from hundreds of patients who had developed expanding annular lesions similar to those of EM. Surprisingly, both ticks have been shown to be incompetent in maintaining and distributing *Bb* s.s. in the laboratory. Yet both species appear to be associated with LD- like illness. Failure to isolate the causative spirochete in BSK II medium does not rule out the possibility of *Ixodes holocyclus* and *Amblyomma americanum* being vectors of closely related yet distinct spirochetes incapable of survival in cultures.

Table 1. Ixodes ricinus/persulcatus complex.

IXODES RICINUS/PERSULCATUS COMPLEX
PALEARCTIC REGION
Ixodes persulcatus Schulze, 1930 *Ixodes ricinus* Nuttall and Warburton, 1911
NEARCTIC REGION
Ixodes (*Ixodes*) *scapularis* Say, 1821 *Ixodes* (*Ixodes*) *pacificus* Cooley & Kohls, 1943 *Ixodes* (*Ixodes*) *jellisoni* Cooley & Kohls, 1938 *Ixodes* (*Ixodes*) *affinis* Neumann, 1899
NEOTROPICS
Ixodes (*Ixodes*) *pararicinus*

Indeed, PCR technology applied to infected *Amblyomma americanum* tissues led to the description of *Borrelia lonestari* as a new species of borrelia capable of infecting humans but so far incapable of being cultivated in BSK II medium (34).

LD-like manifestations (EM rash, mild constitutional symptoms) after bites by *Amblyomma americanum* have also been reported from the state of Maryland (35). Five of 296 *Amblyomma americanum* were found infected with spirochetes that could not be cultivated.

It is safe to speculate that current and future tick/spirochete surveys around the globe will result in identifying additional *Bb*-like, yet distinct, spirochetes. Many isolates distinct from those already described have been placed into groups that await final characterization and nomenclature. It is also becoming apparent that development and behavior of newly described genospecies may differ from those established for *Bb* in its tick vectors, *Ixodes scapularis* or *Ixodes ricinus*. For instance, according to Russian investigators, the percentage of field-collected *Ixodes persulcatus* with systemic infections, including salivary gland tissues, is much higher than that seen in *Ixodes scapularis* (36). Thus, the migration of spirochetes from the midgut into the salivary glands during early feeding, as has been postulated (37) for *Bb* s.s. in *Ixodes scapularis*, is no longer a prerequisite for early transmission via saliva.

For many of the newly-described spirochete/tick associations, the relationship of the spirochetes to their tick vector(s) is still unknown and awaits intensive investigations as to the ticks'ability to maintain and transmit their spirochetes horizontally as well as vertically. In many instances the demonstration of spirochetes in ticks is merely an indication that these ticks did feed on an infective host; it does not reflect the ticks ability to maintain and distribute the spirochetes in nature. In Europe, for instance, *Ixodes trianguliceps* and *Ixodes acuminatus* from western France have been found infected (38), as have 11 of 97 *Dermacentor reticulatus* from eastern Germany (39). In North America, both the Amercian dog tick, *Dermacentor variabilis*, and the brown dog tick, *Rhipicephalus sanguineus*, have been recorded as being infected occasionally with *Bb* (40,41). Under experimental conditions, *Dermacentor variabilis* like the above discussed *Amblyomma americanum* and *Ixodes holocyclus* is an incompetent vector of this spirochete (42).

Finally, reports from China have indicated the presence of *Bb* in *Ixodes granulatus* and in *Ixodes rangtangensis* (16). It is not clear whether these species are efficient vectors to humans.

Considering the complexity of the clinical, ecological, and bacteriological problems in LB it has to be stated that there is still a great need for additional research in the field of this tick-borne spirochetosis.

REFERENCES

1. Lipschütz B. Über eine seltene Erythemform (Erythema chronicum migrans). Arch Derm Syph 1913; 118: 349-56.

2. Weber K, Burgdorfer W. Aspects of Lyme Borreliosis. Heidelberg: Springer-Verlag, 1993.

3. Afzelius A. Verhandlungen der Dermatologischen Gesellschaft zu Stockholm. Arch Derm Syph 1910; 101: 404.

4. Burgdorfer W, Barbour AG, Hayes SF et al. Lyme disease - a tick-borne spirochetosis? Science 1982; 216: 1317-19.

5. Filippova NA. A hypothesis for the palaeogenesis of the distribution of the main vectors of Lyme disease. In: Dusbabeck I, Bukva V (eds.). Modern Acarology. 1991; 109-18.

6. Oliver JH, Jr., Owsley MR, Hutcheson HJ et al. Conspecificity of the ticks *Ixodes scapularis* and *Ixodes dammini* (Acari: Ixodidae). J Med Entomol 1993; 30: 54-63.

7. Liebisch A, Olbrich S, Brand A et al. Natürliche Infektionen der Zeckenart *Ixodes hexagonus* mit Borrelien (*Borrelia burgdorferi*). Tierärztl Umsch 1989; 44: 809-10.

8. Gern L, Toutougi LN, Min HC et al. *Ixodes* (pholeoixodes) *hexagonus*, an efficient vector of *Borrelia burgdorferi* in the laboratory. Med Vet Entomol 1991; 5: 431-35.

9. Anderson JF, Johnson RC, Magnarelli LA et al. Involvement of birds in the epidemiology of the Lyme disease agent *Borrelia burgdorferi*. Infect Immun 1986; 51: 394-96.

10. Olsen B, Jaenson TGT, Noppa L et al. A Lyme Borreliosis cycle in seabirds and *Ixodes uriae* ticks. Nature 1993; 362: 340-42.

11. Olsen B, Duffy DC, Jaenson TGT et al. Transhemispheric exchange of Lyme disease spirochetes by seabirds. J Clin Microbiol 1995; 33: 3270-74.

12. Brown RN, Lane RS. Lyme disease in California: a novel enzootic transmission cycle of *Borrelia burgdorferi*. Science 1992; 256: 1439-42.

13. Anderson JF, Magnarelli LA, LeFebvre RB et al. Antigenically variable *B. burgdorferi* isolated from cottontail rabbits and *Ixodes dentatus* in rural and urban areas. J Clin Microbiol 1989; 27: 13-20.

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

15. Lane RS, Burgdorfer W. Spirochetes in mammals and ticks (Acari: Ixodidae) from a focus of Lyme Borreliosis in California. J Wildl Dis 1988; 24: 1-9.

16. Zhang Z. Survey on tick vectors of Lyme disease spirochetes in China (Chinese). Chung-Hua Liu Hsing Ping Hsueh Tsa Chih 1992; 13: 271-74.

17. Takada N, Ishiguro F, Iida H et al. Prevalence of Lyme borrelia in ticks especially *Ixodes persulcatus* (Acari: Ixodidae) in central and western Japan. J Med Entomol 1994; 31: 474-78.

18. Wills MC, Barry RD. Detecting the cause of Lyme disease in Australia. Med J Australia 1991; 155: 275.

19. Postic D, Assous MV, Grimont PAD et al. Diversity of *Borrelia burgdorferi* sensu lato evidenced by restriction fragment length polymorphism of rrf (5S)-rrL (23S) intergenic spacer amplicons. Int J Syst Bacteriol 1994; 44: 743-52.

20. van Dam NP, Kuiper H, Vos K et al. Different genospecies of *Borrelia burgdorferi* are associated with distinct clinical manifestations of Lyme Borreliosis. Clin Infect Dis 1993; 17: 708-17.

21. Demaerschalck I, Messaoud AB, De Kesel M et al. Simultaneous presence of different *Borrelia burgdorferi* genospecies in biological fluids of Lyme disease patients. J Clin Microbiol 1995; 33: 602-8.

22. Péter O, Bretz AG, Bee D. Spotted distribution of *Borrelia burgdorferi* isolates in Valais (Switzerland). Europ J Epidemiol 1996; (in press).

23. Kawabata M, Baba S, Iguchi K et al. Lyme disease in Japan and its possible incriminated tick vector, *Ixodes persulcatus*. J Infect Dis 1987; 156: 854.

24. Nakao M, Miyamoto K, Uchikawa K et al. Characterization of *Borrelia burgdorferi* isolated from *Ixodes persulcatus* and *Ixodes ovatus* ticks in Japan. Am J Trop Med Hyg 1992; 47: 505-11.

25. Kawabata H, Masuzawa T, Yanagihara Y. Genomic analysis of *Borrelia japonica* sp. nov. isolated from *Ixodes ovatus* in Japan. Microbiol Immun 1993; 37: 843-49.

26. Fukunaga M, Takahashi Y, Tsuruta Y et al. Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. Intern J Bact 1995; 45: 804-10.

27. Stewart A, Glass J, Patel A et al. Lyme arthritis in the Hunter Valley. Med J Australia 1982; 1: 139.

28. Piesman J, Stone BF. Vector competence of the Australian paralysis tick, *Ixodes holocyclus*, for the Lyme disease spirochete, *Borrelia burgdorferi*. Intern J Parasitol 1991; 21: 109-11.

29. Greene RT, Walker RL, Greene CE. Pseudospirochetes in animal blood being cultured for *Borrelia burgdorferi*. J Vet Diagn Invest 1990; 3: 352-53.

30. Hudson BJ, Barry RD, Shafren DR et al. Does Lyme Borreliosis exist in Australia? J Spiroch and Tick-borne Dis 1994; 1: 46-51.

31. Russell RC. Lyme disease in Australia - still to be proven. Emerg Infect Dis 1995; 1: 29-31.

32. Masters EJ, Donnell H, Fobbs M. Missouri Lyme disease: 1989 through 1992. J Spiroch and Tick-borne Dis 1994; 1: 12-17.

33. Fier D, Reppell CA, Ben-Wen LL. Evidence supporting the presence of *Borrelia burgdorferi* in Missouri. Am J Trop Med Hyg 1994; 51: 475-82.

34. Barbour AG, Maupin GO, Teltow GJ et al. Identification of an uncultivable *Borrelia* species in the hard tick *Amblyomma amercianum*: possible agent of a Lyme disease-like illness. J Infect Dis 1996; 173: 403-9.

35. Armstrong PhM, Rich StM, Smith DR et al. A new *Borrelia* infecting lone star ticks. Lancet 1996; 347: 67.

36. Korenberg EI, Moskvitina GG, Vorobyeva NN. Prevention of human borreliosis after infected tick's bite. Proc VI Intern Conf on Lyme Borreliosis 1994: 209-11. (abstract)

37. Piesman J, Mather TN, Sinsky RJ et al. Duration of tick attachment and *Borrelia burgdorferi* transmission. J Clin Microbiol 1987; 25: 557-58.

38. Doby JM, Bigaignon G, Launay H et al. Presence of *Borrelia burgdorferi* in *Ixodes* (Exopalpiger) *trianguliceps* Birula 1895 and *Ixodes* (Ixodes) *acuminatus* Neuman 1901 (Acari: *Ixodidae*) and in *Ctenophthalmus baeticus arvernus* Jordan, 1931, also in *Megabothris turbicus* (Rothschild 1909) (Insects: Siphonapteea), eactoparasites of small mammals in the forests of western France. Bull Soc Franc Parasitol 1990; 8: 311-22.

39. Kahl O, Janetzki C, Gray JS et al. Tick infection rates with *Borrelia*: *Ixodes ricinus* versus *Haemaphysalis concinna* and *Dermacentor reticulatus* in two locations in eastern Germany. Med Vet Entomol 1992; 6: 363-66.

40. Magnarelli LA, Anderson JF. Ticks and biting insects infected with the etiologic agent of Lyme disease, *Borrelia burgdorferi*. J Clin Microbiol 1988; 26: 1482-86.

41. Rawlings JA. Lyme disease in Texas. Zbl Bakt Hyg 1986; 263: 483-87.

42. Piesman J, Sinsky RJ. Ability of *Ixodes scapularis*, *Dermacentor variabilis* and *Amblyomma americanum* (Acari: Ixodidae) to acquire, maintain, and transmit Lyme disease spirochetes (*Borrelia burgdorferi*). J Med Entomol 1988; 25: 336-39.

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