ASSOCIATION OF GENETIC VARIABILITY WITHIN THE *BORRELLIA BURGDORFERI* SENSU LATO WITH THE ECOLOGY, EPIDEMIOLOGY OF LYME BORRELIOSIS IN EUROPE

Markéta Derdáková¹,², Daniela Lenčáková¹

¹Parasitological Institute, Slovak Academy of Sciences, Košice, Slovakia
²Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia


Abstract: Lyme borreliosis (LB) represents the most common vector-borne zoonotic disease in the Northern Hemisphere. The infection is caused by the spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex which circulate between tick vectors and vertebrate reservoir hosts. The complex of *Borrelia burgdorferi* s.l. encompasses at least 12 species. Genetic variability within and between each species has a considerable impact on pathogenicity, clinical picture, diagnostic methods, transmission mechanisms and its ecology. The distribution of distinct genospecies varies with the different geographic area and over a time. In recent years, new molecular assays have been developed for direct detection and classification of different *Borrelia* strains. Profound studies of strain heterogeneity initiated a new approach to vaccine development and routine diagnosis of Lyme borreliosis in Europe. Although great progress has been made in characterization of the organism, the present knowledge of ecology and epidemiology of *B. burgdorferi* s.l. is still incomplete. Further information on the distribution of different *Borrelia* species and subspecies in their natural reservoir hosts and vectors is needed.

Address for correspondence: MVDr. Markéta Derdáková, PhD, Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, 845 06 Bratislava, Slovakia.
E-mail: derdak@saske.sk

Key words: *Ixodes ricinus*, genetic variability, *Borrelia burgdorferi*, Lyme borreliosis, ecology, reservoir hosts.

INTRODUCTION

*Ixodes ricinus* is the most important vector of tick-borne zoonoses in Europe. It transmits viral (e.g. tick-borne encephalitis virus), bacterial (e.g. *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*) as well as protozoan pathogens (e.g. *Babesia microti*, *Babesia divergens*) to humans and animals. In recent years, as a result of global warming, climatic changes and human impact on the environment, the abundance of ticks in Central Europe is increasing [38]. Consecutively this leads to the emergence of the tick-transmitted diseases.

Lyme borreliosis (LB) represents the most common disease transmitted by the *Ixodes ricinus*. Annually, from 14–140 cases/100,000 inhabitants are reported in Europe [5, 82]. The highest incidence of this disease is reported from Central-Eastern Europe. Based on the WHO reports, the highest incidence of LB is in Slovenia and Austria with 120 and 130 cases per 100,000 inhabitants, respectively. On the other hand, in Slovakia the incidence of LB is about 14 cases per 100,000 inhabitants [5]. This number, however, does not have to reflect the real incidence of the disease due to the misdiagnosis and insufficient report system. LB is multisystemic inflammatory disorder caused by spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex [10, 54]. In natural foci, *B. burgdorferi* s.l. circulates efficiently through enzootic cycles involving ticks of the *Ixodes*
ricinus complex and a variety of vertebrate reservoir hosts [33]. Clinical symptoms of human borreliosis commonly occur in stages, and may involve multiple organs and tissues. It usually starts in the skin at the site of the tick bite where typical erythema migrans develops in about 60% of patients. From the primary lesion, spirochetes can disseminate causing multiple erythema migrans or it can affect other organ systems. Positive findings of Borrelia infection have been detected in skin, blood, cerebrospinal fluid, urine, joints, endomyocardial and iris biopsies [11, 26, 97, 105, 118].

At the time of its discovery, it was thought to be a uniform organism [10]. Later, as the culture isolation of the spirochete became more efficient, numbers of isolates were obtained from ticks, reservoir animals and patients. Data from molecular and phenotypic studies of these isolates revealed that the polymorphism is by far exceeding intraspecific variability. Based on morphology, antigen profile and molecular analysis of DNA it belongs to the genus Borrelia, family Spirochaetaceae and order Spirochaetales [54]. B. burgdorferi s.l. complex is subdivided into 11 genospecies: B. burgdorferi sensu stricto (s.s.), Borrelia garinii [6], Borrelia afzelii [12], Borrelia valaisiana [121], Borrelia lusitaniae [68], Borrelia japonica, Borrelia andersonii [74], Borrelia turkii, Borrelia turdæ [32], Borrelia bissetti [96] and Borrelia sinica [75]. Moreover Borrelia burgdorferi strain A14S [120], (according to Richer et al. [99] Borrelia spielmanii sp. nov.), most likely the new Borrelial genospecies was cultured from a erythema migrans of a patient and detected in ticks and small mammals [20, 29, 78, 99, 120]. A high level of intraspecific variability was also observed [7, 20, 78, 104, 125].

Borrelia specific associations with different clinical manifestations have been detected in patients with Lyme borreliosis [117].

In addition, distinct genospecies are also considered to be preferentially associated with different reservoir hosts [50, 61, 63, 64]. Recent studies showed that variability at lower hierarchical level is probably also an important determinant of LB pathogenesis and ecology [7, 48, 104].

Based on this knowledge, the surveillance of the Borrelia prevalence in ticks and reservoir hosts, as well as accurate identification and typing of Borrelia burgdorferi s.l., is the key factor for understanding the ecology and epidemiology of this disease as well as for the establishment effective preventive strategies. Further information on the distribution of the different Borrelia species and subspecies in their natural reservoir hosts and vectors is needed.

**Epidemiology and Ecology of Lyme Borreliosis**

B. burgdorferi s.l. in transmission vectors. Currently, ticks of the I. ricinus complex are the only recognized vectors of B. burgdorferi s.l. [9]. Larvae, nymphs and adult females of ticks can acquire Borreliae when feeding on an infective competent reservoir host [3]. Uninfected ticks can also became infected when feeding together with infected vector ticks on a co-feeding basis in the absence of disseminated infection in the host. This mechanism is well accepted for the tick-borne encephalitis virus [67]. Gern and Rais [36] experimentally proved co-feeding transmission of B. burgdorferi s.l. which seems to play an important route of transmission in some areas of England where sheep feed all 3 stages of I. ricinus. Ticks acquire infection while feeding next to the infected ticks since sheep are not competent reservoir hosts of B. burgdorferi s.l. [83]. In previous studies, authors [36, 98] reported that B. burgdorferi remains in the skin of a host before it disseminates. In some cases, it can take up to 4 weeks. If the uninfected ticks (larvae, nymphs) feed at this site even after the infected tick (source of infection) finishes its bloodmeal they can acquire B. burgdorferi before the systemic infection of a host develops - “localized extended co-feeding” [36, 98]. In unfed ticks, Borrelia is usually located in the midgut [8], although systemic infection affecting different organs of the tick may occur [69]. B. burgdorferi s.l. in ticks is transtadially transmitted to the next instars. Rarely, Borrelia can migrate to the ovaries of a female tick with the systemic infection, which results in transovarial transmission to their larval progeny [50].

B. burgdorferi s.l. have to face more changes in the tick environment related to the temperature and the blood meal. The migration of bacteria within the tick is associated with variable protein expression which, under certain circumstances, evokes the spirochete transmission into an animal host. In the unfed tick, Borrelia are generally located in the midgut where they express outer surface protein A (OspA) but not C (OspC), [28, 103]. On its surface, OspA possesses a receptor for plasminogen of a host organism. After the tick starts to feed on the host, plasminogen changes into plasmin, which facilitates Borrelia migration through the midgut wall and haemocell [15]. During the blood meal, the synthesis of OspC is upregulated and synthesis of OspA is downregulated [84, 102, 103], forming the heterogeneous population of Borrelia expressing both antigens in the midgut. It was thought that Borrelia with the OspC and without the OspA expression enter salivary glands; however, Ohnishi et al. [84] have shown that 80% of the spirochetes in the tick salivary glands do not express either of the proteins. The increased OspC expression is associated with a host immune response in the early stage of the disease [4, 87, 88]. The switch from the OspA to OspC expression is most likely related to the change in temperature in the feeding tick [81, 103].

The tick must be attached to the host for at least 24 hours before transmission starts. The most effective transmission of B. burgdorferi s.s. in North America occurs after 48 hour of the tick attachment [84, 94]. Different results obtained by researchers in Europe indicate that the dynamics of the various Borrelia species
in *I. ricinus* might be different. Systemically infected unfed ticks with *Borrelia* present in salivary glands can transmit spirochetes to the host early after the attachment [69]. Moreover, *B. afzelii* was detected in mice 24 hours after the attachment of infected ticks [18].

The tick-host interaction is also an important factor influencing the *Borrelia* transmission. The attached tick secretes vasoactive mediators and immunomodulators that facilitate the transmission of pathogens to the host [80, 124]. The tick saliva activates the transmission of viruses [66]. This phenomenon, known as salivary activated transmission (SAT), was recently demonstrated also for *B. afzelii* [65, 89].

Based on the review paper by Hubálek and Halouzka [47], the average infection prevalence of questing *I. ricinus* in Europe was 1.9% (0–11%) for larvae, 10.8% for nymphs (2–43%) and 17.4% for adults (3–58%).

The infection prevalence varied geographically and according to the used method of detection [47]. A prevalence of *Borrelia* in *I. ricinus* from eastern Slovakia was studied by PetKo et al., [92] during a 10-year period. Over this period, average prevalence of infection was 12.7%. The prevalence ranged from 2.1–41.7%. The 10-year study showed a considerable local fluctuation in the infectious prevalence of ticks in the individual years, with 3–5 years periods of increased prevalence. The periodic change in *Borrelia* prevalence in ticks was also detected recently by authors in the same area during a 4-year period. The highest prevalence, with 23.9% of ticks infected, was detected in 2001. During 2002 and 2003 the prevalence decreased to 14.1% and 13.5%, respectively. The following year, the percentage of infected ticks rose to 15.3% (authors unpublished). This periodical change in occurrence of *Borrelia* in ticks from Slovakia was already pointed out by Kmety et al. [59]. Štepánová-Tresová et al. [113] surveyed the prevalence of *B. burgdorferi* s.l. in *I. ricinus* during 4 years in 2 distinct geographical regions of eastern Slovakia. The average prevalence rates in 4 consecutive years were 4.8%, 17.2%, 15.5%, 14.2%, respectively.

Besides ticks, mosquitoes [39], horseflies, flies from the family Stomoxydidae [71], fleas, lice and mites [93] were found to be infected with *Borrelia*. Since no experimental or field study proved their competence as vectors, they can only be considered as a carrier species. Carriers might be able to transmit *Borrelia* transstadially, but are unable to transmit the spirochetes to the host [55]. Moreover, the spirochetes detected or isolated from the carriers were not always reliably characterized as a *B. burgdorferi* s.l. [35].

*B. burgdorferi* s.l. in reservoir hosts. Identification of the reservoir hosts participating in a *Borrelia* circulation in natural foci is one of the most important components in understanding the ecology of Lyme borreliosis. The vertebrate hosts that are necessary to maintain tick population might also serve as a source of infection. *Ixodid* ticks infest more than 300 different vertebrates including mammals, birds and reptiles [2]. Competent reservoir host are the animal species that harbour the pathogen and present a long-term infection source for feeding tick vectors. Ticks can become infected while having a blood meal on such an animal, as well as by the already mentioned co-feeding transmission when spirochetes pass from infected to uninfected ticks which feed simultaneously on the same host without systematic infection [33, 83].

Gern et al., [33] reported that 9 small mammals, 7 medium-sized mammals and 16 bird species were competent reservoirs of *B. burgdorferi* s.l. in Europe. The authors listed the methods that should be used for identification of the reservoir host. The most valuable method is xenodiagnosis of captured animal with uninfected larval ticks. However, this method is not always feasible since some animals are difficult to maintain under laboratory conditions. Collection of engorging or engorged larvae from wild animals can be used as an alternative method. Detection of infection in unfed ticks along with blood meal analysis was developed by Kirstein and Gray [57]. This method enables the identification of hosts which have fed in a previous developmental stage. This methodological approach has a considerable potential. Competent rodent reservoirs include the wood mouse (*Apodemus sylvaticus*), yellow-necked field mouse (*Apodemus flavicollis*), black striped mouse (*Apodemus agrarius*), bank vole (*Clethrionomys glareolus*), meadow vole (*Microtus arvalis*) [63, 116], edible dormouse (*Glis glis*) [77], rats (*Norway rat* *Rattus norvegicus* and black rat *Rattus rattus*) [76] and squirrels (red squirrel *Sciurus vulgaris* and grey squirrel *Sciurus carolinensis*) [17, 49]. Among insectivores, the reservoir competence has been demonstrated by shrews (European water shrew *Neomys fodiens*, Eurasian pygmy shrew *Sorex minutus* and Eurasian common shrew *Sorex araneus*) [116] and European hedgehog (*Erinaceus europaeus*) [37]. Hares (brown hare *Lepus europaeus* and mountain hare *Lepus timidus*) [116] also contribute to the maintenance of *B. burgdorferi* s.l. in nature.

Several large-size mammals, such as roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), sika deer (*Cervus nipon yessoensis*), moose (*Alces alces*), fallow deer (*Dama dama*), cattle (*Bos taurus*) and sheep (*Ovis aries*) have been reported as non-significant reservoirs [116]. Even incompetent hosts can participate in the circulation of *B. burgdorferi* s.l. via co-feeding transmission, which has been proved in sheep in Britain [83].

The importance of avian hosts for the maintenance of *Borrelia* infection, especially the role of ground-foraging passerines, is now indisputable. It is also proposed that migratory birds contribute to the wide-spreading of *B. burgdorferi* s.l. spirochetes along their migration routes [85]. Different *Borrelia* species have been detected in ticks (*Ixodes ricinus* and *Ixodes uriae*), blood samples and skin biopsies collected from several avian species throughout Europe [17, 42, 63, 85, 86, 115].
Presence of *B. burgdorferi* by PCR or antibody response against *Borrelia* has been reported also in domestic animals such as dogs [45, 108, 111], horses [73, 110], cattle [109], and cats [72]. Isolation or detection of *Borrelia* from host tissues indicates the host susceptibility to the infection but not ability to transmit *Borrelia* to feeding ticks. Positive serological evidence confirms the exposure to the infection; however, it also does not demonstrate the survival of *Borrelia* in a host and consequent transmitting to the feeding ticks [33].

Results obtained from the studies on reservoir competence for different *B. burgdorferi* s.l. from various geographic areas indicate that distinct genospecies of *B. burgdorferi* s.l. are associated with different reservoir hosts [41, 42, 49, 50, 51, 52, 63]. *B. afzelii* is associated with rodents [41, 52], while *B. garinii* and *B. valaisiana* circulates in birds [42, 51, 63]. However, the invasive strains of *B. garinii* which belong to the OspA type 4 are associated with rodents as reservoir hosts [48]. Recently, Richter et al., [99] reported very specific association of a newly described species *B. spielmani* with garden dormice (*Eliomys quercinus*), which might explain the local findings of this species in the areas where this reservoir host is abundant. Contrary to this, *B. burgdorferi* s.s. does not seem to show the unambiguous relationship to the specific reservoir host. In Switzerland, *B. burgdorferi* s.s. together with *B. afzelii* were isolated from squirrels and the ticks that fed on them [49]. In England, Kurtenbach et al., [63] detected *B. burgdorferi* s.s. in 1.3% of ticks that fed on rodents. Moreover, Kurtenbach et al., [64] showed that experimentally infected pheasants are able to transmit *B. burgdorferi* s.s. on xenodiagnostic ticks. Hanincová, PhD thesis [40] identified *B. burgdorferi* s.s. in 2.2% of the infected larvae collected from *A. flavicollis* in western Slovakia. No *B. burgdorferi* s.s. was detected in ear biopsies from these mice. The hypothesized origin of such specific associations is attributed to the host serum complement. The complement can lysate the different *Borrelial* genospecies depending on the resistance or sensitivity of particular genospecies to the complement of particular host [61]. According to this theory, *B. afzelii*, *B. garinii* (NT29 ribotype, OspA type 4), *B. japonica* and *B. bissetti* are resistant to the rodent complement, that makes rodents appropriate reservoir hosts for these genospecies. *B. garinii* (except the above-mentioned strains) and *B. valaisiana* are resistant to the bird complement. *B. burgdorferi* s.s. has an intermediate resistance to both bird and rodent complement and often seem to be infectious to birds and rodents [61]. In addition, the complement of the host can lyse *Borrelia* that is already present in a feeding tick. For example, if a nymph infected with *B. afzelii* feeds on a bird host, the complement can lyse the *Borrelia* within the feeding tick [61]. This was proven in experimentally infected pheasants. *B. garinii*, *B. burgdorferi* s.s. and *B. valaisiana* survived in ticks that had taken the blood meal on pheasants while *B. afzelii* did not [64]. The deer serum has *Borreliacidal* activity against all the genospecies, which is in accordance with the statement that deers are not competent reservoir hosts for *B. burgdorferi* s.l. [61].

Once spirochetes appear in the mammalian host, they remain in the skin at the bite site for several days. Later, *Borrelia* disseminate to different tissues where they indicate inflammatory reactions. *Borrelia burgdorferi* s.l. species have developed more adaptation strategies in order to escape the immune response of the vertebrate host [4]. This protection involves, e.g. recombination of VlsE protein, which results in wide antigenic heterogeneity and probably help the *Borrelia* to persist in the infected organism [70]. The other mechanism is based on the inhibition of the complement cascade via exported repeated proteins (Erps), e.g. OspE and OspF [79]. Additionally, *Borrelia* start expressing several proteins after spirochaete transmission to the host or even during a tick engorgement. These proteins, including fibronectin-binding protein (Bbk32) [27] and decorin binding proteins DpbA and DpbB [30], seem to be important for the dissemination and localisation of the spirochetes within an infected animal. VlsE, Bbk32 and DpbA are useful antigens in the serology of LB [70]. The function of all proteins expressed during the infection, however, has not been completely explained.

**Distribution of *B. burgdorferi* s.l. in Europe.** Over the past 2 decades, a number of studies have been published on *B. burgdorferi* s.l. prevalence and its genetic variability in Europe [1, 13, 14, 20, 21, 25, 40, 41, 47, 53, 56, 57, 59, 60, 90, 91, 92, 106, 107, 113, 126]. Lyme borreliosis is present all over Europe, except in the hot south (Sicily, Southern Spain) and in the cold north (northern Scandinavia and northern Russia). [123].

The geographic distribution of *B. burgdorferi* s.l. genospecies in Europe is very variable. In the review article of Hubálek and Halouzka [46], information on 1,263 isolates of *B. burgdorferi* s.l. from 26 European countries was provided. 501 isolates were classified as *B. garinii* (39.7%), 469 as *B. afzelii* (37.1%), 201 as *B. burgdorferi* s.s. (15.9%), 85 as *B. valaisiana* (6.7%) and 7 as *B. lusitaniae* (0.6%). Currently, the boundaries of the distribution areas of each genospecies are repeatedly revised as a result of the increased number of prevalence studies and gradual implementation of more performing molecular tools. *B. garinii* and *B. afzelii*, occur alternately as a dominant genospecies in most of the studied European countries [13, 20, 25, 46, 62]; however, distribution of *Borrelial* genospecies can vary even over relatively small areas as well as over the time period [25, 40, 41, 115]. Presence of *B. garinii* in southern Norway is very rare [53]; on the other hand, in neighbouring Sweden it was found in 31% of positive ticks [31]. In Slovakia, *B. garinii* predominates over a 4-year period in ticks from some areas of eastern Slovakia [authors, unpublished results, 113]. In contrast, in western Slovakia *B. afzelii* is the predominant genospecies [34, 41, 62]. *B. burgdorferi* s.s. has been previously reported mostly from western European countries, with rare isolation or detection from...
eastern Europe [46, 95]. However, further studies have shown the presence of *B. burgdorferi* s.s. in *I. ricinus* ticks from the Czech Republic [19, 20, 112], Slovak Republic [34, 62, 113], Poland [106, 126], Bulgaria [14] and Russia [1].

*B. valaisiana*, for the first time isolated in the Switzerland [90], was originally thought to be the less prevalent genospecies, with the exception of Ireland, where it was the most frequently detected genospecies [57]. According to recent studies, *B. valaisiana* belongs to the 3 most prevalent genospecies of the *B. burgdorferi* s.l. complex in Europe [20, 34, 56, 62, 126].

*B. lusitaniae* has been isolated or detected in *I. ricinus* ticks less frequently in a few European countries [13, 34, 41, 95, 126] with the exception of the Iberian Peninsula, where in some regions it represents the only genospecies of the *B. burgdorferi* s.l. complex [21, 62]. *B. spielmani* has been detected in *I. ricinus* ticks from the Czech Republic [20], Germany [78, 99], and France [99], so far.

**Impact of *Borrelia* diversity on pathogenicity and clinical symptoms**. Currently, 3 genospecies within the *B. burgdorferi* s.l. complex are clearly established as pathogenic to humans: *B. afzelii*, *B. garinii* and *B. burgdorferi* s.s. [11, 117]. Hoverer, based on recent publications, *B. valaisiana* and *B. lusitaniae* previously considered as non-pathogenic might cause disease as well. *B. valaisiana* was detected by PCR in erythema migrans (EM) of patients [100], and caused inflammation of joints in experimentally infected mice [24]. Moreover, recently, DNA of *B. valaisiana* was detected in the cerebrospinal fluid of a patient with chronic neuroborreliosis in Greece [23]. *B. lusitaniae* was isolated from a patient with suspected Lyme borreliosis in Portugal [16]. Pathogenicity of *B. bissetti* in North America was not proven, although isolates from EM of patients with genotypic and phenotypic similarities to *B. bissetti* [96] were cultured in Slovenia [114]. In addition, Wang et al. [120] isolated strain A14S from EM of patient in Netherlands, and only recently 4 human isolates were recognized in Germany [29]. This strain had a unique DNA fingerprint pattern, and different from the other *B. burgdorferi* s.l. isolates. The closest relative is *B. spielmani*, recently detected in ticks and rodents [20,78, 99]. *B. afzelii*, *B. burgdorferi* s.s. and *B. garinii* are associated with different clinical symptoms in patients [117, 122]. *B. afzelii* seems to be associated with acrodermatitis chronica atrophicans (ACA), *B. garinii* with neuroborreliosis and *B. burgdorferi* s.s. with arthritis [6, 12, 117, 122]. However, the clinical spectrum caused by different *Borrelia* species can largely overlap [122].

Furthermore, different levels of pathogenicity were recently described among distinct genetic clones within single genospecies. Only 4 clones of 21 major OspC groups of *B. burgdorferi* s.s. are invasive and cause systemic infection [104]. Baranton et al. [7] analyzed OspC sequences of other pathogenic *Borrelia* species. Out of a total of 58 defined groups, only 2 groups of *B. afzelii* and 4 groups of *B. garinii* cause invasive disease

**VACCINE FOR EUROPE**

A wide heterogeneity within the *B. burgdorferi* s.l. complex has been shown to be a crucial problem for the development of an effective vaccine for Europe [125]. The only approved human vaccine was brought out in the USA in 1998. It was withdrawn from the market 4 years after its release, mostly due to unsatisfactory demand [44]. The immunisation worked on blocking the transmission of *Borrelia* from directly in the tick midgut by OspA antibodies produced in the mammalian host [22]. However, this vaccine would probably be less effective in Europe since it is derived from the homologous strain of *B. burgdorferi* s.s. Contrary to this, a European vaccine should represent a cocktail of different strains present in our region [125]. The candidates for successful immunization could be, e.g. polyvalent OspC-based vaccines or vaccines including decorin-binding protein A (DbpA) [43, 44, 101]. Research on this issue is still in progress, though.

**CONCLUSION**

Based on this knowledge, surveillance of the *Borrelia* prevalence in ticks and reservoir hosts, as well as accurate identification and typing of *Borrelia burgdorferi* s.s., is the key factor for understanding the ecology and epidemiology of this disease, as well as for the establishment of the effective preventive strategies. Further information on the distribution of the different *Borrelia* species and subspecies in their natural reservoir hosts and vectors of different geographic areas is continuously needed.

**Acknowledgements**

The work was supported partially by Grants SPVV 2003SP 51/028 08 03/2003 and VEGA No. 2/3213/23 of the Slovak Republic.

**REFERENCES**


7. Baranton G, Seinost G, Theodore G, Postic D, Dykhuaïzen D: Distinct levels of genetic diversity of *Borrelia burgdorferi* are associated


Switzerland.

Hyg

Europe.

Sciurus vulgaris species mice are reservoir hosts of Borrelia burgdorferi isolated from different organs of Ixodes ricinus ticks collected in nature. Zentralbl Bakteriol 1994, 280, 468-475.


