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Review articles

The role of particular ticks developmental stages in the circulation of tick-borne pathogens in Central Europe. 5. Borreliaceae.

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ABSTRACT. The developmental cycles of all *B. burgdorferi* s.l. genospecies present typical, main pattern described in the 90thies. The simple scheme might be modified according to the biology of species and hosts preference. Central European genospecies of *B. burgdorferi* s.l. can be associated with four groups of hosts playing the role of animal reservoirs. The group 1 contains genospecies associated with rodents as primary animal reservoir – *B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto, strains *B. bavariensis* (*B. garinii* OspA serotype 4). The group 2 involves *B. valaisiana* and most of *B. garinii* strains, associated with birds. The group 3 involves *B. spielmanii*, the reservoir hosts are Gliridae, and hedgehogs. The group 4 includes *B. lusitaniae*, the hosts are lizards. *B. miyamotoi* enzootic cycle seems to be similar to *B. burgdorferi* complex, however, differs by the transovarial transmission possibility. The divisions are not extreme; in the hosts group, infected with appropriate *Borrelia* genospecies, very often are found the specimens infected with other genospecies.

Key words: *Borrelia burgdorferi* complex, *Borrelia miyamotoi*, ticks, zoonotic foci

Introduction

Borrelia burgdorferi sensu lato (s.l.) complex is an important group of Gram-negative, helical-shaped, motile bacteria belonging to the Spirochaetales order, family Borreliaceae, genus *Borrelia*; it is the causative agents of tick-borne Lyme disease (borreliosis). The bacterium was detected in 1982 by Burgdorfer et al. [1] and classified as a new species of the *Borrelia* genus by Johnson et al. [2].

It is the bacterium of a quite large in size, the cell is 15.0–20.0 μm in long, 0.2–0.3 μm in width, spiral. The cells are able to move though the presence of two flagella, lying in the periplasmatic area. Due to the two-layer structure of the cell wall, the bacteria are classified as Gram (-), although the ultrastructure and chemical composition differ from typical Gram (-) bacteria [3].

The species from the genus *Borrelia* are obligatory parasites of mammals, as well lizards and birds. They have comparable developmental cycles, but reveal different pathogenicity and display various spectrum of disease symptoms. The human pathogens belonging to *B. burgdorferi* complex cause Lyme Disease (Lyme borreliosis), while others – *Borrelia recurrentis*, *Borrelia hermsi* and *Borrelia miyamotoi* – are the causative agents of louse borne relapsing fever (LBRF) and are classified as the relapsing-fever group spirochetes [3–5].

The variability of *Borrelia burgdorferi* sensu lato

Since time, the results of several studies using the restriction endonuclease analysis, the polymerase chain reaction and reactivity with monoclonal antibodies have shown that this species is heterogenous. The strains differ in hosts preference, pathogenicity for mammals, and at molecular level in chromosome size and number of plasmids [3]. To this time, there have been reported several molecular surveys and phenotypic investigations indicating the occurrence of at least 19 genospecies within *B. burgdorferi* s.l. complex: *B. burgdorferi* sensu stricto, *B. afzelii*, *B. americana*, *B. andersonii*, *B. bavariensis*, *B. bissettii*, *B. californiensis*, *B. carolinensis*, *B. finlandensis*, *B. garinii*, *B. kurtenbachii*, *B. lusitaniae*, *B. japonica*, *B. sinica*, *B. spielmanii*, *B. tanukii*, *B. turdi*, *B. angzte* and *B.valaisiana*. The particular *B. burgdorferi* species vary in their geographic distribution, host specificity and ability to cause disease in humans. Among them, *B. burgdorferi* s.s., *B. afzelii*, and *B. garinii* are most commonly associated with human borreliosis, and much lesser occurrence of other genospecies (*B. bavariensis*, *B. bissettii*, *B. lusitaniae*, *B. spielmanii*, *B. valaisiana*) was also identified in various clinical samples and/or questing ticks [6–10].

Following the genetic diversity, numerous studies evidenced the involvement of genetic variation of the spirochetes with severity and variety of clinical symptoms of Lyme disease in humans. For example, *B. afzelii* is predominantly linked to cutaneous infections and is most frequently linked with skin manifestations (*erythrema migrans*, *acrodermatitis chronica atrophicans*), *B. garinii* and *B. bavariensis* are most often associated with neuroborreliosis, whereas pathogenicity of *B. burgdorferi* s.s. is related to neuroborreliosis and arthritis symptoms [11,12]. Some species, such as *Borrelia lusitaniae*, have only occasionally been associated with human disease while other species, such as *B. valaisiana*, the status is uncertain [4,13].

The distribution of *B. burgdorferi* genospecies is unequal. *B. afzelii*, *B. garinii*, *B. valaisiana*, *B. burgdorferi* s.s. are distributed nearly throughout Europe, other genospecies occur locally or their occurrence is limited to the part of Europe [14]. Moreover, the geographic distribution of the most common *B. burgdorferi* s.l. genospecies appears not to be uniform. In Western Europe, *B. garinii* occurs more frequently, and is followed by *B. afzelii, B. burgdorferi* s.s. and *B. valaisiana*. In Scandinavia and Netherlands, *B. afzelii* is the most common, followed by *B. valaisiana*, *B. burgdorferi* s.s. and *B. garinii* [15]; in Romania predominates *B. afzelii*, followed by *B. garinii* [16]. The recorded proportions in Central Europe are: in Czech *B. afzelii*, followed by *B. valaisiana*, *B. burgdorferi* s.s., *B. garinii* and *B. lusitaniae* [17], in Poland *B. afzelii,* followed by *B. burgdorferi* s.s., and *B. garinii* [18], in Slovakia *B. afzelii,* followed by *B. garinii*, and *B. valaisiana* and *B. burgdorferi* s.s., in Germany *B. afzelii*, followed by *B. garinii*, and *B. valaisiana* and *B. burgdorferi* s.s. [19]. However, the picture is complicated by possible regional differences [20]. There are no data on the season influence on these proportions, in contrast to many other pathogens transmitted by blood-sucking arthropods.

The vectors of *Borrelia burgdorferi* sensu lato

Circulation of *B. burgdorferi* s.l. genospecies in the environment is sustained by enzootic cycles, comprising *Ixodes ricinus* complex (also known as *I. persulcatus* species complex) and numerous vertebrate hosts [14]. In Europe, *I. ricinus* has been considered as the primary vector of *B. burgdorferi* s.l. complex [9], in Asia this role plays *Ixodes persulcatus* [21], in Northern America *Ixodes scapularis* and *Ixodes pacificus* [22].

During the last decade, many authors underlined a tendency to increase the density of *I. ricinus* populations throughout Europe, in parallel with elevations in the infection rates of *B. burgdorferi* s.l. pathogens in questing ticks and their developmental stages [23,24]. Additionally, infection rates of the spirochetes in tick populations varied significantly between different European localities, in dependence on a wide array of environmental factors, such as geographical distribution, habitat type and host cenosis [25–27]. In recent years, it has been demonstrated an increment in the prevalence rates of co-infection of several pathogenic *B. burgdorferi* s.l. genospecies in *I. ricinus* populations throughout Europe [7,28,29]. This rule concern *B. burgdorferi* sensu lato, as well particular genospecies [20,30] (Tables 1 and 2).

All developmental stages of the common ticks (i.e. larvae, nymphs, adult females and males) are able to acquire the spirochetes as a result of blood feeding. Presence of the spirochetes in host-seeking

Table 1. *Borrelia burgdorferi* sensu lato mean prevalence in questing ticks *Ixodes ricinus* in Central European countries

Locality	Prevalence (%)	References
north-western Poland	11.0	Skotarczak et al. 2008 [102]
northern Poland	12.4	Stańczak et al. 2004 [103]
south-eastern Poland	4.9	Wójcik-Fatla et al. 2009 [104]
south-western Poland	1.2	Majláthová et al. 2008* [59]
Luxemburg	11.3	Reye etal. 2010 [78]
Hamburg, Germany,	34.1	May et al. 2015 [105]
Rhine-Main, Germany	9.5	Bingsohn et al. 2013 [106]
Germany	18.4	Kurtenbach et al. 2001 [19]
Praha, Czech Republic	2.8–9.2	Bašta et al. 1999 [107]
Brno, Czech Republic	9.6–13.2	Žákovská et al. 2008 [108]
north Slovakia	29.2	Rusňáková Tarageľová et al. 2016 [109]
Slovakia	25	Subramanian et al. 2012 [110]
Slovakia	40.5	Kurtenbach et al. 2001 [19]

* calculated on the base of published data, according to the prevalence definition "the percent of infected individuals in the whole population".

ticks were mostly identified in the midgut [31], and furthermore, systemic infections of several tick organs including ovarian tissues have been described [32]. Depending on the tick species, in some specimens, the infection restrains in midgut, in other bacteria invades body cavity and tissues. On example, in American *I. pacificus* 32.0% of infected ticks having spirochetes throughout tissues, in *I. dammini* 24.6%, in European *I. ricinus* 5% [33]. The number of spirochetes in ticks is unequal; according to Hubálek et al. [34] about 2.0% of infected ticks have more than 1000 bacteria, 10–12.0% (nymphs and males) to 22.0% (females) have in organism more than 100 spirochetes. In most unfed ticks, the spirochetes inhabit the midgut and during feeding they penetrate the midgut wall and translocate to the salivary glands via the haemolymph. They then pass into the feeding lesion with the saliva. The migration of spirochetes from the gut to the salivary glands during feeding means that most infections do not take place for at least 2 days after attachment [35,36]. The bacteria infected ticks stay in the organism just whole life, and are maintained transstadially during the moulting process.

Detailed molecular surveys demonstrated usually lower prevalence of the spirochetes in larval and nymphal tick individuals compared to adult ones. Prevalence of the spirochetes in *I. ricinus* larvae has been estimated at very low level (reaching up to 5.5%) [25,37]. Several studies evidenced an elevated occurrence of *B. burgdorferi* s.l. DNA in nymphs and adults of *I. ricinus* (with maximal infection rates at approx. 50.0%). The prevalence of infection in the nymphs and adults is highly variable, ranging from 0 to more than 50.0%, with the adults generally showing the higher infection rate in a particular population than nymphs; this rule concerns every *Borrelia* genospecies (Tables 3 and 4) [18,20,28,29,36,38].

The estimation of spirochetes infection is difficult and complicated such by local differentiation even in close places, as the seasonal changes and hosts population fluctuations. The highest parameters of *Borrelia* infection in unfed nymphs are observed in the next year after a population peak in small mammals. Also, annual changes in the parameters of infection in adult ticks and nymphs are synchronous. The following season is characterised by a high *Borrelia* prevalence in unfed adult ticks [39,40].

The ways of transmission

The necessary factor to maintain the circulation of tick-borne pathogens in zoonotic foci is their

Table 2. The prevalence (the percent of infected with particular genospecies in whole populations of investigated ticks) of *Borrelia burgdorferi* genospecies in *Ixodes ricinus* ticks in Central Europe

Locality	Prevalence (%)	References
	B. burgdorferi sensu stricto	
Lower Silesia, Poland	13.0	Kiewra et al. 2014 [20]
eastern Poland	1.7	Sytykiewicz et al. 2015 [29]
south-western Poland	1.24	Majláthová et al. 2008* [59]
Poland	8.1	Stańczak et al. 2000* [18]
southern Poland	9.2	Strzelczyk et al. 2015 [38]
Germany	0.0	Kurtenbach et al. 20* [19]
Germany	3.8	May et al. 2015 [105]
Germany	7	Hildebrandt et al. 2010* [88]
North Slovakia	1.1	Rusňáková Tarageľová et al. 2016 [109]
Slovakia	0.5	Kurtenbach et al. 2001* [19]
Slovakia	5.0	Subramanian et al. 2012 [110]
	B. garinii	
Lower Silesia, Poland	8.7	Kiewra et al. 2014 [20]
south-west Poland	4.1	Majláthová et al. 2008* [59]
eastern Poland	4.7	Sytykiewicz et al. 2015 [29]
southern Poland	3.3	Strzelczyk et al. 2015 [38]
Poland	7.0	Stańczak et al. 2000* [18]
Germany	6.2	Kurtenbach et al. 2001* [19]
Germany	13.8	May et al. 2015* [105]
Germany	13.3	Hildebrandt et al. 2010* [88]
Rhine-Main, Germany	0.7	Bingsohn et al. 2013* [106]
North Slovakia	1.9	Rusňáková Tarageľová et al. 2016 [109]
Slovakia	6.3	Kurtenbach et al. 20* [19]
Slovakia	8.0	Subramanian et al. 2012 [110]
	B. afzelii	
Poland	8.4	Sytykiewicz et al. 2015 [29]
south-western Poland	9.5	Majláthová et al. 2008* [59]
Lower Silesia, Poland	8.7	Kiewra et al. 2014* [20]
southern Poland	3.6	Strzelczyk et al. 2015 [38]
northern Poland	9.9	Stańczak et al. 2000* [18]
Germany	4.2	Hildebrandt et al. 2010* [88]
Germany	8.0	Kurtenbach et al. 2001* [19]
Germany	11.5	May et al. 2015* [105]
Rhine-Main, Germany	4.0	Bingsohn et al. 2013 [106]
North Slovakia	6.0	Rusňáková Tarageľová et al. 2016 [109]
Slovakia	21.4	Kurtenbach et al. 2001* [19]
Slovakia	9.0	Subramanian et al. 2012 [110]

	B. valaisiana	
eastern Poland	1.5	Sytykiewicz et al. 2015 [29]
North Slovakia	1.09	Rusňáková Tarageľová et al. 2016 [109]
south-western Poland	2.5	Majláthová et al. 2008* [59]
Lower Silesia, Poland	2.2	Kiewra et al. 2014 [20]
Germany	2.8	Hildebrandt et al. 2010* [88]
Germany	2.21	Kurtenbach et al. 2001* [19]
Germany	7.4	May et al. 2015* [105]
Rhine-Main, Germany	0.13	Bingsohn et al. 2013* [106]
Slovakia	3.8	Kurtenbach et al. 2001* [19]
Slovakia	3.0	Subramanian et al. 2012 [110]
	B. lusitaniae	
south-west Poland	5.0	Majláthová et al. 2008* [59]
Germany	0.0	Kurtenbach et al. 2001* [19]
Germany	0.2	May et al. 2015* [105]
Germany	0.1	Hildebrandt et al. 2010* [88]
Slovakia	0.0	Kurtenbach et al. 2001* [19]
North Slovakia	10.3–18.34	Rusňáková Tarageľová et al. 2016 [109]
	B. spielmanii	
Hamburg, Germany,	3.45	May et al. 2015* [105]
Germany	0.3	Hildebrandt et al. 2010* [88]
	B. bavariensis	
Rhine-Main, Germany	0.03	Bingsohn et al. 2013 [106]

* calculated on the base of published data, according to the prevalence definition "the percent of infected individuals in the whole population"

Table 3. The prevalence of infection with *Borrelia burgdorferi* s.l. in particular *Ixodes ricinus* tick developmental stages (in %), by various Authors

Larvae	Nymphs	Females	Males	References
nd	1.8	10.1	6.4	Wójcik-Fatla et al. 2009 [104]
nd	7.0	24.3	13.7	Stańczak et al. 2004 [103]
nd	8.1	14.7	15.0	Reye et al. 2010 [78]
nd	9.2–12.0	4.2–28.8	7–14.3	Rosef et al. 2009 [111]
nd	11.7–14.9	17.8–20	12.5–17.6	Venclíková et al. 2014 [17]
nd	16.7	39.3[a]	nd	Richter and Matuschka, 2006 [91]
nd	20.0	28.7	36.1	Hildebrandt et al. 2010 [88]
nd	34.5	36.7	25.9	May et al. 2015 [105]
nd	9.3	11.0	10.3	Bingsohn et al. 2013 [106]
0.0	9.4	56.2	33.3	Faulde and Robbins 2008 [112]
2.9	8.9	25.0	17.8	
0.0	14.6	59.5	25.9	Strzelczyk et al. 2015 [38]
1.0	1.0–15.0	10.0–20.0	nd	Kurtenbach et al. 1995 [45]
1.6	5.6	6.9	10.3	Žákovská et al. 2008 [108]
3.1	12.8	14.5	nd	Zhioua et al. 1994 [113]
5.5	5.8	19.5	4.2	Skotarczak et al. 2002 [37]
6.3	17.2	22.6	23.7	Hubálek et al. 1998 [114]

[a]adults together

Table 4. The prevalence of infection with *Borrelia burgdorferi* genospecies in particular *Ixodes ricinus* tick developmental stages (in %), by various Authors

Genomic groups	Adult	Nymph	References
Borrelia burgdorferi s.s.	8.5	8.0	Stańczak et al. 2000* [103]
	1.8	0.0	Kiewra et al. 2014* [20]
	4.0 f	1.0	Majláthová et al. 2008* [59]
B. afzelii	8.1	9.3	Stańczak et al. 2000* [103]
	16.4	6.6**	Kiewra et al. 2014* [20]
	5.2	11.7	Richter and Matuschka 2006* [91]
	16.0 m 6.7 f	8.1	Majláthová et al. 2008* [59]
B. garinii	7.7	3.7	Stańczak et al. 2000* [103]
	2.7	0.8	Kiewra et al. 2014* [20]
	6.7	3.3	Richter and Matuschka 2006* [91]
	0	5.6	Majláthová et al. 2008* [59]
B. valaisiana	2.3	0.8	Kiewra et al. 2014* [20]
	6.7	3.3	Richter and Matuschka 2006* [91]
	4.0 f	3.0	Majláthová et al. 2008* [59]
B. lusitaniae	19.3	0	Richter and Matuschka 2006* [91]
	12.0 f 5.6 m	4.5	Majláthová et al. 2008* [59]

*calculated on the base of published data; **MIR; f – females; m – males

ability to transovarial and transstadial transmission in ticks [41]. The necessary condition of the possibility of the transovarial transmission is the presence of spirochetes in ovarian tissues. Although the transovarial transmission of *B. burgdorferi* in *I. ricinus* tick has been adjudged as one crucial, it is disputable in the last decades [42] and seems not be essential for the *B. burgdorferi* circulation in Europe. First, in only small part of females the spirochetes penetrate to oocytes, however, the percentage of infected oocytes is quite high andvaries, from 60.0 to 100.0% [33]. Larval infection rates are usually about 1-2.0% and seldom higher, as an *B. burgdorferi* complex, as well particular genospecies (Table 3), so that the larvae are not considered to be a significant source of infection for mammals, and indicating the transovarial transmission rarely occurs [22,43]. There has been no report on the efficiency of transovarially infected *I. ricinus* larvae to transmit spirochetes to a host. On the other hand, due to the aggregating spatial distribution of ticks' larvae in environment [44], a rodent is simultaneously affected by a number of questing larvae. Simultaneous transmission by several larvae could establish an infection [45].

The alternative specific transmission route may take place during co-feeding of numerous ticks on the same host. This mechanism play an important role for many tick-borne pathogens [41], among them for *Borrelia* spirochetes [46]. It occurs especially at high level of tick's infestation and/or bacteremia of the host. It is important to stress that ixodid ticks may feed on the mammals for several days [47]. This mechanism can be explained the percent of *I. ricinus* larvae, infected with *Borrelia*, collected from hosts. In every case, ticks' larvae feed on hosts (small rodent, small birds, lizard) in close proximity, in high number usually. Therefore, the infected larvae can be the results of co-feeding transmission from other, infected specimens, also in the case of uninfected host. Moreover, the accompanied larvae and nymphs showed a greater feeding success than fed alone, and have the higher engorgement weights. It causes that there is simultaneous increased spirochetes transmission efficiency to the nymphs [48].

Animal reservoir

In Central Europe as the main reservoir animals for *B. burgdorferi* s.l. are considered small rodents - black-stripped mouse *Apodemus agrarius*, yellow-necked mouse *Apodemus flavicollis* and bank-vole *Myodes glareolus*, because these are the predominant species among small mammal's population and are abundantly infected by ticks [49,50]. Mice present a higher potential infectivity than voles [43,51]. Other small rodents' species and insectivores can be also

infected [49,52,53]; however, they are not abundant thus their role as the source of infection for ticks is limited.

As reservoir hosts of *Borrelia* spirochetes are considered also small birds, as frequently infested by immature *I. ricinus* ticks. According to Hubálek et al. [34], 28.7% of small birds are infested by preimaginal *I. ricinus* ticks. As the possibly reservoirs are noted European robin *Erithacus rubecola*, Eurasian blackbird *Turdus merula*, Blackcap *Sylvia atricapilla*, Eurasian Chiffchaff *Phylloscopus collybita*, Great tit *Parus major*, Eurasian jay *Garrulus glandarius* [34,50,54].

Larvae and nymphs of ticks feed also on the reptiles – lizards, snakes, turtles [55–57]. It appeared, that lizards are commonly affected by ticks *I. ricinus* and infected by *Borrelia* spirochetes, as well as be the source of infection for ticks' larvae and nymphs [58,59]. The infection with *B. burgdorferi* s.l. was detected in *Lacerta viridis*, *L. agilis*, *Padacris taurica* [57].

Medium-sized animals, wild boars and ruminants are hosts for nymphs and adults *Ixodes* ticks, and thus must be affected with *Borrelia* spirochetes. Cervids – roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and wild boars are frequently affected by ticks, but are not competent susceptible to infection with *Borrelia* [49,60,61], the presence of antibodies is confirmed [62], but there are no documented infections. Therefore, these animals do not play a role of animal reservoir and can be considered as amplifier, because serve as feeding source for adult ticks [63,64]. It is evident, that this same role can play also other animals, being the appropriate hosts for adult ticks. In the absence of deer, the reservoir hosts can be domestic ruminants, for example sheep. In distinction, there is documented the ability of Bovinae to be infected with *B. burgdorferi* – European bison (*Bison bonasus*) [65] and cattle [66]. The role of carnivores is unclear – it is considered that dogs are affected by *B. burgdorferi* [67,68], but there are only single reports about infection of wild carnivores [69]. Similarly, hedgehogs, squirrels and hares are potential animal reservoirs, but their role still needs confirmation [49].

General pattern of *Borrelia burgdorferi* sensu lato zoonotic foci

The structure of zoonotic foci of *B. burgdorferi* was the most often analysed by many Authors and there are several reviews regarding the ecology of *B. burgdorferi* spirochetes, describing in detail host and vector interactions [4,49,63,70]. Thus, the *B. burgdorferi* zoonotic foci structure stay a classic model for building the patterns for many other tick-borne pathogens [41].

Their holding depends on three etiological factors distinguished in 1999 by Siński [70] as the structure pattern of the *B. burgdorferi* zoonotic foci. The components include the animal reservoir, amplifiers and the efficient vector. The vertebrate animal is the hosts of pathogens and the first source of infection for ticks, and thus named the competent reservoir. The competence of an animal to act as a reservoir host for *Borrelia* were considered for rodents, on the base of the fact that rodent populations commonly are numerous, dense and serve as hosts for large numbers of immature ticks. In the last decade, there are add birds, and their role is accented as important component of animal reservoir group. Their activity enables the spread of disease agents and infected ticks to new areas. The second element in the structure of zoonotic foci of *Borrelia* are artiodactyls, such as deer or grazing livestock. They are hosts for nymphs and adult ticks, thereby making it possible for ticks to propagate and maintain the proper size of their population. These animals are not susceptible for infection with *Borrelia*, however, due to their role in tick's population maintenance, serve as amplifiers. Removal of such hosts can significantly reduce exophilic tick abundance [71,72]. On the other hand, ticks feeding on hosts non-susceptible to *Borrelia* infections, can loss the spirochetes [73,74]. Although small and medium sized mammals are considered as reservoir hosts, big mammals as amplifier, every hosts are crucial for ticks' population existence, as hosts for particular developmental stages. The third group of animals, ticks, is considered to be efficient vectors for pathogens. In central Europe for *B. burgdorferi* role serves *I. ricinus*. It is three host tick, *i.e.* it has three feeding stages (larvae, nymphs, adult females) each utilizing a different individual host, in most cases different species. It is essential the preference of immature stages for small to medium-sized vertebrates, for maintaining the bacteria in their natural transmission cycles. The possibility of co-feeding transmission enables the propagation of infection among other tick specimens, feeding on the same hosts. The co-feeding transmission enables

also the infection of tick's larvae, in the absence of transovarial transmission of spirochetes [75]. The transstadial transmission enables to quire of infection by adult ticks. Adult female ticks prefer medium size, such as carnivorous, and large animals, such as deer. These last are considered not susceptible to *B. burgdorferi* infection (Fig. 1).

The diversity of zoonotic foci among the particular *Borrelia burgdorferi* strains

It has been uncovered that numerous *B. burgdorferi* s.l. strains profoundly differ in reservoir host preferences, ability to infect wild animals and humans as well as specific composition of virulence determinants [76]. *B. garinii* seems to have a special

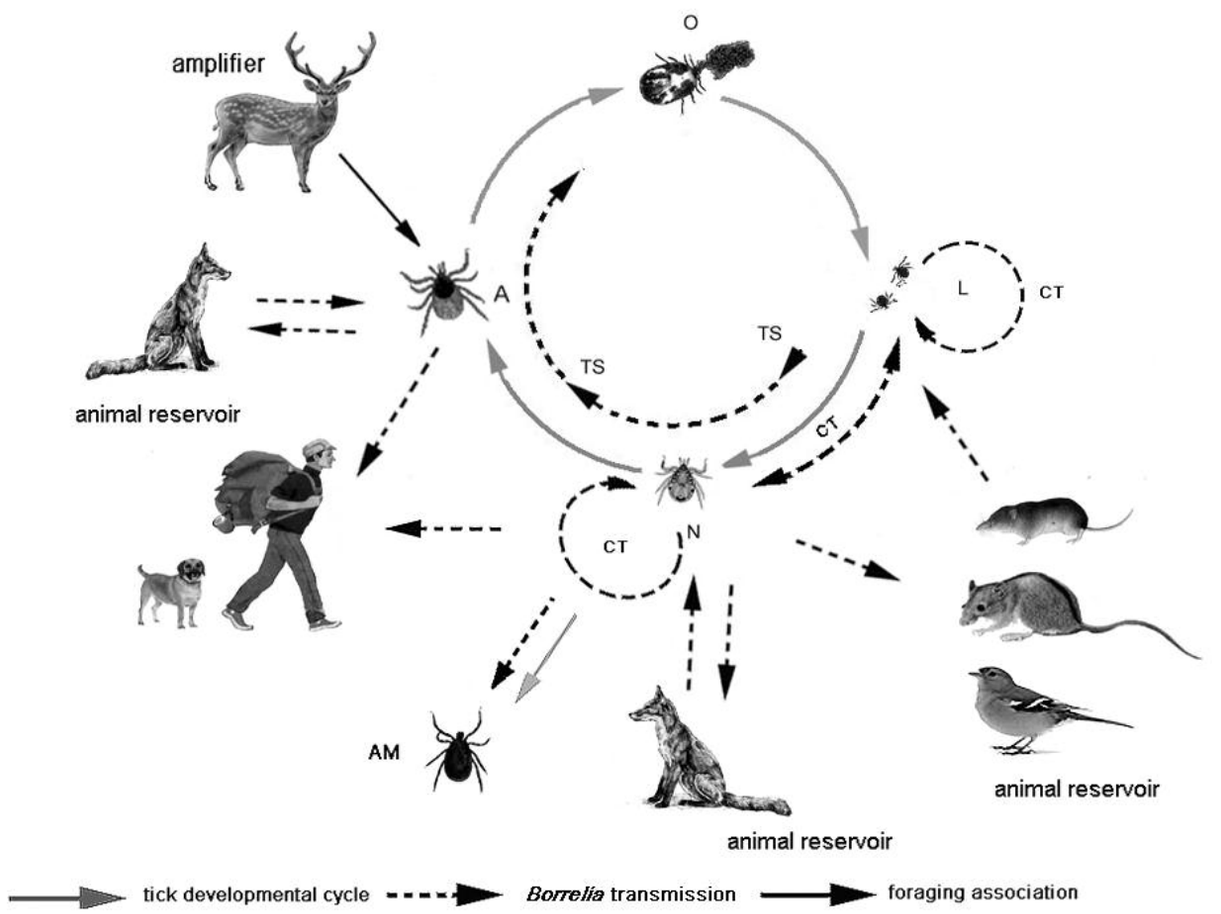

Fig. 1. The general enzootic cycle of *Borrelia burgdorferi* sensu lato
Ixodes ricinus tick is the vector which makes possible the maintenance and the circulation of *B. burgdorferi* sensu lato spirochetes in the environment. The animal reservoir are small mammals – mainly rodents and insectivores, as well birds, possibly lizards and carnivores. Transstadial transmission enable the presence of the agent in adult ticks, but the lack of transovarial mode result, that the circulation goes mainly between nymphs, adult females and their hosts. All ticks' stages acquire the infections from the infected hosts, for larvae and nymphs there is possible also the way of co-feeding transmission. The lack of transovarial transmission causes that there is no possibility for larvae to infect mammal hosts; adult males don't feed, can stay a blind alley for pathogens. Nymphs are crucial element in the spirochetes transmission. Only nymphs and adult females are source of infection for mammals – rodents, dogs and other pests, humans. Red deer and roe deer are not hosts of *B. burgdorferi* sensu lato spirochetes, so they play the role of amplifiers, as hosts of nymphs and adult ticks, maintaining their occurrence. Resuming, pathogen circulates primarily in the cycle: infected rodent (bird) → the tick larva → the nymph → the rodent (bird) → the larva of the tick. The adult ticks become infected by the transstadial route, however this developmental stage stands for the pathogens a blind alley, because hosts of adult ticks are not susceptible for infection, and the transovarial transmission is not there. The tick stages able to effectively infect human are nymphs and adult females, males don't participate in the follow transmission. TS – transstadial transmission; L – larva; N – nymph; A – adult female; AM – adult male; CT – co-feeding transmission.

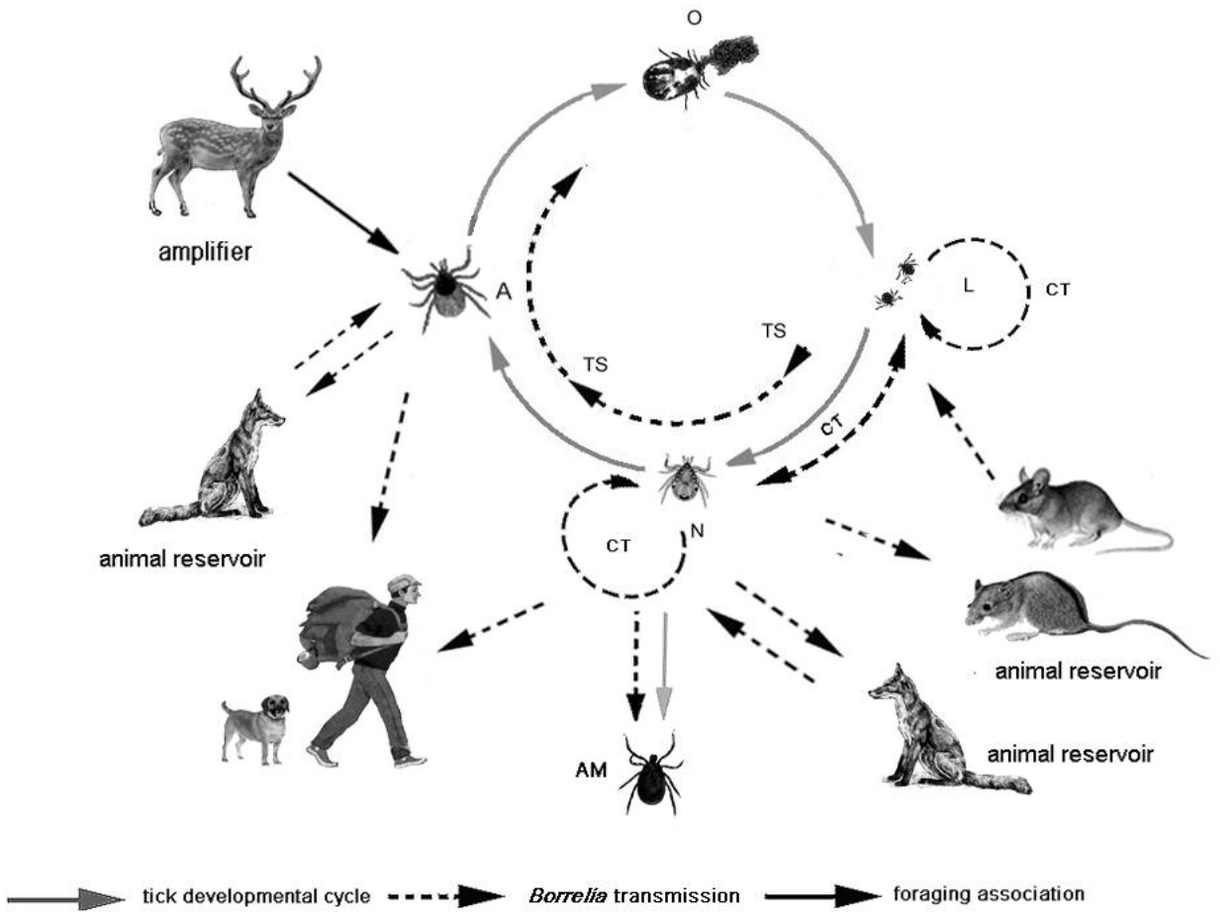

Fig. 2. The enzootic cycle of *Borrelia burgdorferi* genospecies associated with rodents – *B. afzelii*, *B. burgdorferi* s.s. and *B. bavariensis*
Ixodes ricinus tick is the vector which makes possible the maintenance and the circulation of *B. burgdorferi* s.l. pathogenic for human in environment. The animal reservoir for *B. afzelii*, *B. bavariensis* and *B. burgdorferi* sensu stricto are small rodents – in Central Europe mainly *Apodemus flavicollis* and *Myodes glareolus*, lesser role plays other small rodents and insectivore, possibly carnivores. Transstadial transmission enable the presence of the agent in adult ticks, but the lack of transovarial mode result, that the circulation goes mainly between nymphs, adult females and their hosts. Nymphs acquire the infections from the infected hosts, for larvae as well nymphs there is possible also the way of co-feeding transmission. The lack of transovarial transmission causes that there is no possibility for larvae to infect mammal hosts; adult males don't' feed, can stay a blind alley for pathogens. Nymphs are crucial element in the spirochetes transmission. Only nymphs and adult females are source of infection for mammals – rodents, dogs and other pests, humans. Wild boars and deer are resistant for *B. burgdorferi* infection, but affected by ticks; because they maintain the ticks' occurrence, thus play the role of amplifier. Resuming, pathogen circulates primarily in the cycle: infected rodent → the tick larva → the nymph → the rodent → the larva of the tick. The adult ticks become infected by the transstadial route, however this developmental stage stands for the pathogens a blind alley, because hosts of adult ticks are not susceptible for infection, and the transovarial transmission is not there. The tick stages able to effectively infect human are nymphs and adult females, males do not participate in the follow transmission. The infection by larvae are possible, if the transovarial transmission has place. CT – cofeeding transmission; TS – transstadial transmission; L – larva; N – nymph; A – adult female; AM – adult male; O – eggs.

predilection for birds, *B. afzelii* have a special association with rodents, *B. burgdorferi* s.s. is commonly found in both [36]; however, this association is not strongly and denied by some authors [14]. It seems, that some genospecies and hosts appear to exclude other genospecies, result in their local predominance, such as *B. lusitaniae* in sites where green lizards are abundant [77]. The

differentiation of *B. burgdorferi* s.l. for a number of genospecies, and the discovery of their relations with different hosts group result, that the first model of zoonotic foci structure stays out of date. Based on their sensitivity to reservoir host complement, *Borrelia* species have been divided into three ecological groups, associated with different animal reservoir species.

According to available reports, in central Europe, occur 7 genospecies of *B. burgdorferi* s.l., which can be associated with 4 groups of hosts playing the role of animal reservoirs.

Group 1 – *B. afzelii*, *B. burgdorferi* s.s. and *B. bavariensis* and rodents (Fig. 2)

The group 1 contains genospecies and strains associated with rodents as primary animal reservoir. There belong *B. afzelii, B. garinii*, as well *B. burgdorferi* sensu stricto [46,78-80]. To this type can be also joined *B. bavariensis* (previously *B. garinii* OspA serotype 4). Hosts for *B. bavariensis* are rodents, in particular *Apodemu*s spp., and can be also hedgehog, *Myodes* spp., *Microtus* spp. [4,53,81].

In Central Europe, the most abundant small mammals are voles of *Microtus* rodents, *M. glareolus* and mice *A. flavicollis*, *A. agrarius* and *A. sylvaticus*. Shrews, hedgehogs and other small rodents are not such abundant, and don't serve as quantitatively important tick hosts. However, the alone abundance of micromammals does not explain the marked distribution of immature ticks. The equal significance have microclimate factors. The most favourable biotopes characterize by forest communities with plant communities indicating humid and acid conditions, relatively high level of subsoil water and thick leaf litter layer. Because the forest habitats are avoided by *Microtus* voles and *A. agrarius*, only *A. sylvaticus*, *A. flavicollis* and *M. glareolus* are the main mammals pre-digested to the role as hosts for larvae of *I. ricinus*. According to Wodecka et al. [69] the hosts for *B. afzelii* and *B. garinii* can be also badger and raccoon dogs; although their involvement and significance in the *Borrelia* circulation system in environment is not known. *B. burgdorferi* and *B. burgdorferi* s.s. were also isolated and identified in dogs having the clinical symptoms of borreliosis [67,68]. These animals serve as hosts for every stage of *I. ricinus* ticks [82].

Apart the mentioned above biotope factors, the rodent pattern of *B. burgdorferi* zoonotic foci can be divided follow to the patterns associated with Murinae and Microtinae rodents; the reason are the different mode of life of mice and voles and different ratio of infestation with ticks. All rodents are appropriate hosts, however, in Central Europe Murinae rodents are more heavily infested with *I. ricinus* larvae than *M. glareolus*. *Apodemus* mice are preferred hosts for *I. ricinus*, while Microtinae for *Dermacentor reticulatus* and *D. marginatus* ticks [51,83]. These differences can be also caused by different immunological response in Murinae and Microtinae rodents – the voles acquire resistance to repeated infestation with *I. ricinus* larvae, but such phenomena were not observed in *Apodemus* mice [84]. Also among woodland rodents, the role in the transmission cycle is remarkably different. Some differences can be associated with *Borrelia* genospecies. Infection with *B. burgdorferi* sensu stricto is short-lived in mice and voles [85], but in the case of *B. afzelii* it is documented the ability to overwintering in *M. glareolus* and *A. sylvaticus* because once infected rodent remains infective to ticks for whole life [85,86]. According to above – Vostal and Žakovská [87] detected the prevalence of infection in *A. flavicollis* and *A. sylvaticus* mice higher than in *M. glareolus* inhabited this same localization.

Group 2 – *B. valaisiana* and most *B. garinii* strains (OspA serotypes 3, 5, 6, and 7) and birds (Fig. 3)

B. valaisiana and most *B. garinii* strains are found associated in enzootic cycles with birds as main vertebrate hosts [46,78–80,88]. The zoonotic foci pattern is generally similar to the previous, only the animal reservoir is represented by birds mainly, not rodents and insectivores. The role of carnivores in these spirochetes circulation is also not documented, however, as the hosts of nymphs and adult ticks, these can play the role of amplifier. The crucial argument for birds as main hosts is the dominance *B. garinii* and *B. valaisiana* in engorged ticks collected from birds [54]. The most common birds' hosts for ticks able maintain *Borrelia* infections are *Turdus merula*, *T. philomelos*, *Parus major* [4,54]. Co-feeding transmission is not documented yet, although there are no reasons against.

Group 3 – *B. spielmanii*, associated with hedgehogs and Gliridae (Fig. 4)

B. spielmanii – it is thought to be restricted to

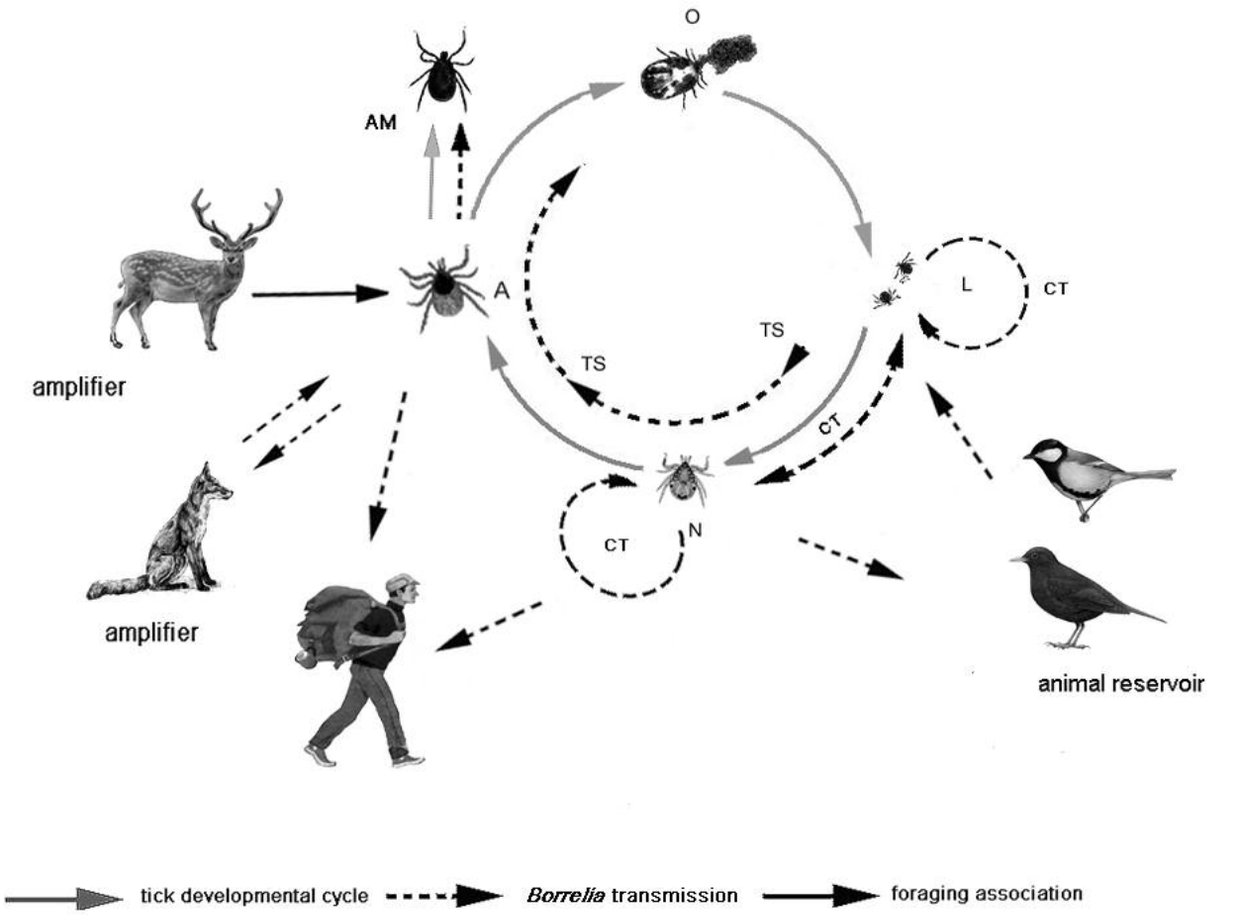

Fig. 3. The enzootic cycle of *Borrelia burgdorferi* genospecies associated with birds – *B. valaisiana* and most *B. garinii* strains (OspA serotypes 3, 5, 6, and 7)
Ixodes ricinus tick is the vector which makes possible the maintenance and the circulation of *B. valaisiana* and *B. garinii* are small birds – in Central Europe mainly belonging to the Turdidae family. Transstadial transmission enable the presence of the agent in adult ticks, but the lack of transovarial mode result, that the circulation goes mainly between nymphs, adult females and their hosts. Nymphs acquire the infections from the infected hosts. The lack of transovarial transmission causes that there is no possibility for larvae to infect birds; adult males don't feed, can stay a blind alley for pathogens. Nymphs are crucial element in the spirochetes transmission. Only nymphs and adult females are source of infection for mammals – rodents and other pests, and humans. Wild boars and deer are resistant for *B. burgdorferi* infection, but affected by ticks; because they maintain the ticks' occurrence, thus play the role of amplifier. Resuming, pathogen circulates primarily in the cycle: infected bird → the tick larva → the nymph → the bird → the larva of the tick. The adult ticks become infected by the transstadial route, however this developmental stage stands for the pathogens a blind alley, because hosts of adult ticks are not susceptible for infection, and the transovarial transmission is not there. The tick stages able to effectively infect human are nymphs and adult females, males do not participate in the follow transmission. The infection by larvae are possible, if the transovarial transmission has place. CT – cofeeding transmission; TS – transstadial transmission; L – larva; N – nymph; A – adult fmale; AM – adult male; O – eggs.

reservoir hosts of the family Gliridae, as dormice *Eliomys quercinus* and *Muscardinus avellanarius* [89,90], as well to hedgehogs [53]. The spread of Gliridae is unequal, thus *B. spielmanii* appears to be prevalent at sites at which these hosts are abundant [90]. In Central Europe occur two hedgehog species – *Erinaceus europaeus* and *Erinaceus roumanicus*, in the part of their areas occur sympatrically. Both species are susceptible for *B. burgdorferi*, *B. bavariensis* and *B. spielmanii*, also in combinations, other genospecies were not recorded [53]. Two species of ticks predominate on hedgehogs – *Ixodes hexagonus* and *I. ricinus*; it is confirmed that larvae can acquire *B. burgdorferi* s.l. infections from

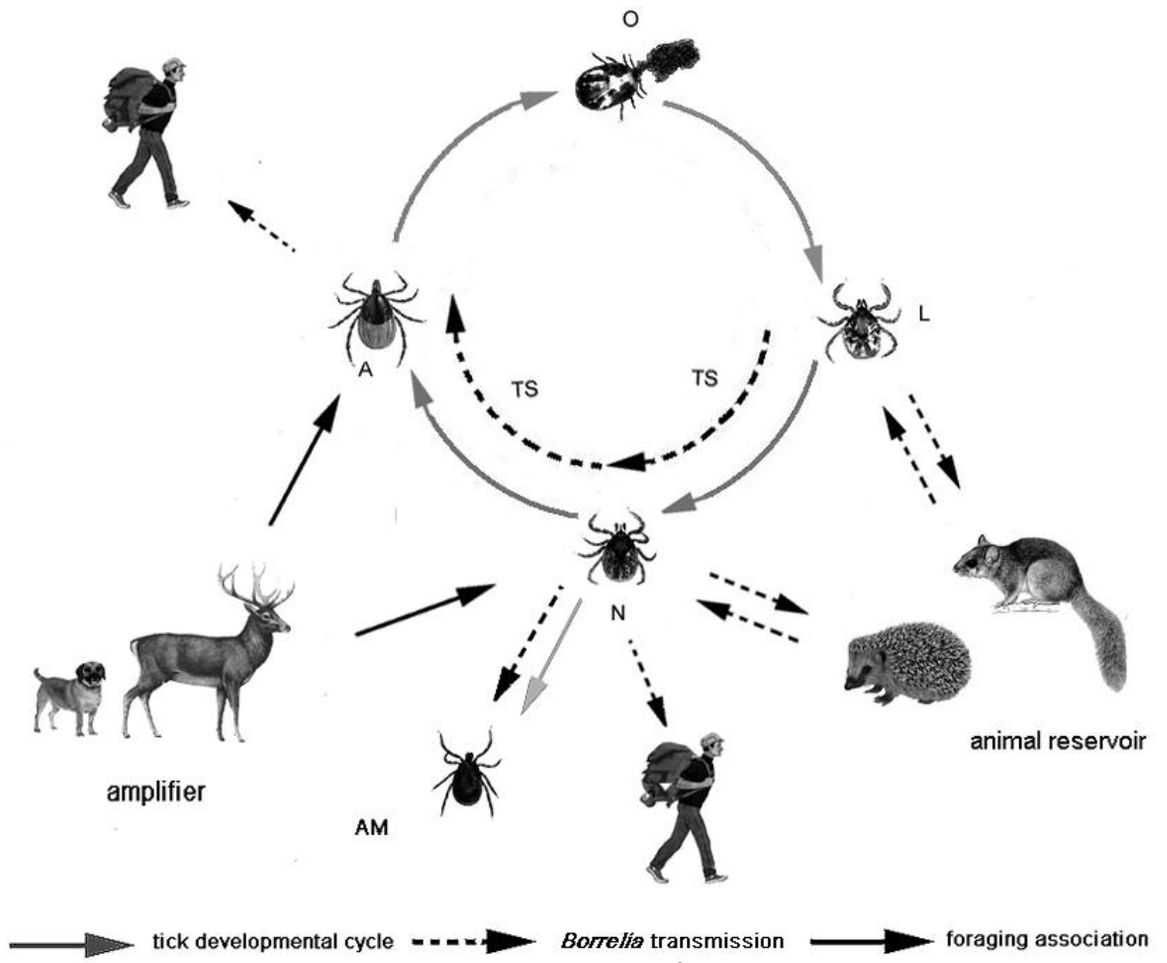

Fig. 4. The enzootic cycle of *Borrelia spielmanii*, associated with hedgehogs and Gliridae
Ixodes ricinus tick is the vector which makes possible the maintenance and the circulation of *B. spielmanii* in the environment. Transstadial transmission enable the presence of the agent in adult ticks, but the lack of transovarial mode result, that the circulation goes mainly between nymphs, adult females and their hosts. Nymphs acquire the infections from the infected hosts. The lack of transovarial transmission causes that there is no possibility for larvae to infect a mammal; adult males don't feed, can stay a blind alley for pathogens. Nymphs are crucial element in the spirochetes transmission. Only nymphs and adult females are source of infection for mammals – rodents, humans. Wild boars and deer are resistant for *B. burgdorferi* infection, but affected by ticks; because they maintain the ticks' occurrence, thus play the role of amplifier. Resuming, pathogen circulates primarily in the cycle: infected glirid → the tick larva → the nymph → the glirid → the larva of the tick. The adult ticks become infected by the transstadial route, however this developmental stage stands for the pathogens a blind alley, because hosts of adult ticks are not susceptible for infection, and the transovarial transmission is not there. The tick stages able to effectively infect human are nymphs and adult females, males do not participate in the follow transmission. The infection by larvae are possible, if the transovarial transmission has place. TS – transstadial transmission; L – larva; N – nymph; A – adult female; AM – adult male; O – eggs.

hedgehogs, confirming their status as reservoir hosts [49]. Co-feeding transmission is not documented yet, although there are no reasons against.

Group 4 – *B. lusitaniae* and lizards (Fig. 5)

The occurrence of *Borrelia lusitaniae* differs from that above. This species is most prevalent in Mediterranean countries. In more northern or eastern countries it has been detected at only a few sites; in central Europe in Slovakia, southern Germany, Poland and Romania [59,77,91].

The main reservoir animals for *B. lusitaniae* are green lizard (*Lacerta viridis*), Balkan wall lizard (*Podarcis taurica*) and wall lizard (*Podarcis*

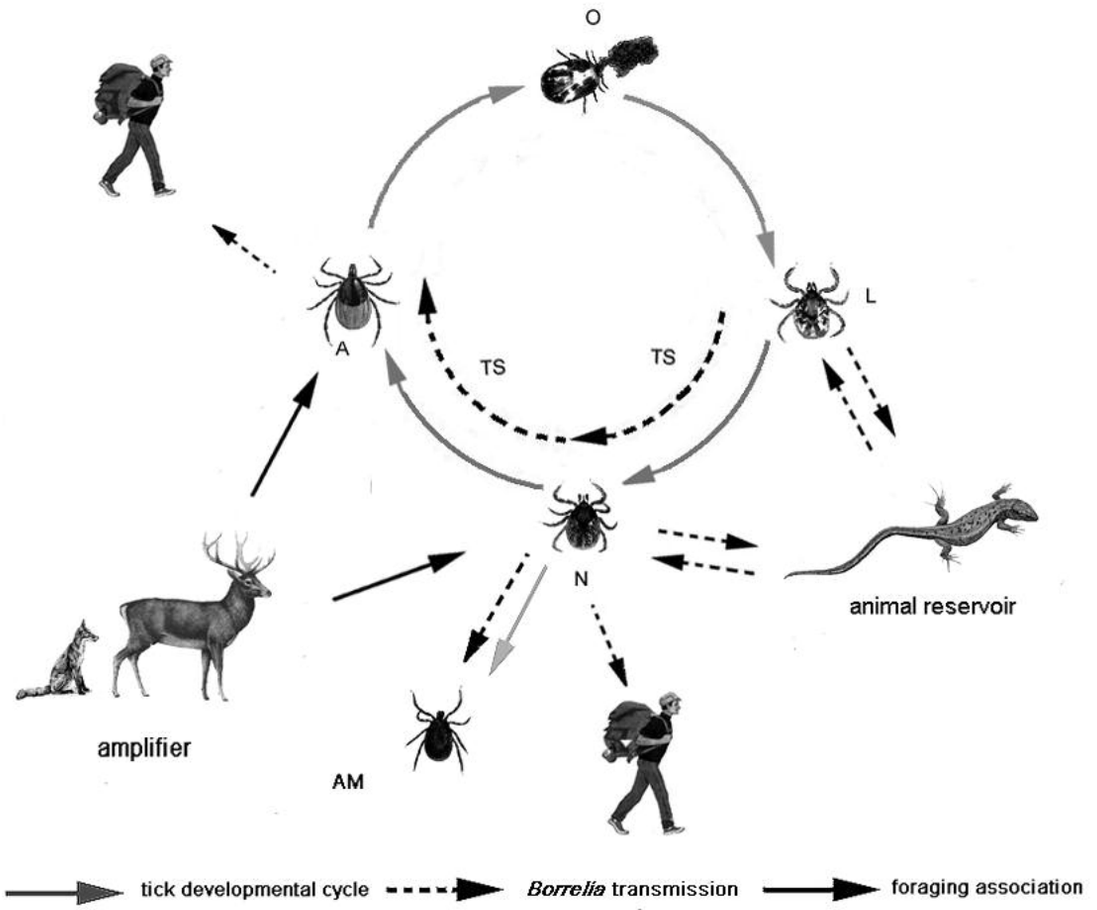

Fig. 5. The enzootic cycle of *Borrelia lusitaniae*, associated with lizards
Ixodes ricinus tick is the vector which makes possible the maintenance and the circulation of *B. lusitaniae* in the environment. Transstadial transmission enable the presence of the agent in adult ticks, but the lack of transovarial mode result, that the circulation goes mainly between nymphs, adult females and their hosts. Nymphs acquire the infections from the infected lizards. The lack of transovarial transmission causes that there is no possibility for larvae to infect a host; adult males don't feed, can stay a blind alley for pathogens. Nymphs are crucial element in the spirochetes transmission. Only nymphs and adult females are source of infection to lizards and mammals – rodents, pets and humans. Wild boars and deer are resistant for *B. burgdorferi* infection, but affected by ticks; because they maintain the ticks' occurrence, thus play the role of amplifier. Resuming, pathogen circulates primarily in the cycle: infected lizard → the tick larva → the nymph → the lizard → the larva of the tick. Nymphs acquire the infections from the infected hosts, for larvae as well nymphs there is possible also the way of co-feeding transmission. The adult ticks become infected by the transstadial route, however this developmental stage stands for the pathogens a blind alley, because hosts of adult ticks are not susceptible for infection, and the transovarial transmission is not there. The tick stages able to effectively infect human are nymphs and adult females, males do not participate in the follow transmission. The infection by larvae are possible, if the transovarial transmission has place. TS – transstadial transmission; L – larva; N – nymph; A – adult female; AM – adult male; O – eggs.

muralis) [57,91]. The northern species - *L. agilis* – is not such intensively infested by ticks; although the infection with spirochete *B. lusitaniae* is confirmed [59,91], the ability to maintain the zoonotic foci in northern countries of Central Europe needs follow study. Because the main host occurrence range is not wide as rodents – *Lacerta viridis*, *Podarcis taurica* and *Podarcis muralis* are absent on the north from Carpathian Mountains – the distribution of this species is also limited [77]. Moreover, it seems that the prevalence in tick populations and the percentage of infected ticks is directly dependent from the lizards' number. According to Majláthová et al. [59], *B. lusitaniae* is predominant *Borrelia* genospecies in ticks' population were lizards were common in Romania and Slovakia, but in Poland, where green lizard is absent and other lizards are not such numerous, the

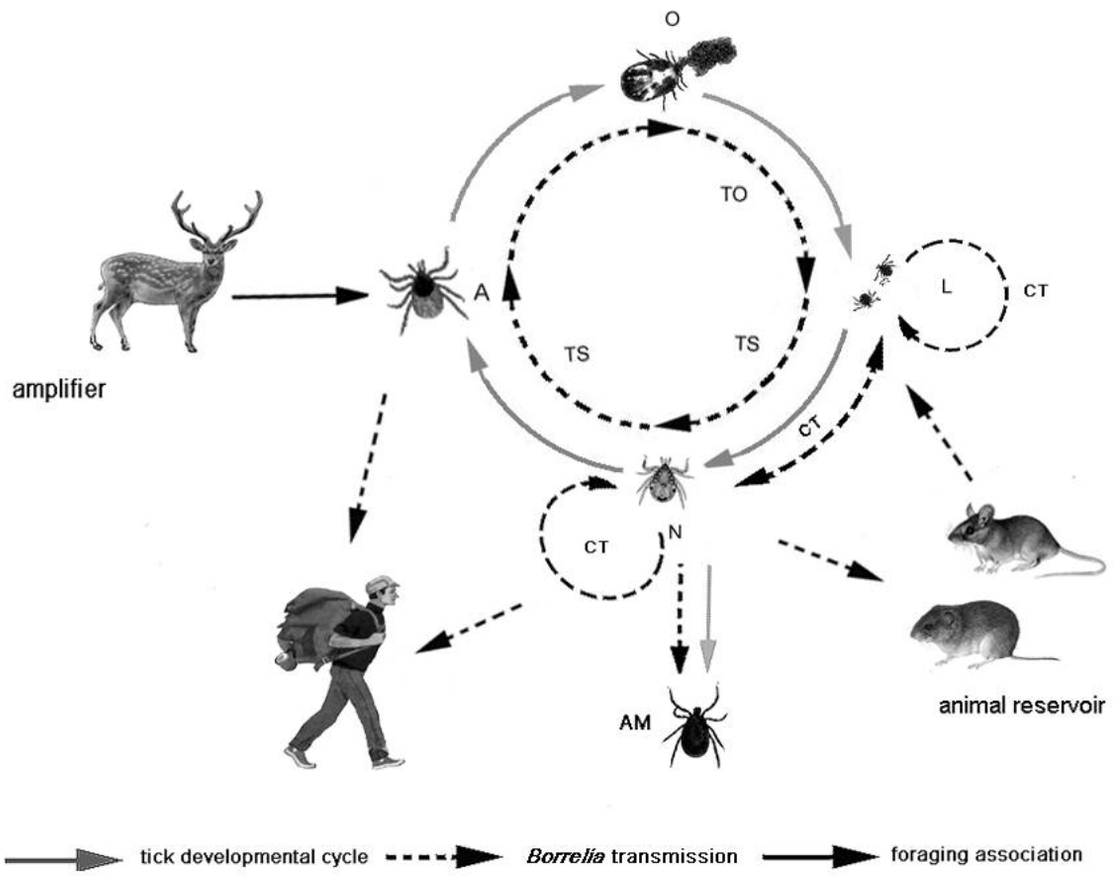

Fig. 6. *Borrelia miyamotoi* zoonotic foci structure
Ixodes ricinus tick is the vector which makes possible the maintenance and the circulation of *B. miyamotoi* spirochetes in the environment. The animal reservoir are small mammals – mainly rodents and insectivores. Transstadial transmission enable the presence of the agent in adult ticks, the possibility of transovarial mode result, that the circulation between larvae, nymphs, adult females and their hosts. All ticks' stages acquire the infections from the infected hosts, for larvae and nymphs there is possible also the way of co-feeding transmission. Larvae, nymphs and adult females can be the source of infection for mammals – rodents, dogs and other pests, humans. Red deer and roe deer are probably not regular hosts of *B. miyamotoi*, so they play the role of amplifiers, as hosts of nymphs and adult ticks, maintaining their occurrence. Males can be infected, but don't feed and are not able to participate in the follow transmission. TO – transovarial transmission; TS – transstadial transmission; L – larva; N – nymph; A – adult female; AM – adult male; CT – co-feeding transmission.

prevalence is low.

Because larvae, as well nymphs commonly feed on lizards, the co-feeding transmission from infected nymphs is the important way of infection and spirochetes circulation in zoonotic foci [57-59].

Borrelia miyamotoi zoonotic foci structure (Fig. 6)

Borrelia miyamotoi is another representative of Borreliaceae, distant genetically to *B. burgdorferi* s.l. complex. Thorp and Tonnetti demonstrated that *B. miyamotoi* is able to survive in human blood components during standard storage conditions [92]. It is the etiological agent of the relapsing fever, the first human case caused by this pathogen was documented in Russia in 2011. Subsequently, increasing number of *B. miyamotoi* infections in Europe, Japan and USA were reported [93]. Fever, headache, fatigue, arthralgia, myalgia were described among the most crucial clinico-pathological manifestations of this disease. Furthermore, few incidents of *B. miyamotoi*-related meningoencephalitis have been diagnosed in immunosuppressed patients [92].

Recently it has been reported the presence in ixodid tick sampled from different habitats in USA, many European countries and Russia. Prevalence rates for this spirochete species in tick specimens

were estimated between 0.2% and 8.2% [27,29,46, 94–97]. *Borrelia miyamotoi* was recognized for the first time in *Ixodes persulcatus* ticks collected in Japan in 1995 [98]. In Poland, prevalence of *B. miyamotoi* in host-seeking *I. ricinus* ticks ranged from 0.3% to 2.0% [20,29,46]. In addition, Wodecka confirmed the occurrence of *B. miyamotoi* DNA in tissue homogenate of one adult female tick (0.8%), collected from the red deer (*Cervus elaphus*) in North-Western Poland [61]. It has been evidenced both transovarial and transstadial transmission modes of *B. miyamotoi* in *I. scapularis* ticks [99]. Tadin et al. [100] established that 3.7% (9/242) of rodents tested in Croatia were infected with *B. miyamotoi*. In Europe, *Apodemus* spp. mice and *M. glareolus* were confirmed as reservoirs for *B. miyamotoi* [101]. Generally, the enzootic cycle of *B. miyamotoi* seems to be similar to *B. burgdorferi* complex, however, differs by the transovarial transmission possibility.

Conclusions

Despite the detail, small differences, the developmental cycles of all *B. burgdorferi* s.l. genospecies present typical, main pattern described in the first part of the review [41]. However, the simple scheme might be complicated, by the co-occurrence of two or more various genospecies in one tick and even various pathogens [15,18,24], which relationship is unknown. The divisions are not extreme; in the hosts group, infected with appropriate *Borrelia* genospecies, very often are found the specimens infected with other genospecies. The example is *B. burgdorferi* s.s. commonly identified in both rodents and birds [46,78-80]. It seems, that the reason is the wide range of *I. ricinus* larvae and nymphs hosts and their three-hosts developmental cycle. Larvae, nymphs and adults can feed on hosts from quite different specimens, species as well phylum. The lack of hosts specificity cause, that there are note the simultaneous infections of ticks with *Borrelia* genospecies representing different pattern – on example, adult ticks infected with *B. garinii*, if it feed on bird as larva, and simultaneously with *B. afzelii*, if it fed on rodent as nymph. There is also possible the infection with three genospecies – it can be explained by the co-feeding transmission, as well by infection of rodent by two genospecies. This mechanism explains also the simultaneous infection with *Borrelia* and other microorganisms, using rodents as animal reservoir [18,29]. On the hosts, as the source of mixed infections, indicate the fact that these are often in adult ticks than in nymphs [29]. It is possible, that the mixed infections in ticks can argued the mixed infections recorded in mammals and birds [53], and it can cause the follow overlapping of described patterns.

The possible factor, which is able to restrict the genospecies mix, is the possibility of pathogens deprivation by ticks. Such phenomena was described in 1992 by Matuschka and Spielman [74], when tick that has acquired spirochete from rodent during its larval blood meal was cleared of spirochetes when feeding as a nymph on a bird.

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