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Review articles

The role of particular ticks developmental stages in the circulation of tick-borne pathogens in Central Europe. 6. *Babesia*.

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ABSTRACT. In the Central European conditions, three species of *Babesia* have epidemiological significance as human pathogens – *Babesia divergens*, *B. microti and B. venatorum.* Tick *Ixodes ricinus* is considered as their main vector, wild mammals as the animal reservoir. The zoonotic cycles of small and large *Babesia* differ in details. Due to the lack of transovarial mode transmission in small species *B. microti*, the circulation goes mainly between immature ticks and vertebrate hosts; pathogen circulates primarily in the cycle: infected rodent → the tick larva → the nymph → the mammal reservoir → the larva of the tick. The tick stages able to effectively infect human are nymphs and adult females, males do not participate in the follow transmission. For large *Babesia* – *B. divergens* and *B. venatorum*, the transovarial and transstadial transmission enable the presence of the agent in adult ticks, moreover, that larvae and nymphs feed on not-susceptible hosts. The tick stages able to effectively infect cattle and other ruminants are adult females. Resuming, pathogen circulates primarily in the cycle the ruminant host – adult female tick – the larva – the nymph – adult female of the next generation – the ruminant. Due to the compound developmental transmission has place after the outflow of a tick began feeding.

Key words: *Babesia*, ticks, zoonotic foci

Introduction

Piroplasmosis is the dangerous tick-borne disease of human and animals, caused by protozoans of the *Babesia* genus. To date, more than 100 known *Babesia* species have been identified worldwide which infect many types of mammalian hosts, and also several avian species.

The taxonomic classification of *Babesia* spp. places them in the phylum Apicomplexa, class Piroplasmea (Aconoidasisa), and the order Piroplasmida [1]. They are grouped informally into the "small" *Babesia* group (trophozoites are from 1.0–2.5 μm in size; i.a. *B. gibsoni*, *B. microti*) and "large" *Babesia* group (2.5–5.0 μm in size; i.a. *B. bovis*, *B. caballi*, *B. canis, B. bigemina*). These morphological classification are generally consistent with the phylogenetic characterisation based on nuclear small subunit-ribosomal DNA sequences, which shows that the large and small babesiae fall into four phylogenetic clusters – firstly, *B. microti*; secondly, *B. duncani* group; thirdly, typical large babesiae; fourthly, a poorly known species from Korea. The exception is *B. divergens*, which morphologically appears small on blood smears (0.4 to 1.5 μm) but is generally related to large *Babesia* [1,2].

Human babesiosis

Human babesiosis has been described to be caused by animal babesiae, but current evidence suggests that the majority of cases are caused by *Babesia divergens* and *B. microti*. Including the first case described by Škrabalo and Deanović in former Yugoslavia in 1957, over 50 cases of human babesiosis have been reported in Europe [3,4]. Babesiosis belongs to opportunistic diseases. Splenectomy or immunodeficiency is the main factor of risk which was found in 86% of the patients [1,4,5]. *Babesia divergens*, a cattle pathogen, was involved in a majority of human babesiosis in Europe, while the cases caused by *B. microti* are seldom. Contrary to Europe, in northern America *B. microti* constitutes the predominant species responsible for human piroplasmosis. Meanwhile, a few human cases have been recognized from outside of the USA and Europe, sporadic infections have been reported from Asia [6,7].

In 2003, another species infecting people was identified – *Babesia* EU1, presently named *B. venatorum*, different in biological and genetic features. This parasite was involved in the first documented cases of human babesiosis in Italy, Austria and Germany [8]. *Babesia venatorum* is closely related to *B. capreoli* and *B. odocoilei*, affecting deer and not infective for people, but clearly distinct from *B. divergens*. European deer are host for *B. capreoli*, the reports about occurrence of *B. odocoilei* in Europe need confirmation. Isolates similar to *B. odocoilei*, parasitizing white-tailed deer (*Odocoileus virginianius*), American elk (*Cervus elaphus canadensis*) and caribou (*Rangifer tarandus caribou*) in northern America [9] have been detected in many European countries; however, their relativity to American strains needs further studies.

Babesia capreoli is not known to be pathogenic for humans or livestock. Due to the high prevalence in deer in natural environment, it influences to the zoonotic foci structure and circulation of pathogenic

Table 1a. *Babesia divergens* sensu lato mean prevalence in questing ticks *Ixodes ricinus* in Central European countries

Locality	Prevalence (%)	References	Notices
Szczecin	3.0	Skotarczak and Cichocka 2001 [5]	
north-western Poland	0.14	Adamska and Skotarczak 2017 [17]	
eastern Poland	0.2	Wójcik-Fatla et al. 2015 [18]	
Germany, Baden	0.9	Hartelt et al. 2004 [19]	**)
southern Germany	1.05	Overzier et al. 2013 [11]	
Germany, Bavaria	0.04	Schorn et al. 2011 [15]	**)
Germany	2.0	Hildebrandt et al. 2010 [16]	**)
Austria	0.7	Schötta et al. 2017 [20]	**)
Hungary	0.0-1.0 (mean 0.5)	Egyed et al. 2012 [21]	

Table 1b. *Babesia divergens* mean prevalence in mammalian hosts in Central European countries

Host	Locality	Prevalence (%)	References	Notices
Capreolus capreolus	eastern Poland	16.6	Welc-Falęciak et al. 2013 [22]	
	north-western Poland	22.3	Sawczuk et al. 2005 [23]	**)
	Slovenia	54.9	Duh et al. 2005 [24]	
		16.7	Duh et al. 2005 [24]	
Cervus elaphus	north-western Poland	33.3	Sawczuk et al. 2005 [23]	**)
	Austria	5.1	Cézanne et al. 2017 [25]	
Bison bonasus	Poland	33.0	Karbowiak et al. 2014 [26]	
Bos taurus	Germany	0.4	Lengauer et al. 2006 [27]	

**) recalculated on the base of data given

species. There are often present mixed infections making the appropriate identification difficult [10–12]. The distinction of deer babesias was based on analysis of the complete 18S rRNA gene [2]. Many reports about the occurrence in environment in Europe and human infections with *B. odocoilei* should be classified as *B. venatorum*. Generally, among 15 species of *Babesia* occurring in Europe, the real epidemiological significance seems to have *B. microti*, *B. divergens* and *B. venatorum*.

The spread of *Babesia* species pathogenic for human in Central Europe

Babesia divergens is a bovine parasite, wide spread in Europe and in western Asia, wherever the vector *Ixodes ricinus* is present [2,7,13]. The northern area of its occurrence reaches Lithuania and Norway [14]. The main host is cattle, however, their common occurrence in many wild ruminants is reported [10]. The infected ticks were reported in Central Europe from Hungary, Austria, Germany, Poland [5,7]. The prevalence of the infection in ticks vary from below 0.1% [15] to 2.0% and, in northern part of Europe even 4.0% [14,16] (Table 1a,b).

Babesia microti occurs in small mammals – rodents and insectivores – in Europe and northern America. Due to the competence of at least two tick species to spread and transmission of *B. microti* in Europe, *I. ricinus* and *I. trianguliceps* [2,6], it seems more prevalent in Western Europe than other parts [28]. It is recorded in all countries of Central Europe in ticks and mammals hosts – Poland [6,19,29–34], Germany [11,16,19,35], Czech Republic (former Czechoslovakia) [36,37], Slovakia [38–40], Hungary [21,28,41] and Austria [42] (Table 2 a,b). Several studies concerned the Holarctic distribution of the parasite, and the molecular investigation show the genetic diversity. Although morphologically various strains are undistinguishable, this species include complex of distinct clades based on the 18S rRNA and β-tubulin genes, with various zoonotic potential [2,57].

Babesia venatorum is the parasite of roe deer [10]. It was described in human babesiosis cases by Herwaldt et al. [8]. The spread of *B. venatorum* is not completely known presently; however, infections in ticks and roe deer are documented throughout Europe, from France and Netherlands on the west, across Switzerland, Austria, Slovakia, Germany to Poland on the east, from Italy and Slovenia on the south to Estonia on the north [7,10,13,15,16,24,58–62] (Table 3a,b).

The vectors of *Babesia* in Central Europe

In distinction to viruses and majority of bacterial pathogens, piroplasms undergo the compound developmental cycle. It comprises not only with hosts exchange; the exchange of host is associated with improvement of quite different morphological forms and different reproduction pattern in particular developmental stages. The compound life cycle strongly influences to the course of circulation of piroplasms in zoonotic foci and transmission ways. During developmental cycle, *Babesia* piroplasms go through at least three stages of reproduction: gamogony – formation and fusion of gametes; sporogony – asexual reproduction; merogony – asexual reproduction, too. Gamogony takes place inside the tick gut, sporogony in tick salivary glands, merogony in the blood of vertebrate host. Thus, the tick is the definitive host, the mammal – intermediate [69].

Babesia sporozoites are inoculated by ticks into the competent vertebrate host during feeding and invade red blood cells (RBCs) where they transform into trophozoites. These grow and divide into two to four merozoites which, in turn, are capable of infecting new RBCs; the division process is then repeated. Merozoite size, the position in the erythrocyte and morphological details are dependent on the parasite species, as well the host species influence. An intermediate phase of multiplication elsewhere in the body has not been observed [1,70–72]. Some merozoites located in red blood cells form into the gamonts. The further development of gamonts is stopped in the vertebrate host; only just swallowed by the tick, release the erythrocytes and in the lumen of the tick's intestine develop into gametocytes and follow gametes, so called ray bodies. Gametes fuse in the lumen of tick's digestive tract, allowing the nuclei to unite, and form the zygote. The zygote invades the cells of intestine, multiply by a series of binary fissions giving the mobile ookinetes. They pass into the hemolymph in the body cavity, disseminate and enter cells of epidermis, Malpighian tubules and musculature. There undergoing a series of binary fission and entering the cells of the salivary glands acini. Here, they further multiply by sporogony and produce sporonts. Sporozoite development begins only when the infected tick attaches to a vertebrate host. In the case of large babesiae, kinetes penetrate

Table 2a. *Babesia microti* mean prevalence in questing ticks *Ixodes ricinus* in Central European countries

Locality	Prevalence (%)	References	Notices
north-western Poland	16.3	Skotarczak and Cichocka 2001 [5,43]	
northern Poland, Tricity	2.3	Stańczak et al. 2004 [44]	
south-western Poland	50.8	Asman at al. 2015 [33]	
eastern Poland	3.1	Sytykiewicz et al. 2012 [45]	
eastern Poland	2.8	Wójcik-Fatla et al. 2015 [18]	
eastern Poland	5.4	Wójcik-Fatla et al. 2006 [46]	
eastern Poland	3.5	Wójcik-Fatla et al. 2009 [47]	
northern Poland	15.2	Asman et al. 2017 [34]	N
Germany, Baden	0.1	Hartelt et al. 2004 [19]	
Germany, Thuringia	2.8	Hildebrandt et al. 2010 [16]	**)
southern Germany	1.5	Overzier et al. 2013 [11]	*), **)
southern Germany	3.5	Eshoo et al. 2014 [48]	**)
Germany	0.27	Overzier et al. 2013 [35]	
Austria	0.7	Schötta et al. 2017 [20]	
Czech	1.5	Rudolf et al. 2005 [37]	N
Slovakia	0.4	Blaňarová et al. 2016 [40]	
Hungary	0.0–0.8 (mean 0.3)	Egyed et al. 2012 [21]	
Hungary	3.5	Kálmán et al. 2003 [41]	

abbreviatious as Table 2b

also ovaries of adult females, where they penetrate the oocytes. They transform into schisonts and do not develop further in the larval tick that hatches from the egg, but when it molts they enter the tissues of salivary gland and continue their development. Development is completed, according to *Babesia* species, within 2–5 days since the tick began fed. The rapidity is necessary since the sporozoites must be transmitted just during the feeding period. The sporozoites break out of the host cell, come to live in the lumen of the gland and are injected into the vertebrate host when tick sucks blood [69–73]. The compound developmental cycle induces one more difference to bacterial and viral pathogens – *Babesia* transmission during blood feeding does not begin at once, but only just after the outflow of a time.

The vectors for *Babesia* piroplasms in Central European conditions are hard ticks of Ixodidae family. Some *Babesia* species, such as *B. bigemina* can infect more than one genus of ticks, whereas *B. microti* can only infect ticks from the genus *Ixodes*. Ticks are not such specific, several tick vectors can carry more than one *Babesia* species, and mixed infections were often noticed [1,18,66]**.** The tick species having importance as vectors of *Babesia* piroplasms for human and animals in Central Europe are *Ixodes ricinus* and *I. trianguliceps*, as well for some species *Rhipicephalus appendiculatus* and *Dermacentor reticulatus* [70,74]. *Ixodes trianguliceps*, as relatively common tick in Western Europe, plays the important role in spread and circulation of *B. microti* in the environment [75]. In contrast, this tick occurs in Central and Eastern Europe seldom and locally, due to it has small significance; this way *I. ricinus* stay the main vector [76]. The role of *D. reticulatus* in spread of human babesiosis is not clear; there is demonstrated the ability of transfer *Babesia canis* piroplasms caused canine babesiosis, but is practically nothing is known about other babesiae. A single reports on this tick species infection with *B. microti* are incidental [18]. Their role is limited also by fact, that *I. ricinus* is common in whole area of Poland and Slovakia, whereas *D. reticulatus* predominates in the part of these countries only [77]. What is more, only *I. ricinus* regularly attacks human, in every active developmental stages, for other tick species human is not an attractive host [1,78,79]. Some authors accent the ability of *Rhipicephalus* ticks in

Table 2b. *Babesia microti* prevalence in mammalian hosts in Central Europe

Hosts	Locality	Prevalence (%)	References
Microtus arvalis	north-eastern Poland	13.8	Siński 1999 [49]
	north-eastern Poland	9.0	Bajer et al. 2001 [50], Pawełczyk et al. 2004 [51]
	north-eastern Poland	33.3	Karbowiak 2004 [30]
	Germany	14.3	Obiegala et al. 2015 [52]
	Austria, Steiermark	8.3	Šebek et al. 1980 [42]
	Czechoslovakia, s. Moravia	0.7	Šebek 1975 [36]
	Czechoslovakia	0.6	Šebek et al. 1977 [28]
Microtus agrestis	south-western Poland	50.0	Karbowiak et al. 1999 [31]
	Germany, Bavaria	38.0	Kramptiz and Bäumler 1978 [53]
	Germany	7–38***)	Krampitz 1979 [54]
	Austria, Steiermark	30.5	Šebek et al. 1980 [42]
	Austria, North Tyrol	6.5	Mahnert 1972 [55]
	Czechoslovakia, s. Bohemia	0.5	Šebek 1975 [36]
	Czechoslovakia	0.5	Šebek et al. 1977 [28]
	Austria, North Tyrol	1.0	Mahnert 1972 [55]
	Czechoslovakia, s. Moravia	0.4	Šebek 1975 [36]
	Czechoslovakia	0.3	Šebek et al. 1977 [28]
	Eastern Slovakia	1.1	Blaňarová et al. 2016 [40]
Microtus eoconomus	eastern Poland	17.6	Karbowiak et al. 1999 [31]
	eastern Poland	7.7	Karbowiak 2004 [30]
	eastern Poland	50.0	Karbowiak 2004 [30]
Myodes glareolus	Germany	0.03	Obiegala et al. 2015 [52]
	north-eastern Poland	0.6	Siński 1999 [49]
	north-eastern Poland	1.0	Bajer et al. 2001 [50]
Pitymys subterraneus	Austria, Steiermark	11.1	Šebek et al. 1980 [42]
	Austria, North Tyrol	18.1	Mahnert 1972 [55]
Apodemus flavicollis	Germany	0.01	Obiegala et al. 2015 [52]
	north-eastern Poland	2.1	Bajer et al. 1998 [29]
	Austria, Steiermark	1.6	Šebek et al. 1980 [42]
	Eastern Slovakia	1.1	Blaňarová et al. 2016 [40]
Apodemus agrarius	Bosnia-Herzegovina	1.1	Šebek et al. 1977 [28]
	Eastern Slovakia	0.8	Karbowiak et al. 2003 [56]
	Eastern Slovakia	7.1–25.0***)	Karbowiak et al. 1999 [31]
	Eastern Slovakia	3.7	Blaňarová et al. 2016 [40]
Sorex araneus Tyrol	Austria, North Tyrol	1.9	Mahnert 1972 [55]

*) sequencing revealed *B. venatorum*, *B. capreoli*, and *B. microti*; **) recalculated on the base of data given; ***) in dependence to the season; N – nymphs only

Table 3a. *Babesia venatorum* mean prevalence in questing ticks *Ixodes ricinus* and hosts in Central European countries

Locality	Prevalence (%)	References	Notices
northern Poland Tri-city	2.3	Stańczak et al. 2015 [65]	
south-eastern Poland	1.2	Wójcik-Fatla et al. 2015 [18]	
northern Poland	0–1.6	Cieniuch et al. 2009 [62]	
southern Germany	1.0	Overzier et al. 2013 [11]	
Germany, Bavaria	0.5	Schorn et al. 2011 [15]	**)
Germany	0.62	Overzier et al. 2013 [11]	**)
Austria	1.4	Schötta et al. 2017 [20]	**)
Czech Republic	0.5	Venclikova et al. 2015 [66]	MIR
Czech Republic	0.1	Rybářová et al. 2017 [67]	
Slovakia	0.4	Švehlová et al. 2014 [63]	
Slovenia	22.2	Duh et al. 2005 [58]	

Table 3b. *Babesia venatorum* prevalences in mammalian hosts in Central Europe

Hosts	Locality	Prevalence (%)	References
Capreolus capreolus	Poland	16.6	Welc-Falęciak et al. 2013 [22]
	southern Germany	9.0	Overzier et al. 2013 [35]
	Germany	25–26	Kauffmann et al. 2017 [68]
	Slovenia	21.6	Duh et al. 2005 [24]
Ovis aries	Germany	1.0–9.0	Kauffmann et al. 2017 [68]

**) – recalculated on the base of the data given; MIR – minimum infection rate

spreading of piroplasms, however this genus as not a permanent component of central European fauna is able to play a marginal role only, and on the areas to the south of Carpathian Mountains [79–81]. Nevertheless, immature stages of all these species are able to maintain the transmission cycle in population of rodents and the presence of zoonotic foci in the environment.

The prevalence rate of ticks infection with *B. divergens* is relatively low; the highest noted prevalence was 3–3.3% in Poland and Germany [5,11], the most common values were reported within the range 0.2–1.05% (Table 1a).

The infection rate of *I. ricinus* ticks with *B. microti* is changeable, depending on the locality, as well the great importance has the season [5,43]. The prevalence in ticks population in Poland varied from 2.8 to 16.3%, rarely higher; moreover, the lowest values were noted in central and northern parts of the country [44,45], the highest in southern and western parts [5,33,34,43]. In Germany the prevalence of infection in ticks was in the range 0.1 to 3.5% [19,48], similar values were noted in Czech Republic and Hungary, 1.5% and 0.33% to 3.5% respectively [21,37,41] (Table 2a).

Interesting, the prevalence infection of ticks with *Babesia microti* has very wide range, sometimes higher than in the case of other pathogens transmitted by ticks. There are recorded such small prevalence, as well high, more than 50.8% [33] (Table 2a). The possible reason can be the seasonal changes, as well the local condition influences [13,21,34]. The possible reason can be seasonal dynamic of *Microtus* voles infection with *B. microti*, the main source of infection for ticks. The highest prevalence rate has place in July-August – beginning of September, reaching over 50% of voles infected [30,32,54,82], just when the ticks larvae and nymphs has the activity peak [83]. The dependence has the character of positive feed-back; ticks larvae fed on voles in June, before prevalence infection peak, have little likelyhood to get

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

a. *Babesia divergens*

Larvae	Nymphs	Females	Males	References
nd	0.54	0.006		Adamska and Skotarczak 2017 [17]
nd	0.4	0	0.4	Wójcik-Fatla et al. 2015 [18]
0.0	0.0	6.7	4.5	Skotarczak and Cichocka 2001 [5]
nd	0.13	0.09		Schorn et al. 2011 [15] **)

b. *Babesia microti*

Larvae	Nymphs	Females	Males	References
3.1	4.2	15.3	9.5	Skotarczak and Cichocka 2001 [5,43]
0.96–12.5	1.21–4.22	9.7–15.33	7.5–9.5	Skotarczak et al. 2005 [23] Skotarczak and Cichocka 2001 [43]*)
nd	1.3	3.0	4.3	Stańczak et al. 2004 [44]
nd	0.9	7.9	2.6	Sytykiewicz et al. 2012 [45]
nd	11.1	59.8	1.25	Asman et al. 2015 [33]
nd	5.2 **)	6.1	2.2	Wójcik-Fatla et al. 2015 [18]
nd	2.9	4.1	1.1	Wójcik-Fatla et al.2015 [18]
nd	0.5	0.3	nd	Blaňarová et al. 2016 [40]

c. *Babesia venatorum*

Larvae	Nymphs	Females	Males	References
nd	0.7	1.9	0.7	Wójcik-Fatla et al. 2015 [18]
nd	0.0	0.0–2.4	0.0–1.1	Cieniuch et al. 2009 [62]
nd	0.5–0.6	0	0	Venclikova et al. 2015 [66]

*) – in dependence to the season and location; **) – MIR; nd – no data

infection, and similarly, the vole has little likelyhood such long as a small number of ticks attacks it. It changes in July, the great number of ticks individuals rapidly increase the infection possibility and their prevalence in rodents population, following the high infection level in new generation of tick nymphs. Such mechanism is common also in the case of other arthropod-borne parasites, such as in rodent's trypanosomes, *Borrelia* spirochetes and bartonellae [84,85].

Babesia divergens infections are generally higher in nymphs than adult females, other proportions are rarely noted [17,18] (Table 4). The highest rate of infection of ticks with *B microti* is noted in adult female ticks (up to 15.5%, range 3.0–59.8%), whereas for males and nymphs the rates of infection constituted only 4.74% (1.1–9.5%) and 3.87% (0.9–11.1%), respectively [34,86].

Similar proportions, although lower values, were noted in Poland for *B. venatorum*. The prevalence of infection of ticks with *B. venatorum* is generally low; the values slightly exceed 1.0% of population were noted in Germany and Poland [11,18,58,61] (Table 3a). The percent of infected nymphs was to 0.7, adult females 1.9 up to 2.4, males 0.7 to 1.1 [18,61]. In Czech Republic, the highest prevalence was noted in nymphs; the mean prevalence of infection in nymphs was about 0.5%, varying from 0.4 to 1.3% [66] (Table 4).

The ways of transmission

As in the case of other tick-borne pathogens, the transstadial and transovarial transmission seems to

be essential in maintenance of the *Babesia* circulation within ticks and mammals populations. In transstadial transmission from nymph to the adult tick, the large club-shaped vermicules formed in the gut epithelium of the nymph and then invaded the internal organs of host. These forms remained in the tissues until the tick emerged and commenced to feed on a new host. Them they changed to motile club-shaped vermicules again, invaded the cells of the salivary gland alveoli, and produced the small infective forms [69,70,87].

There are the differences in the potential of nymphal → adult transstadial transmission between large and small *Babesia*. In the case of *B. microti*, the transmission is not recorded in *I. ricinus* [49,51,69,88]; this way, the larvae theoretically should be free of the pathogen, especially with the addition of the transovarial transmission lack. Also the majority of adult ticks, which as nymphs fed on carnivores and other hosts not susceptible for *B. microti* infection, should not be infected. However, it is possible and documented in *I. trianguliceps*, if the nymphs engorge on the host with the high level of infection [74]. High prevalence and level of infection of *Microtus* voles with *B. microti* in natural conditions are observed in July – August, just then the season of peak prevalence of larvae and nymphs [30,32,51,54]. If this same mechanism has place in the case of *I. ricinus*, this can be the possible explanation of adults infected with *B. microti*. Another explanation gave Gray et al. [89], experimentally demonstrated that transstadial transmission occurs but that the parasite does not persist beyond more than one molt. Resuming, the high prevalence rate of adult females infection with *B. microti* (Table 4b) can be the result of nymphs fed on the *Microtus* rodents intensively infected with piroplasms.

The large babesias, such as *B. divergens*, transmit from nymphs to adult forms practically with no limits [51,72,90].

The transovarial transmission is the next crucial factor enable the circulation of tick-borne pathogens in biotopes [51,64,69,75,85]. Large *Babesia* species, as *B. divergens*, can be transmitted transovarially between tick generations. It is necessary factor – *I. ricinus* is three-hosts tick, and it meets the appropriate for parasite host once during the life only, as adult female. Larvae and nymphs feed on not suitable hosts usually, and without transovarial and transstadial transmission, the transfer of *B. divergens* would be impossible.

Transovarial transmission can result in large numbers of infected ticks in areas where *Babesia* spp. are endemic. In the case of *Ixodes*, babesias surviving up to 4 years without a vertebrate host. The transovarial transmission has been also documented for *B. venatorum* [7,90]. According to above, transovarial transmission appears to be absent in small babesias such as *B. microti* [1,2].

The transmission among ticks during the co-feeding is not reported, and it seems remote. The sporozoites leaved the salivary glands of ticks are morphologically and physiologically adapted to continue their development in the blood of mammals, not in the intestine of the next tick's specimens [70,71].

The poorly known question is the vertical transmission of piroplasms by non-tick-borne route. The preliminary observations has been observed in the case of *B, microti*. There are possibly two alternative ways – oral transmission and congenital. The oral transmission has been observed in rodent trypanosomes and is possible in nature during fighting or in the case of swallow of infected flea [91,92]. The experimental transmission has been conducted by Malagon and Tapia [93]. This possibility has been also observed in the case of *B. gibsoni* and dogs, as blood-to-blood transfer occurring between dogs during fighting [94]. The trace to such possibility for *B. microti* is their ability to the artificial transmission during blood transmission [95] and their ability to infection during syringe passage, commonly used in laboratories for *B. microti* maintenance.

The ability to vertical transmission via congenital route in natural conditions has been observed in many parasite and host species. Vertical transmission occurs by the transfer of the parasite from mother to offspring during pregnancy, child birth or lactation. Vertical transmission has been documented for many other Apicomplexa parasites, such *Neospora caninum*, *Plasmodium* spp., *Toxoplasma gondii* [96,97]. Transmission of *Babesia* spp. from a female to her offspring has been reported in mammals: in bovines (*B. divergens*), sheep (*B. ovis*), dogs (*B. gibsoni*) and horses (*B. caballi*). They are documented the abortion cases result from gestational babesiosis due to *B. bovis* [87]. In addition, several reports indicated congenital *B. microti* infections in humans. The ability of *B. microti* vertical transmission among rodents has been confirmed also in natural conditions [98,99].

Animal reservoir

Babesia divergens and *Babesia venatorum*

The natural host for *B. divergens* are big ruminants – cattle, deer and bisons [1,10,26,72]. The infections of ruminants are noted throughout Europe and possibly into North Africa, which corresponds with the distribution of the vector, *I. ricinus*. Although domestic cattle are the principal host, infections have been detected also in wild cervids [7,73]. *Babesia divergens* host range (described from experimental infections) overlaps that of *B. capreoli*, often parasitizing this same hosts, but the two species can be differentiated by experimental infections of gerbils (*Meriones unguiculatus*) or cattle [100–102], neither of which are susceptible to *B. capreoli*.

The presence of *B. divergens* or *B. divergens*-like parasites in naturally infected roe deer has been recently reported in Slovenia [24] and Poland [23], and in red deer in Slovenia [24]. The noted prevalence in Slovenia was 54.9% in roe deer, and 16.7% of red deer [24]. Also other ruminants can be infected, the recorded prevalence of infection is about 29% in fallow deer [103]. However, the identity of *B. divergens* from wild ungulates based solely on sometimes partial 18S rDNA sequencing is questionable.

Deer are hosts for many *Babesia* species. Three species of *Babesia* have been described to date in wild European cervids: *B. divergens*, *B. capreoli* and *B. venatorum*. The variety of *Babesia* infections in deer is great in different countries, such as the prevalence of infection, as well species proportions in different hosts [67,68]. *Babesia divergens* and *B. capreoli* are species closely related and morphologically similar. The two species could not be clearly differentiated on the basis of morphology or serology [10]. There is indeed a gap between the early studies (up until 1991) based on morphology and biological characterization of *B. capreoli* and recent studies (from 2005 onwards) where identification has been solely based on molecular comparisons of sometimes only partially sequenced 18S rDNA amplified from ticks or wild ungulates. The two species only differ at three nucleotide positions at the 18S rRNA gene (99.83% nucleotide similarity). Thus, there is possible that the study conducted before are not reliable [10].

The type host for *B. capreoli* is roe deer (*Capreolus capreolus*), and this *Babesia* species predominates in that host. *Babesia capreoli* has also been reported in asymptomatic and naturally infected red deer (*Cervus elaphus*) [104,105], sika deer (*Cervus nippon*) [100], Père David's deer (*Elaphurus davidianus*) and probably reindeer (*Rangifer tarandus*) [105]. This species has also been identified in blood from roe deer in Slovenia, but not from red deer [24]. The confirmed cases of babesiosis have been described with parasitemia as high as 20–25% in roe deer and reindeer [105]. There are possible mixed infections in deer species and ticks with *B. capreoli* and *B. divergens* [11], *B. venatorum* and *B. capreoli* in ticks [66] and other configurations.

Roe deer are the natural host of *B. venatorum* and infected deer have been reported in Central Europe from Slovenia, France and Italy [10,24,58,60]. Prevalence in roe deer are generally high, above 20% [7,24].

Babesia microti

As main zoonotic reservoir of *B. microti* serve many species of small mammals – rodents and insectivores. The occurrence of some *Babesia* in natural environment in central European countries is documented from various regions in the wide range of mammal hosts; there is documented ability of at least 16 European species of insectivores and rodents to be host of this piroplasma [28,77] (Table 2b). Microtinae rodents are considered to be the main reservoir of *B. microti* in natural environment in Europe. The analysis of data collected from various research centres shows that the prevalence rate of infection in Microtinae voles is much higher than in other rodents [77]. In Poland, the prevalence of infection is the highest in *Microtus* voles: in common vole *M. arvalis* the prevalence is 9–33% [30,50,51]; in field vole *M. agrestis* reaches almost 50% [31], in root vole *M. oeconomus* 7.7–50% [31,32]. The lesser role as zoonotic reservoir play *Myodes* voles, *Apodemus* mice and shrews; the prevalence of infections in these mammals does not exceed 2.0% usually (Table 2b) [24,29,30,32,50. 51,106]. The important aspect of *Babesia* infections in mammals is their great dependence on the season; the seasonal variations of the prevalence shows a rise in the summertime and a minimum in winter [54].

The infection of *Microtus* voles with *B. microti* resulted in a dramatically enlarged spleen [30,54,107]. This phenomenon has not been observed with other common hemoparasite infections in rodents, such as *Trypanosoma* or

Hepatozoon. However, apart splenomegaly symptoms, natural *Babesia* infections have not any visible signs, so it is evident that piroplasmas cause chronic avirulent infections in their natural hosts [53,82,108]. In blood smears *B. microti* could not be detected all times, however, the parasitaemia last for months [109].

Particular circulation schemes

Babesia microti and *B. divergens* are parasites wide spread in northern hemisphere. The various geographic localities differ not only with the parasite strain, but also in different possible vectors, hosts species and hosts biology. *Babesia divergens* maintenance and circulation depends on *I. ricinus* tick as vector, on the whole area of this pathogen occurrence, so it seems to present this same pattern elsewhere. For *B. microti*, there are present few species of vectors and animal reservoir on different geographic locations. Therefore, the structure of *Babesia* spp. zoonotic foci in Northern America can be different in details in comparison to Europe [2,30], which effect that human babesiosis caused by *Babesia microti* is more often in United States.

In the USA the white-footed mouse *Peromyscus leucopus*, is considered the main reservoir host and the vector is the human-biting *I. scapularis*, the deer or black-legged tick [110]. Other vectors of various strains of *B. microti* are *I. spinipalpis*, *I. angustus* and *I. muris* which transmit the parasites to various species of voles. However, these ticks do not bite humans and the zoonotic potential of the strains of *B. microti* that they transmit is unknown. The vectors and reservoirs of the main west coast US zoonotic babesia, *B. duncani*, are not known. European strains of *B. microti* also parasitize a variety of Microtine rodents. Two vectors are involved. *Ixodes trianguliceps* is a specialised rodent tick that rarely if ever bites man and is probably responsible for the transmission of *B. microti* throughout Europe. *Ixodes ricinus*, which is closely related to *I. scapularis*, and is well known to infest humans, was identified as a vector of *B. microti* in Germany by Walter [109], with the field vole, *Microtus agrestis* as the natural reservoir [111].

Also in Western Europe the circulation scheme can differ to central part, by the common occurrence of the second vector – *Ixodes trianguliceps* [2,75].

***B. microti* (Fig. 1)**

Babesia microti piroplasma is associated with rodents as primary animal reservoir. In central Europe *I. ricinus* tick is the vector which makes possible the maintenance and the circulation of *B. microti* in environment. The animal reservoir in Central Europe are small mammals, mainly voles of *Microtus* genus: *M. arvalis* and *M. economus*. *Myodes glareolus* and mice *Apodemus flavicollis*, *A. agrarius* and *A. sylvaticus* play lesser role, also in the places were cohabitate this same area. Shrews, hedgehogs and other small rodents are not such abundant, and do not serve as quantitatively important tick hosts [83]. However, the alone abundance of micromammals does not explain the marked distribution of immature *I. ricinus* ticks. The equal significance have microclimate factors. The most favorable 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 predigested to the role as hosts for larvae of *I. ricinus*. However, as mentioned above, the highest prevalence of infection with *B. microti* is noted in Microtinae voles – in Central Europe *M. arvalis* and *M. oeconomus*, associated with grasslands. This can explain, that the percent of ticks infected by *B. microti* is lower, in comparison to other tick-borne pathogens usually.

The transstadial transmission enable the presence of the *B. microti* in nymphs and possibly adult ticks, but the lack of transovarial mode result, in addition to foraging associations, that the circulation in biotope goes mainly between larvae and nymphs [88]. These stages are crucial element in the *B. microti* circulation and transmission. Larvae acquire the infection from rodents, pathogen is transmitted from the larval phase of the tick to the nymphal phase, and the ticks as nymphs infect the either rodent hosts. There is no possibility for larvae to infect mammal hosts; adult females use as hosts bigger mammals, males do not feed. Wild boars and deer are resistant for *B. microti* infection, but affected by ticks; because they maintain the ticks' occurrence, thus play the role of amplifier.

Summing up, *B. microti* circulates primarily in the cycle: infected rodent → the tick larva → the nymph → the rodent → the larva of the tick. The adult ticks sometimes become infected by the

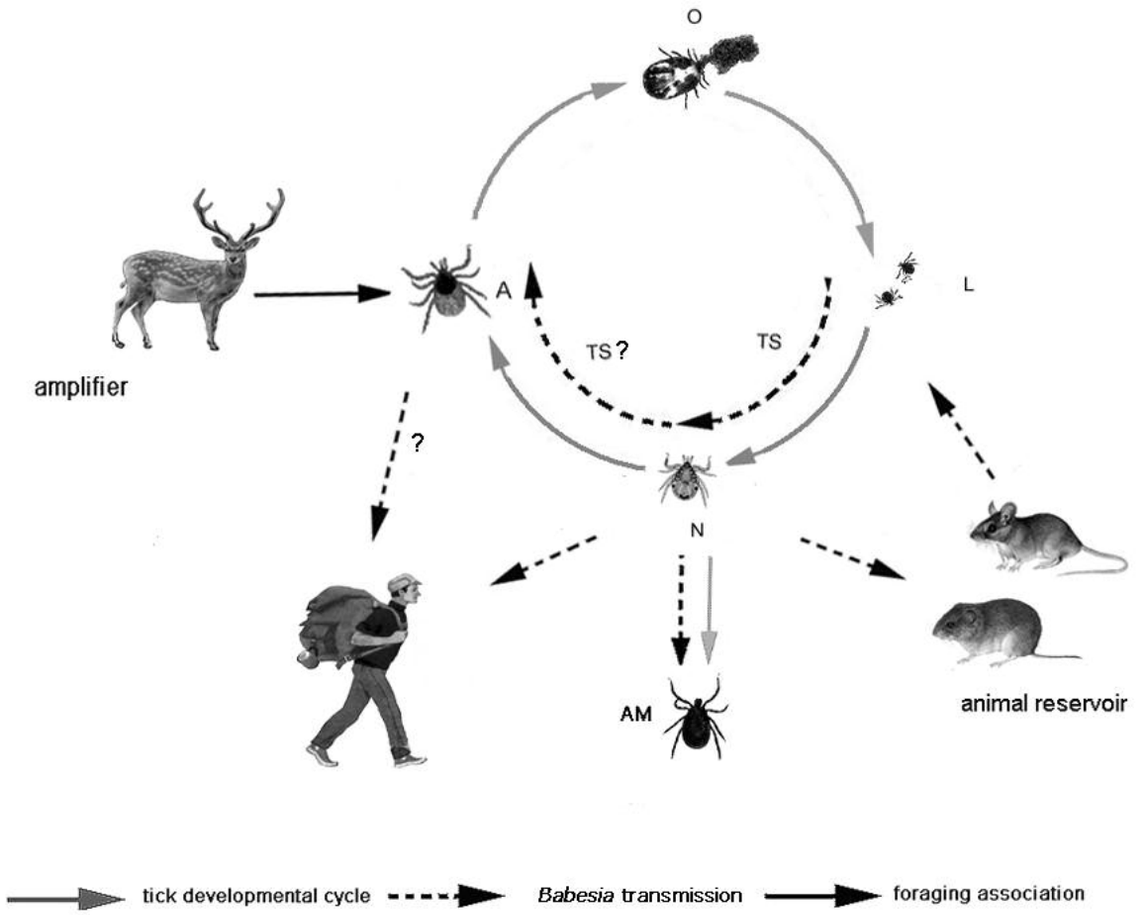

Fig. 1. The enzootic cycle of *Babesia microti*, the species associated with rodents
Ixodes ricinus tick is the vector which makes possible the maintenance and the circulation of *Babesia microti* in environment. The animal reservoir in Central Europe are mainly Microtinae – *Microtus arvalis* and *M. oeconomus* and lesser role plays other small rodents – *Myodes glareolus* and *Apodemus* mice.
The transstadial transmission enable the presence of the agent in nymphs and adult ticks, but the lack of transovarial mode result, in addition to foraging associations, that the circulation goes mainly between larvae and nymphs. These stages are crucial element in the *B. microti* circulation and transmission. Larvae acquire the infection from rodents, and as nymphs infect the next rodent host. There is no possibility for larvae to infect mammal hosts; adult females use as hosts bigger mammals, males do not feed. Adult females and males in collateral circumstances can be infected, but the foraging association with not-rodent hosts, are the blind valley for parasite. Wild boars and deer are resistant for *Babesia microti* 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.
CT – cofeeding transmission; TS – transstadial transmission; L – larva; N – nymph; A – adult female; AM – adult male; O – eggs.

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.

Larvae, nymphs and adult ticks can all feed on humans, but the nymph is the primary vector of *B. microti* to humans. It is evident, that nymphs are more efficient in propagation of *B. microti* than adults. Although the transstadial transmission of piroplasms from nymph to adult is not such efficient than from larvae to nymph, moreover, nymphal salivary glands are more intensely parasitized than

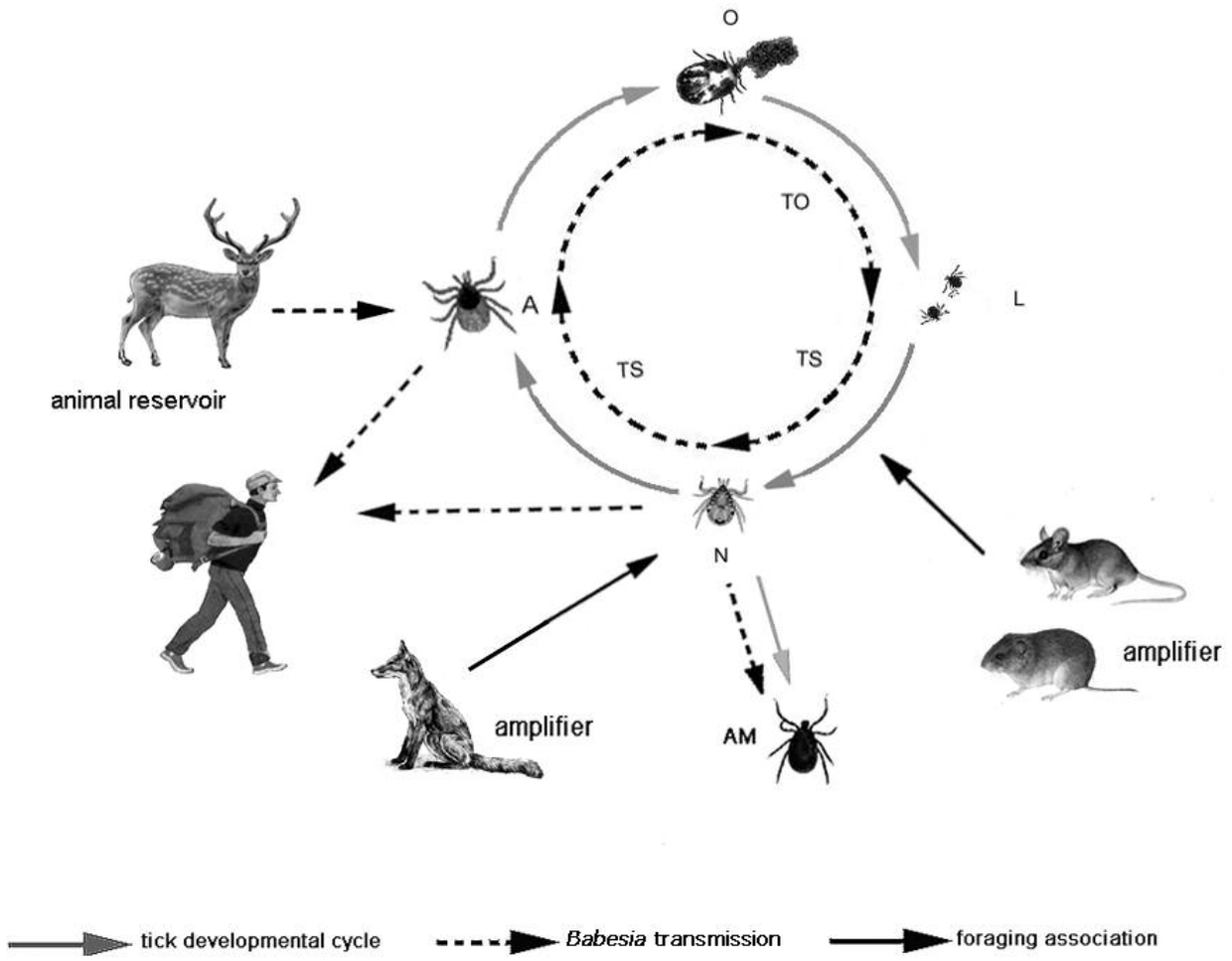

Fig. 2. The enzootic cycle of *Babesia divergens,* genospecies associated with ruminants
Ixodes ricinus tick is the vector which makes possible the maintenance and the circulation of *B. divergens* in the environment. The animal reservoir are cattle, red deer and roe deer, possibly European bison. Small rodents are not hosts of *B. divergens*, so they play the role of amplifiers, as hosts of ticks larvae and nymphs and maintaining their occurrence. Also as amplifiers serve medium sized mammals, as hosts of nymphs and adult ticks. Transovarial and transstadial transmission enable the presence of the agent in adult ticks, moreover, that larvae and nymphs feed on not-susceptible hosts. The tick stages able to effectively infect cattle and other ruminants are adult females. Resuming, pathogen circulates primarily in the cycle: the ruminant host → adult female tick → the next tick's generation → the ruminant. Adult males are blind valley for pathogen. The co-feeding transmission is not documented. Due to the transovarial and transstadial transmission during all developmental stages, human can be infected by every active tick's forms.
TO – transovarial transmission; TS – transstadial transmission; L – larva; N – nymph; A – adult female; AM – adult male; O – eggs.

are adult salivary glands [2,112]. All three stages of ticks may feed on the deer, which is not infected by *B. microti*. Thus, the deer appears to be a necessary host for the tick, while the parasite is maintained in a rodent reservoir.

Adult females and males in collateral circumstances can be infected, but due to the foraging association with not-rodent hosts, are the blind valley for parasite. They feed on the no susceptible hosts, and the transovarial transmission of small *Babesia* species has no place. The absence of transovarial transmission suggests that only nymphs and adults can transmit the parasite, despite some reports of PCR-positive larvae.

This way can be explained the proportions of larvae, nymphs and adult females infections. Rodent host is the source of infection for larvae and for nymphs, which does not been infected earlier. The percent of infected adults is the sum of previously high infected specimen, which enquired infection as

larvae and nymphs.

Ticks are the host enable to survive the winter for parasite and maintain their population. There are two crucial factors indicated – first, the infection of rodent stands four-six weeks and became extinct, or run into chronic infection, with the number of parasite to low to infect tick. Moreover, only a small part of rodent population survive the winter. Second factor is the biology of *I. ricinus* immatures. A minority of engorged larvae transform into nymph and feed as nymph the same year; a majority undergo diapause and feed the next year, being the source of infection for the next generation of rodents [79]. This is another reason of the seasonal incidence – the period from November to March is enough to eradicate the infection with *Babesia* by rodent's organisms, and the individuals born in October have no chance to acquire the parasite. In result, on spring the rodents are practically free of piroplasms, and the first infections are possible just late March, when the overwintering nymphs begin to feed.

***B. divergens* (Fig. 2)**

Ixodes ricinus tick is the vector which makes possible the maintenance and the circulation of *B. divergens* in the environment. Transovarial and transstadial transmission enable the presence of the agent in adult ticks [60].

The tick stage feed on ruminants is adult female, nymphs feed on the ruminants seldom [77], thus their role is lesser than adult forms; majority of larvae and nymphs feed on not-susceptible hosts. Therefore, the tick stages able to effectively infect cattle and other ruminants are mainly adult females. The contrary, each tick developmental stage appear to be able to be infected and to transmit *B. divergens*. However, by the reason mentioned above, adult females are the most often infected with *B. divergens*, and by the transovarial route deliver the infection to the next generation.

The animal reservoir are cattle, red deer and roe deer, possibly European bison. Small rodents are not hosts of *B. divergens*, so they play the role of amplifiers, as hosts of ticks larvae and nymphs and maintaining their occurrence. Also as amplifiers serve medium-sized mammals, as hosts of nymphs and adult ticks. This way, pathogen circulates in zoonotic foci primarily in the cycle: the ruminant host → adult female tick → the next tick's generation → the ruminant. Adult males are blind valley for pathogen. The co-feeding transmission is not documented. Due to the transovarial and transstadial transmission during all developmental stages, human can be infected by every active tick's forms.

Because the infection persists through molts of tick (transstadial maintenance) and is transmitted transovarially, it appears that the vertebrate host is not essential in the short term for maintaining the parasite in the tick population. Ticks are not only the vectors of. *B. divergens*, but also its most important non-bovine reservoir [24,73]. The infection may be retained in the tick population even if the absence of a bovine host [73].

***B. venatorum* (Fig. 3)**

Babesia venatorum seems to phylogenetically lie in a sister group with *B. divergens*. Transovarial and transstadial transmission of *B. venatorum* by *I. ricinus* ticks has been documented [60]. The circulation scheme is very similar to *B. divergens*, with two differences – the only animal reservoir is roe deer *C. capreolus*, and there is higher role of nymphs in the circulation. *C. capreolus* is lower than other cervids, and the percent of *I. ricinus* nymphs on this host is greater.

Conclusions

In comparison to other tick-borne diseases affecting human, the structure, circulation and transmission mode of babesiosis show the variability across in Europe. The reason are the differences in the spread and number of tick vectors – *I. trianguliceps* and *I. ricinus*, the variability in mean winter and summer temperatures, affecting the different seasonal dynamic. Moreover, there are more several factors to sort the Central European countries from Western, Northern or Southern parts – Germany, Poland, Austria, Czech Republic, Slovakia, Hungary, Slovenia [113]. It is the possible reason to separately describe of the phenomena occur in the foci of zoonoses in various part of Europe.

The agents of bovine babesiosis – *B. divergens* and human babesiosis – *B. microti* and *B. venatorum* – co-occur in particular sites of Central Europe, where they perpetuate in a cycle involving common *I. ricinus* tick vector. There are recorded also mixed infection of ticks with the *Babesia* species. However, the zoonotic cycles of small and large babesiae are different in details, due to transovarial transmission possibility and different

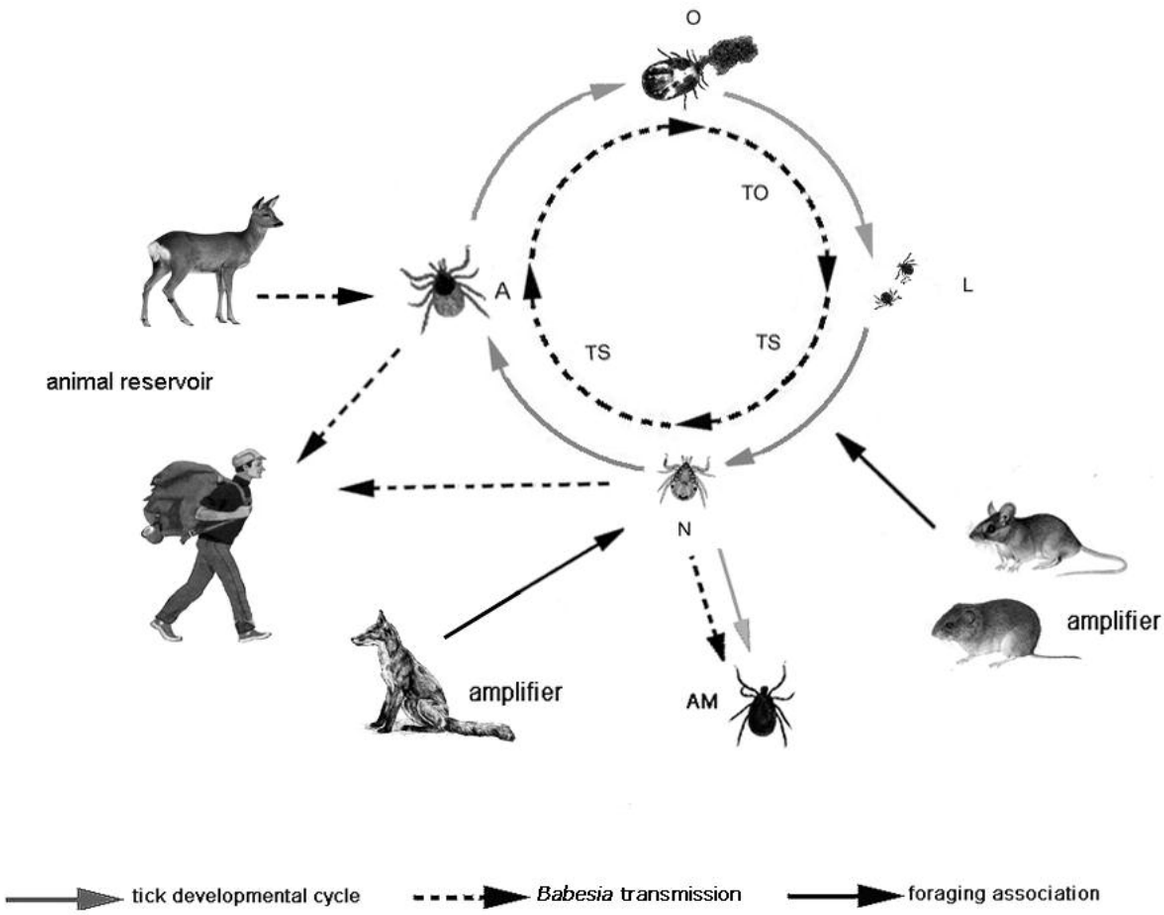

Fig. 3. The enzootic cycle of *Babesia venatorum,* species associated with roe deer
Ixodes ricinus tick is the vector which makes possible the maintenance and the circulation of *B. divergens* in the environment. The animal reservoir are roe deer. Small rodents are not hosts of *B. divergens*, so they play the role of amplifiers, as hosts of ticks larvae and nymphs and maintaining their occurrence. Also as amplifiers serve medium sized mammals, as hosts of nymphs and adult ticks. Transovarial and transstadial transmission enable the presence of the agent in adult ticks, moreover, that larvae and nymphs feed on not-susceptible hosts. The tick stages able to effectively infect cattle and other ruminants are adult females. Resuming, pathogen circulates primarily in the cycle: the ruminant host → adult female tick → the next tick's generation → the ruminant. Adult males are blind valley for pathogen. The co-feeding transmission is not documented. Due to the transovarial and transstadial transmission during all developmental stages, human can be infected by every active tick's forms.
TO – transovarial transmission; TS – transstadial transmission; L – larva; N – nymph; A – adult female; AM – adult male; O – eggs.

biology as well expectation of life of the hosts.

The zoonotic foci structure of *B. microti* in Central Europe differ in some details from analogical in Western Europe and Northern America. In Western Europe *Microtus* rodents are affected by *I. trianguliceps* and *I. ricinus*. Both tick species are abundant and are able to transfer and maintenance *B. microti* among rodents; moreover, adult *I. trianguliceps*, feeding on rodents, participate in the parasite circulation [75]. In Central Europe, *I. trianguliceps* is relatively rare and occurs locally, but in many localities occurs *Dermacentor reticulatus* and on the south of Carpathian Mountains, *D. marginatus* [81].

All rodents occur in Central Europe are appropriate hosts for *I. ricinus*; however, Murinae rodents are more heavily infested with *I. ricinus* larvae than *M. glareolus*. *Apodemus* mice are preferred hosts for *I. ricinus*. *Microtus* spp. are preferable host for immature *D. reticulatus* and *D. marginatus* [83,114], moreover, *Ixodes* ticks avoid the open areas. 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 [115]. Therefore, only a part of *I. ricinus* larvae and nymphs feed on infected *Microtus*. This fact can explain, that the prevalence of *I. ricinus* infection with *B. microti* is lower than with *B. divergens* and other tick-borne pathogens.

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