Annals of Parasitology 2016, 62(4), 267–284
doi: 10.17420/ap6204.62

Review articles

The role of particular ticks developmental stages in the circulation of tick-borne pathogens in Central Europe. 4. Anaplasmataceae.

Grzegorz Karbowiak[1], Beata Biernat[2], Joanna Stańczak[2], Joanna Werszko[1], Piotr Wróblewski[1], Tomasz Szewczyk[1], Hubert Sytykiewicz[3]

[1]W. Stefański Institute of Parasitology, Polish Academy of Sciences, ul. Twarda 51/55, 00-818 Warszawa, Poland
[2]Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine, Medical University of Gdańsk, ul. Powstania Styczniowego 9B, 81-519 Gdynia, Poland
[3]Department of Biochemistry and Molecular Biology, University of Natural Sciences and Humanities, ul. Prusa 12, 08-110 Siedlce, Poland

Corresponding Author: Grzegorz Karbowiak; e-mail: grzgrz@twarda.pan.pl

ABSTRACT. In Central European conditions, two species of Anaplasmataceae have epidemiological significance – *Candidatus* Neoehrlichia micurensis and *Anaplasma phagocytophilum*. Tick *Ixodes ricinus* is considered as their main vector, wild mammals as the animal reservoir. There is presented the transstadial transmission in ticks, due to the lack of transovarial mode the circulation goes mainly between immature ticks and 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. The summary of available data of different *A. phagocytophilum* strains associations with different hosts revealed at least few distinct enzootic cycle, concern the same ticks species and different mammal hosts. It is possible to reveal in Central Europe the existence of at least three different epidemiological transmission cycles of *A. phagocytophilum*. The first cycle involves strains pathogenic for human and identical strains from horses, dogs, cats, wild boars, hedgehogs, possibly red foxes. The second cycle involves deer, European bison and possibly domestic ruminants. The third cycle contains strains from voles, shrew and possibly *Apodemus* mice. In Western Europe voles might be involved in separate enzootic cycle with *Ixodes trianguliceps* as the vector.

Key words: *Anaplasma phagocytophilum*, *Candidatus* Neoehrlichia micurensis, ticks, zoonotic foci

Introduction

Ehrlichiosis and anaplasmosis are zoonotic tick-borne diseases caused by small, gram-negative, obligate intracellular bacteria of the family Anaplasmataceae, order Rickettsiales. Among others, there belong the genera *Ehrlichia* and *Anaplasma,* which include the range of tick-borne human pathogens.

There are three serious zoonotic diseases associated with these pathogens: human monocytic ehrlichiosis (HME) caused by *Ehrlichia chaffeensis*, granulocytic form of ehrlichiosis in dogs and humans caused by *Ehrlichia ewingii*, and human granulocytic anaplasmosis (HGA) caused by *Anaplasma phagocytophilum*. These agents were all formerly classified within the genus *Ehrlichia*, and the diseases caused by them were broadly referred to as ehrlichiosis. However, recent taxonomic revisions have reclassified the agent formerly called *Ehrlichia phagocytophila* under the genus *Anaplasma* as a single species *Anaplasma phagocytophilum* [1]. In Europe, HGA has bigger public health importance than infections caused by *Ehrlichia*, reported mainly from America [2–4]. In the Central European conditions, two species of Anaplasmataceae have great epidemiological significance – *Candidatus* Neoehrlichia micurensis and *Anaplasma phagocytophilum*.

The enzootic cycle of *Candidatus* Neoehrlichia micurensis

Candidatus Neoehrlichia micurensis – (*N. mikurensis*) is small (0.5–1.5 μm), gram-negative, pleomorphic coccus which grows in membrane-bound inclusions within the cytoplasm of endothelial cells [5]. This bacterium appeared as pathogenic for humans and was identified in the blood of febrile patients in different countries in Europe [6] and in China [7]. In Europe it was noted in Sweden, Germany, Switzerland, Austria and Czech Republic [7,8].

Tick *Ixodes ricinus* is considered as the main vector for *Ca.* Neoehrlichia mikurensis in Central Europe. It has been detected so far in ticks from at

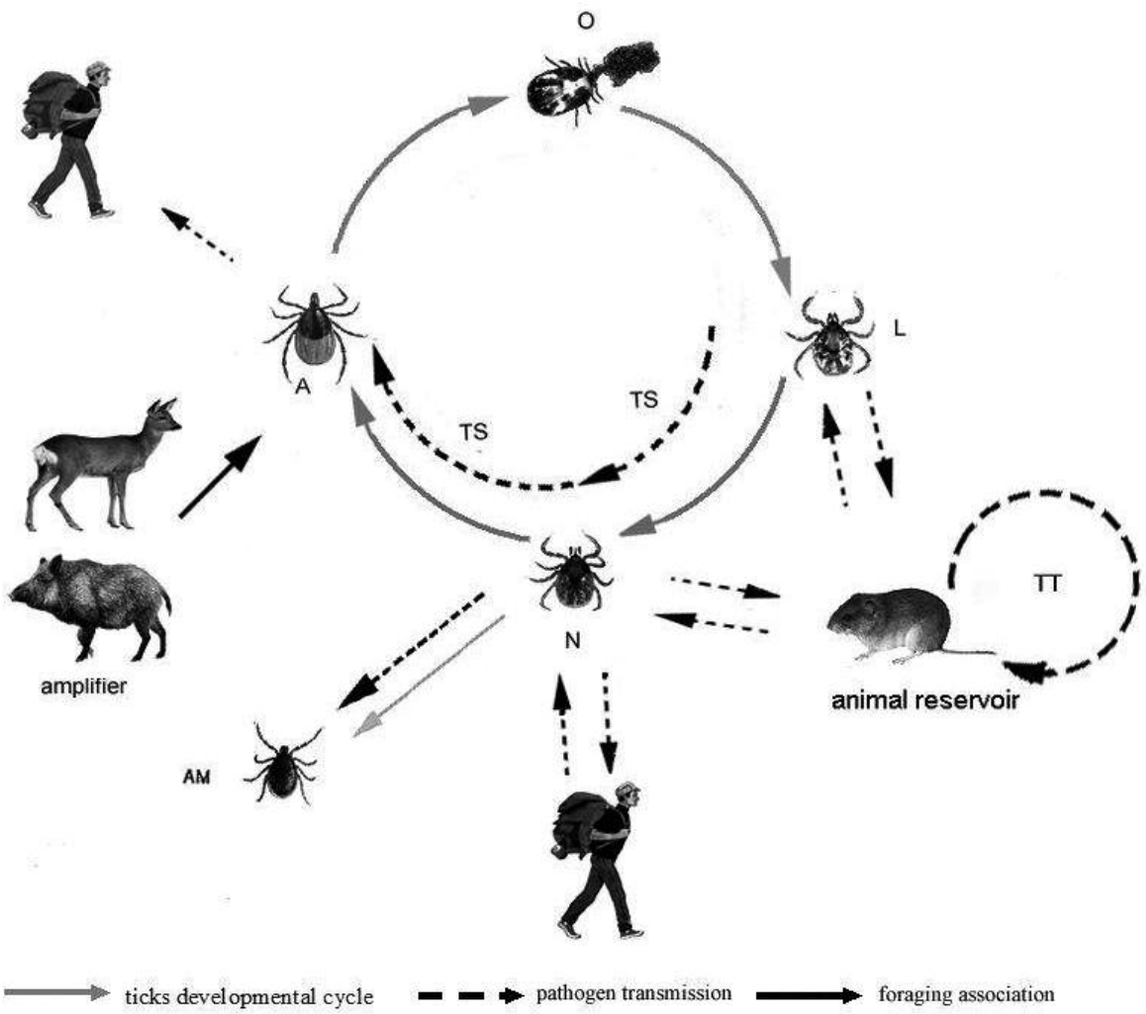

Fig. 1. The possible zoonotic cycle of *Candidatus* Neoehrlichia mikurensis
Ixodes ricinus serve as the vector which makes possible the maintenance and the circulation of *Ca.* N. micurensis in environment. The animal reservoir are small rodents, mainly from the subfamily Arvicolinae. The transplacental transmission enable the infection of young rodents without the vector participation. Large mammals play the role of amplifier and maintain the tick population. Transstadial transmission enable the presence of the agent in adult ticks, but the lack of transovarial mode, cause that the circulation goes mainly between immature ticks and rodents – larva acquires infection during feeding on the infected host, and as nymph infects another rodent specimen. 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 patogen 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; TT – transplacental transmission; L – larva; N – nymph; A – adult; AM – adult male; O – eggs

least 16 European countries [9]. The prevalence from various countries is within the range of the 0.3–24.2% of positively tested ticks, the mean 6.6, and median 5.3 [9–12]. Apart *I. ricinus*, *Ca.* Neoehrlichia mikurensis was also isolated from *I. frontalis* ticks fed on one migratory bird [13], *I. persulcatus* in Asian Russia, China and Japan [8,14] and *I. ovatus* in Japan [14]. The highest prevalence of infection was recorded in nymphs of *I. ricinus* (6.1%) in comparison to adult females and males (2.4%); larvae are not found to be infected [11,15]. However, Venclíková et al. [16] notes the prevalence rate 4.4 to 9.9 and 2.2 to 9.8 in adults and nymphs respectively, and accent that the differences varied significantly between study sites and years, but not between developmental stages except a single studied location. It is known nothing about the co-feeding transmission possibility. Although few investigations on the transovarial transmission of *Ca.* N. mikurensis have been undertaken [15], the transovarial transmission is not revealed. Moreover, the lack of agent in ticks' larvae and simultaneously their presence in rodents also suggest that transovarial transmission of *Ca.* N. mikurensis does not occur.

The animal reservoir is poorly known, however, available data indicate the role of small rodents [9,14,17]. In Europe, *Ca.* N. mikurensis has been detected in tissues of the six rodent species – *Apodemus agrarius*, *A. flavicollis*, *A. sylvaticus*, *Myodes glareolus*, *Microtus agrestis* and *M. arvalis* [9,17]. *Myodes glareolus* was the most frequently infected species with an average of 9.1% positive voles (range 1.8% up to 52.7% in various countries). Even if the prevalence of infection was relatively low, not exceeding 3.9%, the results from xenodiagnostic experiments with ticks have shown that rodents are competent reservoir hosts and are able to infect tick's larvae [18]. Further study reported observations of transplacental transmission in rodents [19]. The infection was also detected in dog in Germany [18]. The study of wild ruminants (roe deer, red deer and fallow deer) surveyed in Europe gave negative results [9,20].

The scheme of enzootic cycle of *Candidatus* Neoehrlichia mikurensis in environment is presented in Fig. 1.

The enzootic cycle of *Anaplasma phagocytophilum* – general aspects

Anaplasma phagocytophilum is an obligatory intracellular parasite, targets mainly granulocytes in its mammalian hosts and localise in membrane-bound vacuoles in the cytoplasm. This bacteria is spread over a spacious territory of the USA, Europe and Asia. It causes granulocytic anaplasmosis in humans (HGA) as well tick-borne fever in ruminants, equine anaplasmosis in horses and severe febrile diseases in dogs and cats [12,21–27]. The infection has been detected in several mammalian species, including humans, in areas of northern hemisphere with endemic occurrence of *Ixodes* ticks.

The first cases of human granulocytic anaplasmosis were reported from the USA in 1994 [28]. Since, human anaplasmosis have been occasionally reported throughout Europe [25]. The majority of confirmed HGE cases have been recorded in the USA; the prevalence of infection in Europe is significantly lower. The first European case was reported from Slovenia in 1995, later series of confirmed human cases have been reported in the majority of European countries [29–33].

Anaplasma phagocytophilum is transmitted by ticks belonging to the *Ixodes persulcatus* complex, which are mainly found in the Northern hemisphere – *I. scapularis* in the Northern America, *I. persulcatus* in northern Asia and *I. ricinus* in Europe. The prevalence of infection of ticks with *A. phagocytophilum* is very differentiated, from 1.0% [34] to 26.6% [35]. Many authors [16,36–38] accent, that prevalence of infection varied significantly among the study sites, years and it differed between tick stages. Apart in *I. ricinus*, *A. phagocytophilum* has been often found in *I. trianguliceps* [4,39] and in some populations of *D. reticulatus* [40–42], but their role is marginal and has local significance. However, Paulauskas et al. [43] detect unique *A. phagocytophilum* 16S rRNA sequence in a *D. reticulatus* tick collected in Lithuania which differed from other previously identified *A. phagocytophilum* sequences in the GenBank database.

Infection rates reported in many studies are usually higher in adult *I. ricinus* ticks than in nymphs, with the percentage of females infected with *A. phagocytophilum* significantly greater compared to males. On the data available from various countries of Europe, females are infected with *A. phagocytophilum* significantly more often than males and nymphs, which indicate the key role of tick females in the pathogen transmission to humans and animals. The mean percentage, range

Table 1. The prevalence of infection with *Anaplasma phagocytophilum* in particular tick *Ixodes ricinus* developmental stages (%)

Nymphs	Females	Males	References
nd	3.4	1.7	Sixl et al. 2003 [44][a]
2.1	23.3	4.2	Grzeszczuk et al. 2004 [31][a]
2.0	47.6	8.6	Stańczak et al. 2004 [46]
0.1	1.1	1.8	Stańczak et al. 2015 [69]
4.1	27.8	nd	Grzeszczuk 2006 [32]
4.4	24.6	6.5	Chmielewska-Badora et al. 2007 [37]
1.14	5.67	4.0	Silaghi et al. 2008 [47]
0.8	6.1	10.9	Overzier et al. 2013 [48]
1.0	1.2	1.2	Glatz et al. 2014 [11]
	6.5 – 11.1[b]		Venclíková et al. 2016 [16]

[a] calculated on the base of published data; [b] adults together

and median of infection of adult males, females and nymphs (recalculated from the original records) is 7.5% (1.6–33.5, mean 4.4), 19.5% (1.1–47.6, mean 5.7), 4.6% (0.1–27.7, mean 2.0) respectively [11,31,37,44–48]. There is no official record about the larvae infection with *A. phagocytophilum* (Table 1). Moreover, the important aspect is the role of *I. ricinus* males. Although many authors report their infections with *A. phagocytophilum*, they do not feed principally [49] and the pathogen has no possibility to take account in the follow circulation in the environment.

It seems allowed, that general mode of transmission and circulation scheme is similar to other tick-borne pathogens from Anaplasmataceae family. When tick feeds on infected host, pathogens first enter the tick midgut epithelium, where their primary replication takes place. Then the pathogens move to the tick salivary glands to invade the epithelial cells. In the salivary epithelial cells, bacteria undergo the second replication cycle and enter the salivary gland secretion when the tick feeds on the next vertebrate host [8]. Transstadial transmission of the bacterium occurs; the agent survives in infected ticks for over a year while the tick is awaiting a new host. In contrast to other Rickettsiales, the transovarial transmission has not been demonstrated [8,25] and larvae are considered free of *A. phagocytophilum*. A non-systematic co-feeding transmission of the pathogen from infected to uninfected ticks while feeding on hosts at common sites has not been yet reported for *Anaplasma* in contrast to viruses or spirochetes.

The study conducted in Europe confirms the wide range of mammals to be competent animal reservoir for *A. phagocytophilum*, varied according to geographical regions. As the most competent reservoir is considered roe deer (*Capreolus capreolus*). It is show the prevalence rates reaching up to 98.9%, according to data from southern Germany [48]; also the high prevalence, from 13.3 up to 72%, has been recorded in a fallow deer (*Dama dama*) from Great Britain, Czech Republic, Slovakia and Poland [45,50–56]. Other deer species also seem to contribute to the endemic cycles in Europe, and may also constitute efficient reservoir hosts, although they role is lower. The pathogen has been detected in red deer (*Cervus elaphus*) from 13.3 with up to 87.0% prevalence [45,52–54], and in sika deer (*Cervus nippon*) with up to 50.0% [25]. Apart deer, the organism was also detected in wild boar (*Sus scrofa*), mouflon (*Ovis aries*), European bison (*Bison bonasus*), elk (*Alces alces*) and chamois (*Rupicapra rupicapra*) [3,31,45,52,53,57, 58]. Medium sized mammals are poorly recognized as reservoirs; however, *A. phagocytophilum* was found in red foxes (*Vulpes vulpes*) in Czech Republic, Germany and Poland and in hares (*Lepus europaeus*) in Czech Republic [45,59,60].

The role of rodents as animal reservoir of *A. phagocytophilum* in Europe is not clear. Small mammals are hosts for both nymphal and larval ixodid ticks, so they could support endemic cycles of *A. phagocytophilum*. The yellow-necked mice (*Apodemus flavicollis*) were found to be infected with ranges from <1.0 to 15.0%, wood mice

(*Apodemus sylvaticus*) from <1.0 to 11.0%, bank-voles (*Myodes glareolus*) from 5.0 to 22% [4,25], root-vole (*Microtus oeconomus*) to 6.6% [61]. The higher prevalence (21.9%) has been noted in *M. glareolus* in Finland [62]. Hulínská et al. [45] found the infection in the range of rodents, and noted that the percentage of *Anaplasma*-positive animals was significantly higher in mice (15.0%) than in bank voles (13.3%). However, many studies showed the absence of infection in rodents, for example Svitálková et al. [63] during study conducted in Bratislava detected *A. phagocytophilum* in bank voles (prevalence 2.5%), while in cohabitating yellow-necked mouse the pathogen was not detected (Table 2).

The genetic variability of *Anaplasma phagocytophilum*

The schemes of HGE agent circulation in environmental and the structure of zoonotic foci complicate due to the great genetic variability of *A. phagocytophilum*, described in the last decade. Many studies demonstrate the different virulence and different hosts preference of particular *A. phagocytophilum* strains. However, the host specificity of strains seems to be restricted, and the multiple infections with different strains are often. Farm and large wild animals, small mammals and ticks were especially prone to carrying multiple genetic variants. In humans and domestic animals double infections are not so frequent [64].

The first records about the genetic, pathogenic and host preference diverse were made in northern America [65]. To this time, the studies of the zoonotic reservoir of anaplasmosis have shown small rodents, in northern America the white-footed mouse (*Peromyscus leucopus*), as a competent reservoir. In the follow investigations, on the basis of serological assays or molecular detection of *A. phagocytophilum* DNA in blood or tissue samples of more rodents and insectivores (mice, voles, shrews), medium sized small mammals (e.g., squirrels, raccoons) and large mammals (e.g., white-tailed deer, horses) are proved [25]. The DNA sequencing of the *A. phagocytophilum* 16S rRNA gene amplified from *I. scapularis* ticks and from white-tailed deer (*Odocoileus virginianus*) showed that novel genetic variant of *A. phagocytophilum*, AP-variant 1, differed from that originally described by Chen et al. [28] and named AP-ha. Moreover, the novel variant has never been amplified from a confirmed human infection and in contrast, the human agent, AP-ha, has not been detected in a white-tailed deer. The field and laboratory studies showed, that both strains are transmitted by ticks, but differ in the infectivity for mice [65]. Later investigations confirmed the variability and that different epidemiological context are associated with considerable strain, e.g., AP-ha strain, which is pathogenic for humans, can also infect cattle, sheep and mice, whereas the AP-Varian 1, which is not infectious for humans, infect goat and deer, but not mice, similarly [21,65].

Similar range of variable strains and isolates associated with the particular group of hosts, is observed in Europe. During several investigations, multiple genetic variants of the bacterium have been characterized, on the base of some genetic markers. To monitor the environment for the incidence of *A. phagocytophilum* there are involved both classical and nested PCR. As markers, fragments of the 16S rRNA, *ankA, groESL, msp2*, or *msp4* genes are used for analyses usually. Amplification is based on a one-step PCR and nested PCR protocol for the genes *msp2*, *ankA*, or 16S rRNA, or a two-step PCR assay, which is recommended for the *groESL* or *msp4* genes [66,67].

Ribosomal 16S rRNA genes are highly conserved among all bacterial species; therefore, they are a useful tool in the identification of pathogen species and in phylogenetic research [67,68]. As mentioned above, the testing of different primer pair variants has allowed the differentiation of five northern American strains of *A. phagocytophilum* denoted as AP-ha, AP-variant 1, AP-variant 2, AP-variant 3 and AP-variant 4, with the most common variant being AP-ha [67,68]. Sequence analyses have proven that the differences between variants are small and fall within the range of 1–4 nucleotides; however enough to distinguish subpopulation within the species are probably associated with different hosts [67]. Rar and Golovljova [8] by analysing of the 16S rRNA gene nucleotide sequences discriminated 15 worldwide variants differing in a variable fragment located near the 5'end of the gene. Two are pathogenic for human and abundant all over the world, one is non-pathogenic for humans and common in the USA and Europe in cattle and deer, three of them were found in Europe only, eight is very rare and recorded only in a few samples. As in case of mammal hosts, also the investigations of *I. ricinus* ticks conducted in Europe identified several 16S rRNA gene variants

Table 2. The prevalence of *Anaplasma phagocytophilum* infection in wild mammals in Central Europe

Host species	Prevalence (%)	Locality	References
Sus scrofa	4.35	Moravia, Czech Republic	Hulínská et al. 2004 [45]
	6.0	west-northern Poland	Skotarczak et al. 2008 [53]
	12.0	west-northern Poland	Michalik et al. 2012 [57]
	16.7	Slovakia	Víchová et al. 2014 [17]
Deer			
Capreolus capreolus	12.5	Moravia, Czech Republic	Hulínská et al. 2004 [45]
	30.0	Bohemia, Czech Republic	Zeman and Pecha 2008 [52]
	23.5–35.9	Slovakia	Stefanidesova et al. 2008 [54]
	98.9	Germany	Overzier et al. 2013 [48]
	38.7	east-northern Poland	Hapunik et al. 2011 [55]
	53.6	west-northern Poland	Skotarczak et al. 2008 [53]
	23.6	west-northern Poland	Adamska 2006 [51]
	9.6	western Poland	Michalik et al. 2009 [81]
	37.3	Poland	Welc-Falęciak et al. 2013 [56]
Cervus elaphus	13.33	Moravia, Czech Republic	Hulínská et al. 2004 [45]
	86.0	Bohemia, Czech Republic	Zeman and Pecha 2008 [52]
	10.2	western Poland	Michalik et al. 2009 [81]
	68	west-northern Poland	Skotarczak et al. 2008 [53]
	50.9	east-northern Poland	Hapunik et al. 2011 [55]
	19.2–24.5	Slovakia	Stefanidesova et al. 2008 [54]
	17.5	Slovakia	Víchová et al. 2014 [17]
Dama dama	13.33	Moravia, Czech Republic	Hulínská et al. 2004 [45]
	20.5	western Poland	Michalik et al. 2009 [81]
	1.5	east-northern Poland	Hapunik et al. 2011 [55]
Other ruminants			
Bison bonasus	62.0	eastern Poland	Grzeszczuk et al. 2004 [31]
	66.7	eastern Poland	Karbowiak et al. 2015 [58]
Ovis aries	4.0	Bohemia, Czech Republic	Zeman and Pecha 2008 [52]
Alces alces	20.0*	Poland	Karbowiak et al. 2015 [58]
Rodents and insectivores			
Microtus oeconomus	6.6	eastern Poland	Grzeszczuk et al. 2006 [61]
Apodemus flavicollis	15.0	Moravia, Czech Republic	Hulínská et al. 2004 [45]
Apodemus agrarius	21.5	western Poland	Gajda et al. 2013 [91]
Erinaceus europaeus	25.8*	Germany	Skuballa et al. 2010 [92]
Other			
Vulpes vulpes	4.0*	Moravia, Czech Republic	Hulínská et al. 2004 [45]
	2.7	Poland	Karbowiak et al. 2009 [59]
Lepus europaeus	12.5*	Moravia, Czech Republic	Hulínská et al. 2004 [45]

* statistically insignificant

of *A. phagocytophilum* [43,65,69,70]. The reason of such variability is the generalist feeding behaviour of *I. ricinus* nymphs and adults that facilitates the continuous exchange of *A. phagocytophilum* variants between the different vertebrate species. Investiga - tions of strain in mammal hosts revealed that the genetic diversity is associated with several hosts species. Analysis of 16S rRNA gene using the method of multiple-locus variable number tandem repeat (VNTR), obtained from strains isolated from big mammals (cattle, cervides, horses, dog) and ticks in France confirmed that strains origin from roe deer and cattle belong to two different clusters [21]. In Slovenia, sequences obtained from roe deer cluster separately to obtained from humans [71]. Hulínská et al. [45] constructed the phylogenetic tree with respect to the *Ehrlichia equi* sequence, homologous to the human granulocytic ehrlichiosis

agent, on the base of sequences obtained from ticks, deer, rodents, cows and horses. Two *E. equi* variants from two horses were 100% homologous to human strain, and 99.5 % homologous to selected samples from other animals. Overzier et al. [48] differentiated four genetic variants of *A. phagocytophilum* isolated from wild mammals. The most common is named X, and it seems mainly associated with wild cervid species such as roe deer; moreover, it had been also detected in small mammals, sheep (*Ovis aries*), European bison *(Bison bonasus*), cattle (*Bos taurus*), red deer (*Cervus elaphus*), chamois (*Rupicapra rupicapra*) and mouflon (*Ovis orientalis musimon*) and is known to cause tick-borne fever in cattle and sheep. Other different variants are I, V, and O, which are also associated with these hosts, but occur more rarely. It is obvious, that strain isolated from sheep and field voles in Great Britain, differs by a single nucleotide substitution from the prototype HZ strain (accession number U02521) [39].

The variability of *A. phagocytophilum* variants in *I. ricinus* ticks is great as well, that suggest that all can be transmitted by this tick species. Blaňarová et al. [4] detected four 16S rRNA genetic variants in questing *I. ricinus*, feeding *I. trianguliceps* ticks and in rodents in one study. Two identical strains were detected in *I. ricinus*, *I. trianguliceps* and *M. glareoulus*. Paulauskas et al. [43] described three types of 16S rRNA gene sequences that were obtained from the *I. ricinus* ticks collected in the Baltic countries, and 5 types of gene sequences obtained from ticks collected in Norway. There were designated 7 variants. Three (1-3) were identified in host seeking ticks and were 100% identical to the published GenBank sequences that were derived from dog, domestic cat, roe deer, cow and *I. ricinus* ticks. In the samples from Norway, two different variants (2 and 3) were detected in questing *I. ricinus* ticks, two others from tick specimens collected from hosts. One gene variants was 100% identical to the prototype sequence of the HGA agent; another variant showing a 100% identity with a sequence from a horse. Stańczak et al. [69] differentiated four genetic variants of the partial 16S rRNA of *A. phagocytophilum* detected in *I. ricinus* in Poland, none equalled in the amplified part the human pathogenic prototype variant. The most prevalent variant 1 has been already detected for example in ticks, dogs and cats; variant 2 was found in *I. ricinus* feeding on birds and red foxes; variant 3 has been previously detected in roe deer and in ticks feeding on them; variant 4 was found in *I. ricinus* and elk *Alces alces*. Far greater diversity was found by von Loewenich et al. [6] in a population of *A. phagocytophilum* isolated from *I. ricinus* ticks. They described as many as 9 types of the gene encoding the small subunit of the ribosome.

The heat shock operon *groESL* codes for the expression of heat shock proteins (HSP) and is another conserved fragment employed in *A. phagocytophilum* studies. The operon is composed of two genes, *groES*, encoding a 10–20 kDa protein, and *groEL*, encoding a 58-65 kDa protein, separated by a non-coding sequence of variable length depending on the bacterial species [67,72]. The sequencing of the *groESL* has facilitated the observation of a high degree of variability within the operon, although most of the variants were of neutral character for the fitness of cells, and thus do not undergo selection. The presence of different variants of heat shock operon in *A. phagocytophilum* in ticks or wild animals considered to be reservoirs of the bacterium has been confirmed many times by independent researchers. First research revealed the presence of two mean different bacterial strains [6,66,70,73–75]. The sequence analysis suggest, that human pathogenic *Anaplasma* variant has the closest similarity (more than 99.0%) to strains identified in wild boar as well domestic animals – horse and dog [44,70,76,77]. The sequences of strains isolated from red deer also clustered with those obtained from humans [71]. Follow studies revealed greater variability. Rar and Golovljova [8] differentiated over 50 different variants of groESL heat-shock operon, and among them are three separate groups of sequences from small mammals, from a separate cluster differing from the clusters associated with large mammals. Welc-Falęciak et al. [56] distinguished in Poland 6 genetic variants, differed in hosts preferences and geographic range: variant A, show 99.6–99.8 similarity to sequences from *I. ricinus*, and roe deer from Czech Republic; variant B, differs from human pathogenic strains isolated from Slovenia, Poland and Italy by 2 nucleotides; variant X, homology to strains from goat (*Capra aegagrus hircus*); variant Y, identical to strains from goat and mouflon; variant V from dog (*Canis lupus familiaris*) and hedgehog (*Erinaceus europaeus*); variant W, present in the high range of various mammals.

Other genes employed in the detection and identification of *A. phagocytophilum* are *msp2* and

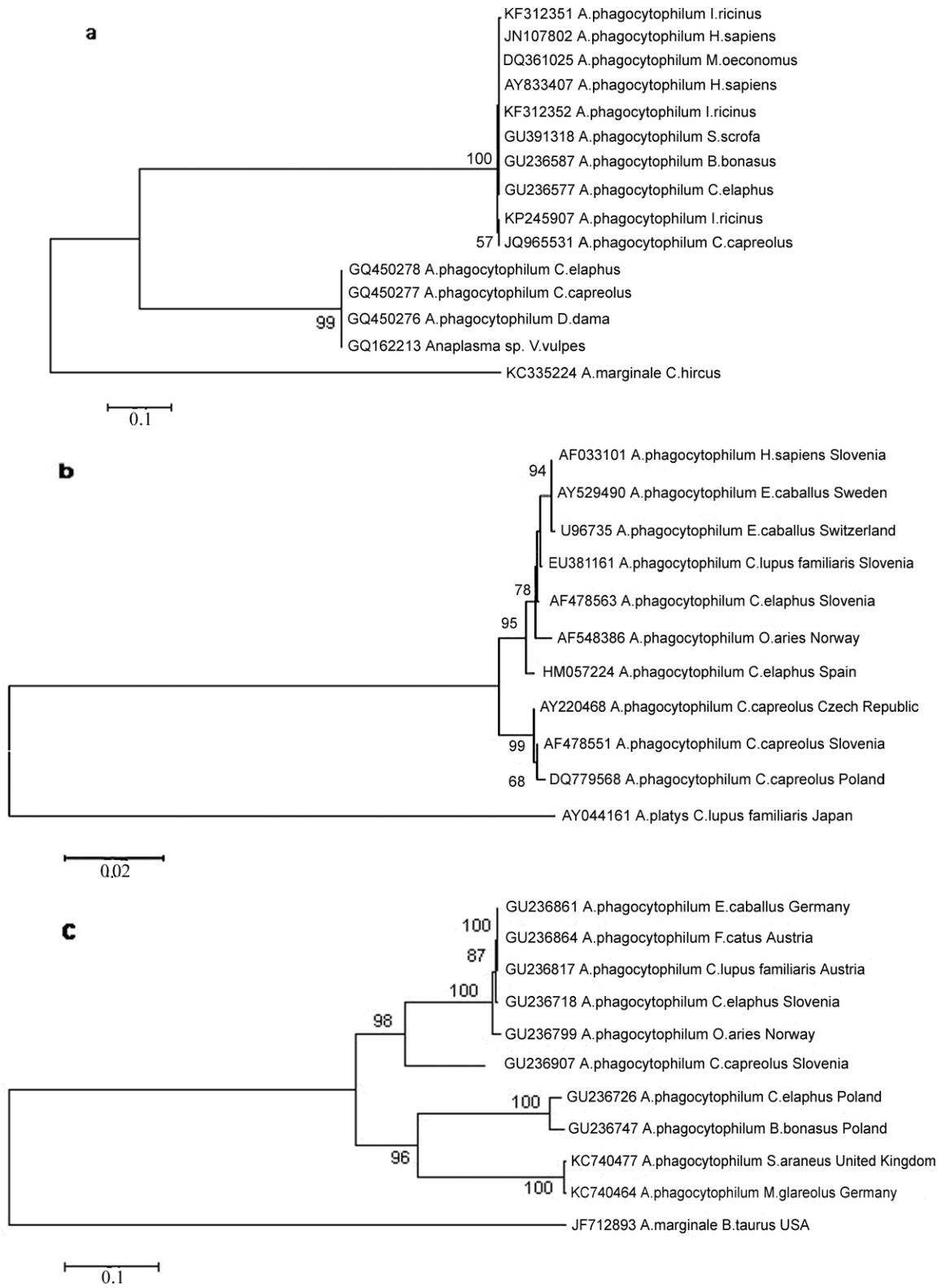

Fig. 2. The phylogenetic trees of different *A. phagocytophilum* strains chosen from GenBank and origin from Central European countries, with associations to mammal hosts
a – based on the fragment of the 16sRNA gene; b – based on the fragments of groESL heat shock operon; c – based on the fragments of *ankA* gene

msp4 genes belonging to the OMP-1/MSP-2/P44 superfamily, characteristic of Anaplasmataceae [66,67]. In the genome of *A. phagocytophilum* three *omp-1*, one *msp-2,* two *msp-2*-homologs, one *msp4,* and 113 *p44* loci were found within this superfamily. The high variability of *msp2*, which includes intra-species variability, with consequent protein polymorphism and the generation of antigenic variations facilitate bacterial survival in diverse hosts. The correlation of different variants of the MSP2 protein with geographical localization has also been observed [66,78]. Another recent study indicated that variants which infect ruminants in Europe could be differentiated from variants which infect dogs, horses, and humans by sequencing *msp4* [79], and follow sequences of *A. phagocytophilum* isolate from a dog and from a horse showed an identity of 100% [60].

The *ank*A gene encodes a protein antigen of molecular weight between 150–160 kDa and which has repeated ankyrin motifs at the amino terminus. Analysis of the ankA protein indicates localization in the bacterial cytoplasm and not in the membrane [64,67]. PCR and subsequent sequencing allowed a structural comparison of this genomic region in *Anaplasma* originating from various geographical locations and different hosts; moreover, the AnkA protein sequences showed multiple mutations [80]. Hartwig et al. [60] demonstrated, that isolates from foxes were identical to variants AF36712 and U02521 known from human and animal infections. Scharf et al. [80] noted the 100% identity of *ankA* gene sequences of strains isolated from dog an horses in Germany, meanwhile, isolates from roe deer are different and group into two separate clusters; other ruminants, as from European bisons, can be ranked to all clusters. Michalik et al. [57,81] isolated strains from wild boar and deer, corresponded to *A. phagocytophilum* strains caused human disease. Majazki et al. [82] described new, separate cluster with sequences restricted to voles and shrews.

The great problem in the interpretation of *A. phagocytophilum* strains diversity is simultaneous differentiation associated with hosts preferences and geographic location. To confirm the particular strains preferences to mammal hosts without local influences, Authors made analysis of strains deposited in GenBank and origin from Central European countries only, if possible. The deposited sequences were analyzed using GenDoc 2.7.0. software [83] and compared using NCBI BLAST [84]. The phylogenetic analysis was performed using the neighbor-joining (NJ) and maximum parsimony (MP) methods in PAUP [85]. Molecular distances for neighbor-joining tree were evaluated using Kimura two-parameter model and followed by 1000 bootstrap replications. Whereas for the maximum parsimony followed by 1000 bootstrap replications using the Tree-Bisection-Reconnection algorithm were used. The available sequences of 16S rRNA gene groups into three lines, associated with different cervids and fox, human and several mammal hosts from every group, and separate from *C. capreolus* respectively (Fig. 2a). Sequences of *groESL* operon group into one separate line associated with *C. capreolus*, and second, diverse into groups associated with human and horse, *C. elaphus*, *Canis lupus*, *Ovis aries* respectively (Fig. 2b). The available sequences of *ankA* gene present the separate lines associated with rodents and insectivores, *C. elaphus* and *B. bonasus*, *C. capreolus*, and group of related strains from horse, carnivores and *O. aries* (Fig. 2c).

The high genetic variability of *A. phagocyto - philum* strains shortly described above exclude a simple scheme of enzootic HGA foci, composed from competent vector, animal reservoir and amplifier. Due to the hosts preferences of different strains, some of wild animals can serve as reservoir hosts for granulocytic anaplasmosis in humans, some of them not. Moreover, the strains divergence made on the basis of different genetic markers and some Authors analysis covered partially only. The attempt to sum up the genetic variability is made by Huhn et al. [64], using method of multilocus sequence typing (MLST) to analysis of 400 strains from human and animals mostly from Europe and USA. The advantage of the analysis is independent of the sequences as it takes into account only the allelic profiles. Authors identified eight clonal complexes, and among them three contained more than 10 variable strains. These are complex 1, containing typeable strains from human, dogs, horses, hedgehogs, wild boars, cats, red foxes; complex 2, containing typeable strains from voles and shrews; complex 3, containing human and canine strains origin from USA. Other smaller complexes contained variable strains from cattle, European bison and goat; two variable lines from sheep, and strains from roe and red deer, sheep and cattle. The phylogenetic analysis revealed that strains from human, horses, dogs, hedgehogs and wild boars are uniform, but strains from sheep,

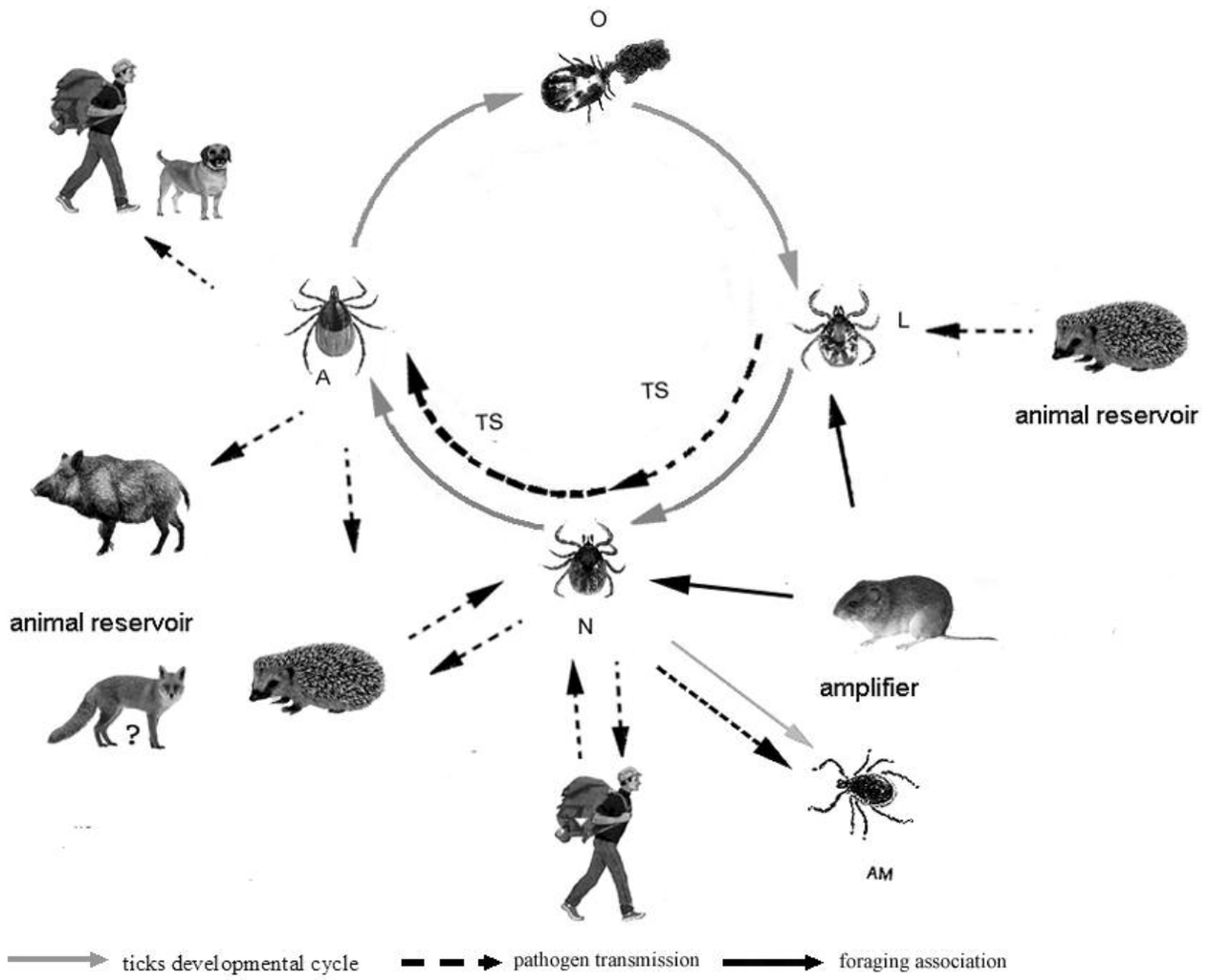

Fig. 3. The enzootic cycle of *Anaplasma phagocytophilum* variants pathogenic for human
Ixodes ricinus tick is the vector which makes possible the maintenance and the circulation of *A. phagocytophilum* variants pathogenic for human in environment. The animal reservoir are hedgehogs and wild boars, possibly carnivores. Small rodents are not hosts of human affected strains, so they play the role of amplifiers, as hosts of ticks larvae and nymphs, and maintain their occurrence. Transstadial transmission enable the presence of the agent in adult ticks, but the lack of transovarial mode result, that the circulation goes mainly between immature ticks, adults and their hosts. Hedgehogs, as hosts for every *I. ricinus* developmental stages can be the source of infection for larvae, and be also infected by nymphs and adults. The tick stages able to effectively infect human are nymphs and adult females, males do not participate in the follow transmission.
TS – transstadial transmission; L – larva; N – nymph; A – adult; AM – adult male; O – eggs

cattle, European bison, roe deer, red deer show a higher degree of heterogenity. In total, among the complexes three major clusters can be distinguished. Cluster 1 contains strains according to the strains distinguished in complex 1. The strains from human, horses, hedgehogs, wild boars cluster together in the *ankA* gene and groESL operon sequences. Moreover, human, canine and equine strains Cluster 2, containing strains from roe deer and part of strains from red deer. The strains are not as uniform as those from humans and domestic animals and show a higher diversity; similarly, a higher degree of strain diversity was found for *A. phagocytophilum* strains from cattle, red deer and European bison. Cluster 3 contains strains from voles and shrews. These are distantly related to all other strains. It is significant, that the association of variable *A. phagocytophilum* strains with different hosts group is supported by other Authors remarks. Majazki et al. [82] revealed, that

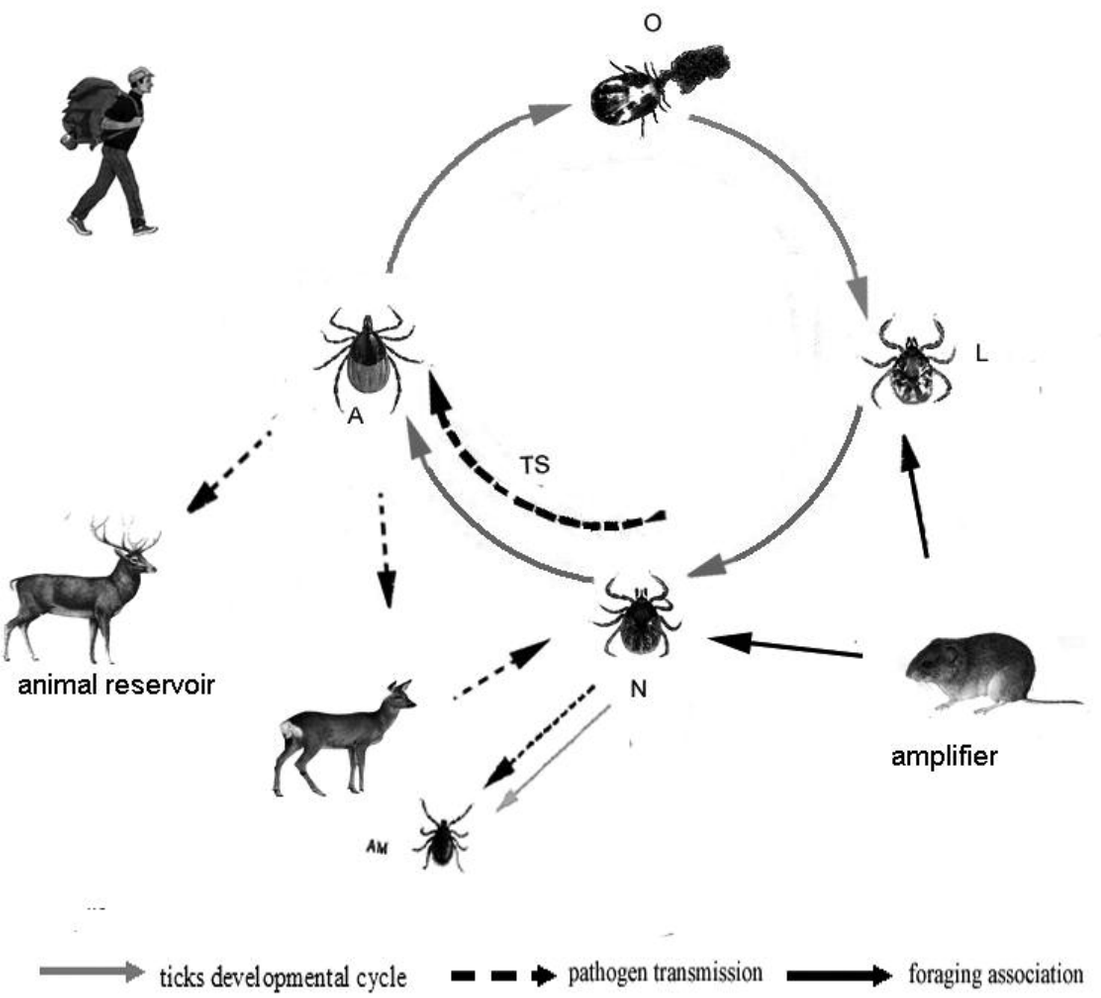

Fig. 4. The enzootic cycle of *Anaplasma phagocytophilum* variants pathogenic for ruminants
Ixodes ricinus tick is the vector which makes possible the maintenance and the circulation of *A. phagocytophilum* variants pathogenic for ruminants in the environment. The animal reservoir are red deer and roe deer, possibly European bison. Small rodents are not hosts of *A. phagocytophilum* strains affecting ruminants, so they play the role of amplifiers, as hosts of ticks larvae and nymphs and mainaining their occurrence. 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. The tick stages able to effectively infect cattle are adult females. Due to the lack of transovarial transmission, there is no possibility for larvae to aquire the infection. By the route the source of infection for nymphs are mammal hosts only, so they are able to infect next hosts as adult stages only. Resuming, pathogen circulates primarily in the cycle the nymph – adult female tick – the ruminant – the nymph. Adult males are blind alley for pathogen. The co-feeding transmission is not documented. Human is not affected by this *A. phagocytophilum* variant.
TS – transstadial transmission; L – larva; N – nymph; A – adult; AM – adult male; O – eggs

strains from small mammals belong to a distinct *ankA* gene cluster; on the separate character of rodent's strains of *A. phagocytophilum* show also the analysis of Víchová et al. [17] – the sequences of strain isolated from voles and *Apodemus* mice in Slovakia were identical in overlapped regions of 16S rRNA and *groEL* genes, and clustered together.

The summary of available data of different *A. phagocytophilum* strains associations with different hosts revealed at least few distinct enzootic cycle, concern the same ticks species and different mammal hosts. Bown et al. [39] suggest the occurrence of a few co-existing yet distinct enzootic cycles, involving deer as hosts and another with voles as hosts. They notified that *A. phagocytophilum* strains associated with rodents circulate in

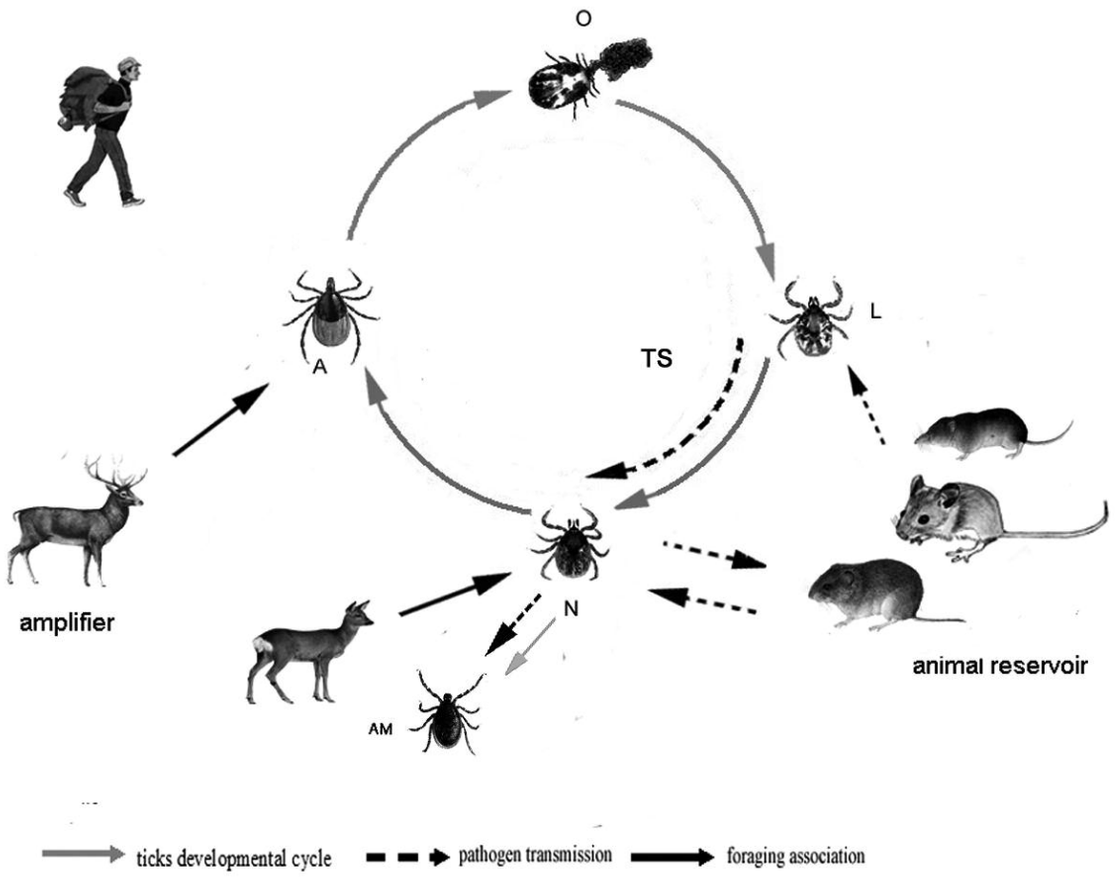

Fig. 5. The enzootic cycle of *Anaplasma phagocytophilum* variants affecting rodents and shrews

Ixodes ricinus tick is the vector which makes possible the maintenance and the circulation of *A. phagocytophilum* variants pathogenic for rodents in the environment. The only hosts are small mammals, whereas ruminants and insectivores are not hosts of this *A. phagocytophilum* strains, so they play the role of amplifiers, as hosts of adult ticks and nymphs and maintaining their occurrence. Transstadial transmission enable the presence of the agent in adult ticks, but the lack of transovarial mode result, that the circulation goes mainly between larvae, nymphs and their hosts. Resuming, pathogen circulates primarily in the cycle small mammal – tick's larva – nymph – small mammal. The co-feeding transmission is not documented. The adult ticks become infected by the transstadial route, however this development stage stands for the patogen a blind alley, because hosts of adult ticks are not susceptible for infection, and the transovarial transmission is not there. In the case of *I. trianguliceps*, all developmental stages participate in the *A. phagocytophilum* circulation, as well, both small rodents and shrews are engaged [89,91]. Human and domestic animals are not affected by this *A. phagocytophilum* variant.
TS – transstadial transmission; L – larva; N – nymph; A – adult; AM – adult male; O – eggs

enzootic cycles separately from non-rodent associated strains and that they are transmitted by *I. trianguliceps* but not *I. ricinus* ticks. *Myodes glareolus*, *A. flavicollis* and *A. agrarius* were infected with the same genotype of *A. phagocytophilum* those areas where *I. trianguliceps* were present. On that base authors suggest, that rodents strains are transmitted by *I. trianguliceps*, not *I. ricinus*; this hypothese is able to explain the high variability of *A. phagocytophilum* infections among rodents; however does not explain the presence of infections in rodents in localities in Eastern Europe, where *I. trianguliceps* ticks is rare or absent. Such situation has place in north-eastern Poland, were *A. phagocytophilum* has been detected in rodents but *I. trianguliceps* was not found at the same time and localization [61]. This hypothesis can be supported by observation of Liz et al. [86] as well Blaňarová et al. [4] who demonstrated the presence of *A. phagocytophilum* in rodents and collected

from these animals ticks larvae. The partial 16S r RNA gene sequences of bacteria from rodents and ticks showed a high degree of homology, in contrast, *groESL* sequence analysis showed a strong divergence, between samples derived from rodents and those from questing ticks. Woldehiwet [3] suggest that free-living rodents harbour TBF variant (tick-borne fever) of *A. phagocytophilum*, infecting for domestic and free-living ruminants. Jahfari et al. [15] describe four ecotypes, created on the basis of the *groEL*-gene sequences and ecological factors. Ecotype I concerns human isolates from Genbank and the published papers. Ecotype II is present in roe deer, *I. ricinus*, and deer keds. Ecotype II may circulate between roe deer via *I. ricinus*, or deer keds, or both. Ecotype III is represented by strains isolated from rodents. Two variants of this ecotype were found in two different tick species, *I. trianguliceps* and *I. ricinus*, feeding on wood mouse. Ecotype III was not found in questing *I. ricinus* or in any other wildlife, except rodents. Ecotype IV is associated with one or more bird species, but not with other vertebrates. Ecotype IV was not found or in any other animal species. As ecotype IV was not found in questing *I. ricinus* either, it might be adapted to a life cycle involving exclusively birds and a bird-specific vector, such as *I. frontalis*.

Conclusions

Summary the above, it is possible to reveal in Central Europe the existence of at least three different epidemiological transmission cycles of *A. phagocytophilum*, associated with the genetic diversity of these bacteria and variable hosts preferences of different strains.

The first cycle may involve *A. phagocytophilum* strains pathogenic for human and identical strains from horses, dogs, cats, wild boars, hedgehogs, possibly red foxes. According to many authors [48,64,77] wild strains from boars and hedgehogs are equally part of this same clonal complex that human, this suggest that these animals may serve as reservoir hosts for HGA in humans and domestic animals. Strasek Smrdel et al. [77] and Michalik et al. [57] accent the potential role of wild boar as animal reservoir of HGE.

The proposed scheme and detailed description of enzootic cycle of variant of *A. phagocytophilum* pathogenic for human is presented in Fig. 3.

The second cycle might involve roe deer, red deer, European bison and possibly domestic ruminants, as either accidental hosts. These strains are distinctly related to strains from human and small domestic animals. Therefore deer and Bovinae are unlikely to serve as reservoir hosts for granulocytic anaplasmosis in humans. The scheme of enzootic cycle of variant of *A. phagocytophilum* affecting ruminants is presented in Fig. 4.

The third cycle contains strains from voles, shrew and possibly *Apodemus* mice. These are distantly related to other strains, and therefore small mammals are unlikely to serve as reservoir hosts for granulocytic anaplasmosis in humans, domestic and farm animals. In Western Europe voles and shrews might be involved in separate enzootic cycle with *Ixodes trianguliceps* as the vector [87]. It suggests also Blaňarová et al. [4] in Slovakia, who demonstrates that at sites where *I. trianguliceps* was absent, they did not detect *A. phagocytophilum* in rodents. In the light of the biological features of *Ixodes trianguliceps* tick, it is possible that in the circulation of the rodent's *A. phagocytophilum* strains are engaged small rodents and shrews both. Larve and nymphs of *I. trianguliceps* are hosts specific and prefer shrews, whilst adult ticks prefer rodents [88]. Although it is no evidence of *A. phagocytophilum* in shrew in central Europe, Bown et al. [89] demonstrate the 18.7% infection rate in England.

On the other hand, in many part of central Europe *I. trianguliceps* is not such common, or their occurrence is restrained to specific habitats and thus absent in many localities [88,90].

The scheme of enzootic cycle of variant of *A. phagocytophilum* circulating among rodents and shrews is presented in Fig. 5.

References

[1] Dumler J.S., Barbet A.F., Bekker C.P.J., Dasch G.A., Palmer G.H., Ray S.C., Rikihisa Y., Rurangirwa F.R. 2001. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila. International Journal of Systematic and Evolutionary Microbiology* 51: 2145-2165.

[2] Demma L.J., Olman R.C., Mcquiston J.H., Krebs J.W., Swerdlow D.L. 2005. Epidemiology of human

ehrlichiosis and anaplasmosis in the United States, 2001-2002. *American Journal of Tropical Medicine and Hygiene* 73: 400-409.

[3] Woldehiwet Z. 2010. The natural history of *Anaplasma phagocytophilum*. *Veterinary Parasitology* 167: 108-122.

[4] Blaňarová L., Stanko M., Carpi G., Miklisová D., Víchová B., Mošanský L., Bona M., Derdáková M. 2014. Distinct *Anaplasma phagocytophilum* genotypes associated with *Ixodes trianguliceps* ticks and rodents in Central Europe. *Ticks and Tick-borne Diseases* 5: 928-938.

[5] Kawahara M., Rikihisa Y., Isogai E., Takahashi M., Misumi H., Suto C., Shibata S., Zhang C., Tsuji M. 2004. Ultrastructure and phylogenetic analysis of '*Candidatus* Neoehrlichia mikurensis' in the family Anaplasmataceae, isolated from wild rats and found in *Ixodes ovatus* ticks. *International Journal of Systematic and Evolutionary Microbiology* 54: 1837-1843.

[6] von Loewenich F.D., Geissdörfer W., Disqué C., Matten J., Schett G., Sakka S.G., Bogdan C. 2010. Detection of „*Candidatus* Neoehrlichia mikurensis" in two patients with severe febrile illnesses: evidence for a European sequence variant. *Journal of Clinical Microbiology* 48: 2630-2635.

[7] Li H., Jiang J.F., Liu W., Zheng Y.C., Huo Q.B., Tang K., Zuo S.Y., Liu K., Jiang B.G., Yang H., Cao W.C. 2012. Human infection with *Candidatus* Neoehrlichia mikurensis, China. *Emerging Infectious Diseases* 18: 1636-1639.

[8] Rar V., Golovljova I. 2011. *Anaplasma*, *Ehrlichia*, and "*Candidatus Neoehrlichia*" bacteria: pathogenicity, biodiversity, and molecular genetic characteristics, a review. *Infection, Genetics and Evolution* 11: 1842-1861.

[9] Silaghi C., Beck R., Oteo J.A., Pfeffer M., Sprong H. 2016. Neoehrlichiosis: an emerging tick-borne zoonosis caused by *Candidatus* Neoehrlichia mikurensis. *Experimental and Applied Acarology* 68: 279-297.

[10] Kowalec M., Welc-Falęciak R., Karbowiak G., Szewczyk T., Bajer A., Siński E. 2013. The coexistence of pathogenic microparasites *Babesia* spp. and '*Candidatus* Neoehrlichia mikurensis' in *Ixodes ricinus* ticks from forests under different levels of anthropopression. *Annals of Parasitology* 59 (suppl.): 175.

[11] Glatz M., Müllegger R.R., Maurer F., Fingerle V., Achermann Y., Wilske B., Bloemberg G.V. 2014. Detection of *Candidatus* Neoehrlichia mikurensis, *Borrelia burgdorferi* sensu lato genospecies and *Anaplasma phagocytophilum* in a tick population from Austria. *Ticks and Tick-borne Diseases* 5: 139-144.

[12] Welc-Falęciak R., Kowalec M., Karbowiak G., Bajer A., Behnke M.J., Siński E. 2014. Rickettsiaceae and Anaplasmataceae infections in *Ixodes ricinus* ticks from urban and natural forested areas of Poland. *Parasites and Vectors* 7: 121.

[13] Movila A., Alekseev A.N., Dubinina H.V., Toderas I. 2013. Detection of tick-borne pathogens in ticks from migratory birds in the Baltic region of Russia. *Medical and Veterinary Entomology* 27: 113-117.

[14] Naitou H., Kawaguchi D., Nishimura Y., Inayoshi M., Kawamori F., Masuzawa T., Hiroi M., Kurashige H., Kawabata H., Fujita H., Ohashi N. 2006. Molecular identification of *Ehrlichia* species and '*Candidatus* Neoehrlichia mikurensis' from ticks and wild rodents in Shizuoka and Nagano Prefectures, Japan. *Microbiology and Immunology* 50: 45-51.

[15] Jahfari S., Coipan E.C., Fonville M., van Leeuwen A.D., Hengeveld P., Heylen D., Heyman P., van Maanen C., Butler C.M., Földvári G., Szekeres S., van Duijvendijk G., Tack W., Rijks J.M., van der Giessen J., Takken W., van Wieren S.E., Takumi K., Sprong H. 2014. Circulation of four *Anaplasma phagocytophilum* ecotypes in Europe. *Parasites and Vectors* 15: 365.

[16] Venclíková K., Mendel J., Betášová L., Blažejová H., Jedličková P., Straková P., Hubálek Z., Rudolf I. 2016. Neglected tick-borne pathogens in the Czech Republic, 2011-2014. *Ticks and Tick-borne Diseases* 7: 107-112.

[17] Víchová B., Majláthová V., Nováková M., Stanko M., Hviščová I., Pangrácová L., Chrudimský T., Čurlík J., Peťko B. 2014. *Anaplasma* infections in ticks and reservoir host from Slovakia. *Infection, Genetics and Evolution* 22: 265-272.

[18] Burri C., Schumann O., Schumann C., Gern L. 2014. Are *Apodemus* spp. mice and *Myodes glareolus* reservoirs for *Borrelia miyamotoi*, *Candidatus* Neoehrlichia mikurensis, *Rickettsia helvetica*, *R. monacensis* and *Anaplasma phagocytophilum*? *Ticks and Tick-borne Diseases* 5: 245-251.

[19] Obiegala A., Pfeffer M., Pfister K., Tiedemann T., Thiel C., Balling A., Karnath C., Woll D., Silaghi C. 2014. *Candidatus* Neoehrlichia mikurensis and *Anaplasma phagocytophilum*: prevalences and investigations on a new transmission path in small mammals and ixodid ticks. *Parasites and Vectors* 4: 563.

[20] Beck R., Čubrić Čurik V., Račić I., Šprem N., Vujnović A. 2014. Identification of '*Candidatus* Neoehrlichia mikurensis ' and *Anaplasma* species in wildlife from Croatia. *Parasites and Vectors* 7 (suppl. 1): O28.

[21] Dugat T., Chastagner A., Lagrée A-C., Petit E., Durand B., Thierry S., Corbičre F., Verheyden H., Chabanne L., Bailly X., Leblond A., Vourc'h G., Boulouis H-J., Maillard R., Haddad N. 2014. A new multiple-locus variable-number tandem repeat analysis reveals different clusters for *Anaplasma phagocytophilum* circulating in domestic and wild

ruminants. *Parasites and Vectors* 7: 439.
[22] Łukaszewska J., Adaszek Ł., Winiarczyk S. 2008. Obraz krwi w przebiegu anaplazmozy granulocytarnej u psów i koni [Hematological changes in granulocytic anaplasmosis in dogs and horses]. *Życie Weterynaryjne* 83: 827-831.
[23] Adaszek Ł., Kotowicz W., Klimiuk P., Górna M., Winiarczyk S. 2011. Ostry przebieg anaplazmozy granulocytarnej u psa – przypadek własny [Severe granulocytic anaplasmosis in a dog – a case study]. *Weterynaria w praktyce* 8: 59-62.
[24] Adaszek Ł., Policht K., Górna M., Kutrzuba J., Winiarczyk S. 2011. Pierwszy w Polsce przypadek anaplazmozy (erlichiozy) granulocytarnej u kota [The first case of anaplasmosis (ehrlichiosis) in cat in Poland]. *Życie Weterynaryjne* 86: 132-134.
[25] Stuen S., Granquist E.G., Silaghi C. 2013. *Anaplasma phagocytophilum* – a widespread multihost pathogen with highly adaptive strategies. *Frontiers in Cellular and Infection Microbiology* 3: 31.
[26] Čabanová V., Pantchev N., Hurníková Z., Miterpáková M. 2015. Recent study on canine vector-borne zoonoses in southern Slovakia – serologic survey. *Acta Parasitologica* 60: 749-758.
[27] Slivinska K., Víchová B., Werszko J., Szewczyk T., Wróblewski Z., Peťko B., Ragač O., Demeshkant V., Karbowiak G. 2016. Molecular surveillance of *Theileria equi* and *Anaplasma phagocytophilum* infections in horses in some regions of Ukraine, Poland and Slovakia: preliminary study. *Veterinary Parasitology* 215: 35-37.
[28] Chen S-M., Dumler J.S., Bakken J.S., Walker D.H. 1994. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *Journal of Clinical Microbiology* 32: 589-595.
[29] Lotrič-Furlan S., Avsic-Zupanc T., Petrovec M., Nicholson W.L., Sumner J.W., Childs J.E., Strle F. 2001. Clinical and serological follow-up of patients with human granulocytic ehrlichiosis in Slovenia. *Clinical and Diagnostic Laboratory Immunology* 8: 899-903.
[30] Parola Ph. 2004. Tick-borne rickettsial diseases. *Comparative Imunology, Microbiology and Infectious Diseases* 27: 297-304.
[31] Grzeszczuk A., Stańczak J., Kubica-Biernat B., Racewicz M., Kruminis-Łozowska W., Prokopowicz D. 2004. Human anaplasmosis in north-eastern Poland: seroprevalence in humans and prevalence in *Ixodes ricinus* ticks. *Annals of Agricultural and Environmental Medicine* 11: 99-103.
[32] Grzeszczuk A. 2006. *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks and human granulocytic anaplasmosis seroprevalence among forestry rangers in Białystok region. *Advances in Medical Sciences* 51: 283-286.
[33] Socolovschi C., Mediannikov O., Raoult D., Parola P. 2009. Update on tick-borne bacterial diseases in Europe. *Parasite* 16: 259-273.
[34] Hartelt K., Oehme R., Frank H., Brockmann S.O., Hassler D., Kimmig P. 2004. Pathogens and symbionts in ticks: prevalence of *Anaplasma phagocytophilum* (*Ehrlichia* sp.), *Wolbachia* sp., *Rickettsia* sp., and *Babesia* sp. in Southern Germany. *International Journal of Medical Microbiology* 293 (suppl. 37): 86-92.
[35] Asman M., Nowak M., Cuber P., Strzelczyk J., Szilman E., Szilman P., Trapp G., Siuda K., Solarz K., Wiczkowski A. 2013. The risk of exposure to *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato, *Babesia* sp. and co-infections in *Ixodes ricinus* ticks on the territory of Niepolomice Forest (southern Poland). *Annals of Parasitology* 59: 13-19.
[36] Grzeszczuk A., Ziarko S., Prokopowicz D., Radziwon P. M. 2004. Zakażenie żubrów z Puszczy Białowieskiej bakteriami *Anaplasma phagocytophilum* [Evidence of *Anaplasma phagocytophilum* infection of European bisons in the Białowieża Primewal Forest, Poland]. *Medycyna Weterynaryjna* 60: 600-601.
[37] Chmielewska-Badora J., Zwoliński J., Cisak E., Wójcik-Fatla A., Buczek A., Dutkiewicz J. 2007. Prevalence of *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks determined by polymerase chain reaction with two pairs of primers detecting 16S rRNA and *ankA* genes. *Annals of Agricultural and Environmental Medicine* 14: 281-285.
[38] Sytykiewicz H., Karbowiak G., Hapunik J., Szpechciński A., Supergan-Marwicz M., Goławska S., Sprawka I., Czerniewicz P. 2012. Molecular evidence of *Anaplasma phagocytophilum* and *Babesia microti* co-infections in *Ixodes ricinus* ticks in central-eastern region of Poland. *Annals of Agricultural and Environmental Medicine* 19: 45-49.
[39] Bown K.J., Lambin X., Ogden N.H., Begon M., Telford G., Woldehiwet Z., Birtles R.J. 2009. Delineating *Anaplasma phagocytophilum* ecotypes in coexisting, discrete enzootic cycles. *Emerging Infectious Diseases* 15: 1948-1954.
[40] Duh D., Slovák M., Saksida A., Strašek K., Petrovec M., Avšič-Županc T. 2006. Molecular detection of *Babesia canis* in *Dermacentor reticulatus* ticks collected in Slovakia. *Biologia* 61: 231-233.
[41] Wirtgen M., Nahayo A., Linden A., Garigliany M., Desmechtl D. 2011. Detection of *Anaplasma phagocytophilum* in *Dermacentor reticulatus* ticks. *Veterinary Record* 168: 195.
[42] Karbowiak G., Víchová B., Slivinska K., Werszko J., Didyk J., Peťko B., Stanko M., Akimov I. 2014. The infection of questing *Dermacentor reticulatus* ticks with *Babesia canis* and *Anaplasma phagocytophilum* in the Chernobyl exclusion zone. *Veterinary Parasitology* 204: 372-375.
[43] Paulauskas A., Radzijevskaja J., Rosef O. 2012.

Molecular detection and characterization of *Anaplasma phagocytophilum* strains. *Comparative Immunology Microbiology and Infectious Diseases* 35: 187-195.

[44] Sixl W., Petrovec M., Marth E., Wüst G., Stünzner D., Schweiger R., Avšič-Županc T. 2003. Investigation of *Anaplasma phagocytophila* infections in *Ixodes ricinus* and *Dermacentor reticulatus* ticks in Austria. *Annals of the New York Academy of Sciences* 990: 94-97.

[45] Hulínská D., Langrová K., Pejčoch M., Pavlásek I. 2004. Detection of *Anaplasma phagocytophilum* in animals by real-time polymerase chain reaction. *APMIS* 112: 239-247.

[46] Stańczak J., Gabre R.M., Kruminis-Łozowska W., Racewicz M., Kubica-Biernat B. 2004. *Ixodes ricinus* as a vector of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia microti* in urban and suburban forests. *Annals of Agricultural and Environmental Medicine* 11: 109-114.

[47] Silaghi C., Gilles J., Höhle M., Fingerle V., Just F.T., Pfister K. 2008. *Anaplasma phagocytophilum* infection in *Ixodes ricinus*, Bavaria, Germany. *Emerging Infectious Diseases* 14: 972-974.

[48] Overzier E., Pfister K., Herb I., Mahling M., Böck G. Jr., Silaghi C. 2013. Detection of tick-borne pathogens in roe deer (*Capreolus capreolus*), in questing ticks (*Ixodes ricinus*), and in ticks infesting roe deer in southern Germany. *Ticks and Tick-borne Diseases* 4: 320-328.

[49] Siuda K. 1993. Ticks of Poland (Acari: Ixodida). Polish Parasitological Society, Warszawa, Poland. (in Polish)

[50] Alberdi M.P., Walker A.R., Urquhart K.A. 2000. Field evidence that roe deer (*Capreolus capreolus*) are a natural host for *Ehrlichia phagocytophila*. *Epidemiology and Infection* 124: 315-323.

[51] Adamska M. 2006. DNA *Anaplasma phagocytophilum* we krwi saren oraz w pozyskanych z nich klesz - czach [Detecting *Anaplasma phagocytophilum* DNA in blood of roe deer and in ticks]. *Medycyna Wetery - naryjna* 62: 201-203.

[52] Zeman P., Pecha M. 2008. Segregation of genetic variants of *Anaplasma phagocytophilum* circulating among wild ruminants within a Bohemian forest (Czech Republic). *International Journal of Medical Microbiology* 298 (suppl. 1): 203-210.

[53] Skotarczak B., Adamska M., Sawczuk M., Maciejewska A., Wodecka B., Rymaszewska A. 2008. Coexistence of tick-borne pathogens in game animals and ticks in western Poland. *Veterinarni Medicina* 53: 668-675.

[54] Stefanidesova K., Kocianova E., Boldis V., Kostanova Z., Kanka P., Nemethova D., Spitalska E. 2008. Evidence of *Anaplasma phagocytophilum* and *Rickettsia helvetica* infection in free-ranging ungulates in central Slovakia. *European Journal of Wildlife Research* 54: 519-524.

[55] Hapunik J., Víchová B., Karbowiak G., Wita I., Bogdaszewski M., Peťko B. 2011. Wild and farm breeding cervids infections with *Anaplasma phagocytophilum*. *Annals of Agricultural and Environmental Medicine* 18: 73-77.

[56] Welc-Falęciak R., Werszko J., Cydzik K., Bajer A., Michalik J., Behnke J.M. 2013. Co-infection and genetic diversity of tick-borne pathogens in roe deer from Poland. *Vector Borne and Zoonotic Diseases* 13: 277-288.

[57] Michalik J., Stańczak J., Cieniuch S., Racewicz M., Sikora B., Dabert M. 2012. Wild boars as hosts of human-pathogenic *Anaplasma phagocytophilum* variants. *Emerging Infectious Diseases* 18: 998-1001.

[58] Karbowiak G., Víchová B., Werszko J., Demiaszkiewicz A.W., Pyziel A.M., Sytykiewicz H., Szewczyk T., Peťko B. 2015. The infection of reintroduced ruminants – *Bison bonasus* and *Alces alces* – with *Anaplasma phagocytophilum* in northern Poland. *Acta Parasitologica* 60: 645-648.

[59] Karbowiak G., Víchová B., Majláthová V., Hapunik J., Peťko B. 2009. *Anaplasma phagocytophilum* infection of red foxes (*Vulpes vulpes*). *Annals of Agricultural and Environmental Medicine* 16: 299-300.

[60] Härtwig V., von Loewenich F.D., Schulze C., Straubinger R.K., Daugschies A., Dyachenko V. 2014. Detection of *Anaplasma phagocytophilum* in red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) from Brandenburg, Germany. *Ticks and Tick-borne Diseases* 5: 277-280.

[61] Grzeszczuk A., Karbowiak G., Ziarko S., Kovalchuk O. 2006. The toot-vole *Microtus oeconomus* (Pallas, 1776): a new potential reservoir of *Anaplasma phagocytophilum*. *Vector-Borne and Zoonotic Diseases* 6: 240-243.

[62] Kallio E.R., Begon M., Birtles R.J., Bown K.J., Koskela E., Mappes T., Watts P.C. 2014. First report of *Anaplasma phagocytophilum* and *Babesia microti* in rodents in Finland. *Vector borne and Zoonotic Diseases* 14: 389-393.

[63] Svitálková Z., Haruštiaková D., Mahríková L., Berthová L, Slovák M., Kocianová E., Kazimírová M. 2015. *Anaplasma phagocytophilum* prevalence in ticks and rodents in an urban and natural habitat in South-Western Slovakia. *Parasites and Vectors* 8: 276.

[64] Huhn C., Winter C., Wolfsperger T., Wüppenhorst N., Strašek Smrdel K., Skuballa J., Pfäffle M., Petney T., Silaghi C., Dyachenko V., Pantchev N., Straubinger R.K., Schaarschmidt-Kiener D., Ganter M., Aardema M.L., von Loewenich F.D. 2014. Analysis of the population structure of *Anaplasma phagocytophilum* using multilocus sequence typing. *PLoS One* 9: e93725.

[65] Massung R.F., Priestley R.A., Miller N.J., Mather

T.N., Levin M.L. 2003. Inability of a variant strain of *Anaplasma phagocytophilum* to infect mice. *Journal of Infectious Diseases* 188: 1757-1763.

[66] Rymaszewska A. 2008. Divergence within the marker region of the *groESL* operon in *Anaplasma phagocytophilum*. *European Journal of Clinical Microbiology and Infectious Diseases* 27: 1025-1036.

[67] Rymaszewska A. 2011. PCR for detection of tick-borne *Anaplasma phagocytophilum* pathogens: a review. *Veterinarni Medicina* 56: 529-536.

[68] Massung R.F., Lee K., Mauel M., Gusa A. 2002. Characterization of the rRNA genes of *Ehrlichia chaffeensis* and *Anaplasma phagocytophila*. *DNA and Cell Biology* 21: 587-596.

[69] Stańczak J., Cieniuch S., Lass A., Biernat B., Racewicz M. 2015. Detection and quantification of *Anaplasma phagocytophilum* and *Babesia* spp. in *Ixodes ricinus* ticks from urban and rural environment, nothern Poland, by real-time polymerase chain reaction. *Experimental and Applied Acarology* 66: 63-81.

[70] Strašek Smrdel K., von Loewenich F.D., Petrovec M., Avšič Županc T. 2015. Diversity of *ankA* and *msp*4 genes of *Anaplasma phagocytophilum* in Slovenia. *Ticks and Tick-borne Diseases* 6: 164-166.

[71] Petrovec M., Bidovec A., Sumner J.W., Nicholson W.L., Childs J.E., Avšič-Županc T. 2002. Infection with *Anaplasma phagocytophila* in cervids from Slovenia: evidence of two genotypic lineages. *Wiener Klinische Wochenschrift* 114/113: 641-647.

[72] Sumner J.W., Nicholson W.L., Massung R.F. 1997. PCR amplifcation and comparison of nucleotide sequences from the groESL heat shock operon of *Ehrlichia* species. *Journal of Clinical Microbiology* 35: 2087-2092.

[73] Petrovec M., Sixl W., Schweiger R., Mikulasek S., Elke L., Wüst G., Marth E., Strasek K., Stünzner D., Avsic-Zupanc T. 2003. Infections of wild animals with *Anaplasma phagocytophila* in Austria and the Czech Republic. *Annals of the New York Academy of Sciences* 990: 103-106.

[74] Shukla S.K., Aswani V., Stockwell P.J., Reed K.D. 2007. Contribution of polymorphisms in ankA, gltA, and groESL in defining genetic variants of *Anaplasma phagocytophilum*. *Journal of Clinical Microbiology* 45: 2312-2315.

[75] Katargina O., Geller J., Alekseev A., Dubinina H., Efremova G., Mishaeva N., Vasilenko V., Kuznetsova T., Järvekülg L., Vene S., Lundkvist A., Golovljova I. 2012. Identification of *Anaplasma phagocytophilum* in tick populations in Estonia, the European part of Russia and Belarus. *Journal of Clinical Microbiology and Infections* 18: 40-46.

[76] Stuen S., Moum T., Petrovec M., Schouls L.M. 2006. Genetic variants of *Anaplasma phagocytophilum* in Norway. *International Journal of Medical Microbiology* 296 (suppl. 1): 164-166.

[77] Strasek Smrdel K., Bidovec A., Malovrh T., Petrovec M., Duh D., Avsic Zupanc T. 2009. Detection of *Anaplasma phagocytophilum* in wild boar in Slovenia. *Clinical Microbiology and Infection* 15 (suppl. 2): 50-52.

[78] Dumler J.S., Choi K.S., Garcia-Garcia J.C., Barat N.S., Scorpio D.G., Garyu J.W., Grab D.J., Bakken J.S. 2005. Human granulocytic anaplasmosis and *Anaplasma phagocytophilum*. *Emerging Infectious Diseases* 11: 1828-1834.

[79] de la Fuente J., Massung R.F., Wong S.J., Chu F.K., Lutz H., Meli M., von Loewenich F.D., Grzeszczuk A., Torina A., Caracappa S., Mangold A.J., Naranjo V., Stuen S., Kocan K.M. 2005. Sequence analysis of the *msp4* gene of *Anaplasma phagocytophilum* strains. *Journal of Clinical Microbiology* 43: 1309-1017.

[80] Scharf W., Schauer S., Freyburger F., Petrovec M., Schaarschmidt-Kiener D., Liebisch G., Runge M., Ganter M., Kehl A., Dumler J.S., Garcia-Perez A.L., Jensen J., Fingerle V., Meli M.L., Ensser A., Stuen S., von Loewenich F.D. 2011. Distinct host species correlate with *Anaplasma phagocytophilum ankA* gene clusters. *Journal of Clinical Microbiology* 49: 790-796.

[81] Michalik J., Stańczak J., Racewicz M., Cieniuch S., Sikora B., Szubert-Kruszyńska A., Grochowalska R. 2009. Molecular evidence of *Anaplasma phagocytophilum* infection in wild cervids and feeding *Ixodes ricinus* ticks from west-central Poland. *Clinical Microbiology and Infection* 15: 81-83.

[82] Majazki J., Wüppenhorst N., Hartelt K., Birtles R., von Loewenich F.D. 2013. *Anaplasma phagocyto - philum* strains from voles and shrews exhibit specific ankA gene sequences. *BMC Veterinary Research* 9: 235.

[83] Nicholas K.B., Nicholas Jr H.B., Deerfield D.W. 1997. GeneDoc: analysis and visualization of genetic variation. *Embnew. News* 4: 144.

[84] Altschul S.F., Gish W., Miller W., Myers E.W., Lipman D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403-410.

[85] Swofford D.L., Waddell P.J., Huelsenbeck J.P., Foster P.G., Lewis P.O., Rogers J.S. 2001. Bias in phylogenetic estimation and its relevance to the choice between parsimony and likelihood methods. *Systematic Biology* 50: 525-539.

[86] Liz J.S., Anderes L., Sumner J.W., Massung R.F., Gern L., Rutti B., Brossard M. 2000. PCR detection of granulocytic ehrlichiae in *Ixodes ricinus* ticks and wild small mammals in western Switzerland. *Journal of Clinical Microbiology* 38: 1002-1007.

[87] Bown K.J., Begon M., Bennett M., Birtles R. J., Burthe S., Lambin X., Telfer S., Woldehiwet Z., Ogden N.H. 2006. Sympatric *Ixodes trianguliceps* and *Ixodes ricinus* ticks feeding on field voles (*Microtus agrestis*): Potential for increased risk of

Anaplasma phagocytophilum in the United Kingdom? *Vector-Borne and Zoonotic Diseases* 6: 404-410.

[88] Stanko M., Krasnov B.R., Miklisova D., Morand S. 2007. Simple epidemiological model predicts the relationships between prevalence and abundance in ixodid ticks. *Parasitology* 134: 59-68.

[89] Bown K.J., Lambin X., Telford G., Heyder-Bruckner D., Ogden N.H., Birtles R.J. 2011. The common shrew (*Sorex araneus*): a neglected host of tick-borne infections? *Vector Borne and Zoonotic Diseases* 11: 947-953.

[90] Nowak-Chmura M., Siuda K. 2012. Ticks of Poland: review of contemporary issues and latest research. *Annals of Parasitology* 58: 125-155.

[91] Bown K.J., Begon M., Bennett M., Woldehiwet Z., Ogden N.H. 2003. Seasonal dynamics of *Anaplasma phagocytophila* in a rodent-tick (*Ixodes trianguliceps*) system, United Kingdom. *Emerging Infectious Diseases* 9: 63-70.

[92] Gajda E., Woźniak M., Buńkowska-Gawlik K., Perec-Matysiak A., Hildebrand J., Kváč M. 2013. *Anaplasma phagocytophilum* in wild rodent populations from areas of Lower Silesia, Poland. *Annals of Parasitology* 59 (suppl.): 170.

[93] Skuballa J., Petney T., Pfäffle M., Taraschewski H. 2010. Molecular detection of *Anaplasma phagocyto - philum* in the european hedgehog (*Erinaceus europaeus*) and its ticks. *Vector-Borne and Zoonotic Diseases* 10: 1055-1057.

Received 10 July 2016
Accepted 23 September 2016