ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2011, 18, 73–77

WILD AND FARM BREEDING CERVIDS INFECTIONS WITH *ANAPLASMA PHAGOCYTOPHILUM*

Joanna Hapunik[1], Bronislava Víchová[2], Grzegorz Karbowiak[1], Irena Wita[1], Marek Bogdaszewski[1], Branislav Peťko[2, 3]

[1] W. Stefański Institute of Parasitology of the Polish Academy of Sciences, Warsaw, Poland
[2] Parasitological Institute of the Slovak Academy of Sciences, Košice, Slovakia
[3] Faculty of Natural Sciences Comenius University, Bratislava, Slovakia

Hapunik J, Víchová B, Karbowiak G, Wita I, Bogdaszewski M, Peťko B: Wild and farm breeding cervids infections with *Anaplasma phagocytophilum*. *Ann Agric Environ Med* 2011, **18**, 73–77.

Abstract: The main goal of our study was to determine the prevalence of *Anaplasma phagocytophilum* infections in wild cervids living in north-eastern part of Poland. Material used in the study was gathered between the years 2004–2008. The blood samples from 106 red deer (*Cervus elaphus*), 32 sika deer (*Cervus nippon hortulorum*), 130 fallow deer (*Dama dama*) and 31 roe deer (*Capreolus capreolus*) were collected. DNA was isolated using Genomic Mini AX blood kit (A&A Biotechnology). Molecular detection of *A. phagocytophilum* was based on nested PCR amplification of a species-specific 16S rRNA fragment gene of *A. phagocytophilum*. The highest prevalence of infection was detected in *Cervus elaphus*, *Capreolus capreolus*, *Cervus nippon hortulorum*, there were 50.9%, 38.7%, 34.37% of infected animals, respectively. The lowest rate of infection was found in fallow deer (*Dama dama*) – only 1.5%.

Address for correspondence: Joanna Hapunik, W. Stefański Institute of Parasitology of the Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw, Poland.
E-mail: asiahapunik@wp.pl

Key words: ***Anaplasma phagocytophilum*, cervidae.**

INTRODUCTION

The north-eastern part of Poland is considered a preference area of high risk of tick-borne diseases infection [38]. Especially in recent years there has been an observed increase cases of infection in humans as well as animals, caused especially by Lyme borreliosis, TBE and HGE agents [10, 11, 34, 47, 50]. The most important reason for the high risk of infection is exposure to ticks attack. Moreover, the increase in ticks population, and the spreading of ticks to new geographic areas has been observed [17]. There are two main factors significantly favourable for tick's expansion. The most important one is global warming, the signs symptoms of which are increases in average temperatures in winter months, along with shorter winter periods [5, 15]. Another factor is the growth of the deer population as well as small mammals which are the ticks' hosts.

The problem of increasing incidence of Lyme borreliosis and TBE sick rate is commonly known and studied. However, human granulocytic anaplasmosis is a fairly new issue. It has been studied in Poland only in the last decade [21, 41, 50]. The etiological agent of HGE is *Anaplasma phagocytophilum*. This is a Gram(-) bacteria belonging to Anaplasmataceae family. It is an obligatory parasite which grows and replicates in the membrane-bound vacuoles in the cells of vertebrate and invertebrate host [14]. Neutrophils are the cells mainly infected.

A. phagocytophilum was discovered for the first time in European ruminants in 1932 and described as *Cytoecetes phagocytophila* [18]. For over 70 years it was considered as a pathogen of domestic and free living ruminants in

Received: 10 January 2011
Accepted: 19 April 2011

Europe [48]. However, as a result of phylogenetic analysis of 16S rRNA gene and operons groESL, significant changes were made in the taxonomy of Anaplasmataceae. *Ehrlichia equi*, *Ehrlichia phagocytophila* and HGE agents have been unified into a single species and classified as the *A. phagocytophilum* [14]. Human infections with *A. phagocytophilum* were detected between the years 1990–1994 in the USA for the first time, among inhabitants of the states of Wisconsin and Minnesota [9]. In Poland, the first case of HGE infections was diagnosed by PCR analysis in 2001 [45].

In the environment, species from Cervidae family serves the animal reservoir for *A. phagocytophilum*. In Europe these species are: roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), elk (*Alces alces*) [1, 6, 12, 36, 44, 48]. Moreover, infections with *A. phagocytophilum* were also detected in chamois (*Rupicapra rupicapra*), mouflon (*Ovis musimon*) boars (*Sus scrofa*) [29, 42]. In Poland, *A. phagocytophilum* was also detected in the blood of the European bison (*Bison bonasus*) free living in Białowieża Primeval Forest [22] and red foxes (*Vulpes vulpes*) in Mazovia Province [24].

Apart from the above, many small mammals such as: wood mouse (*Apodemus sylvaticus*), yellow–necked mouse (*Apodemus flavicollis*), field vole (*Microtus agrestis*), bank vole (*Myodes glareolus*), and common shrew (*Sorex araneus*), participate in the circulation of *A. phagocytophilum* in natural zoonotic foci. Among domestic animals and pets, *A. phagocytophilum* infections have been detected in cattle, goats and sheep [7, 20, 29, 35, 40].

The presence of two distinct genetic variants of *A. phagocytophilum* has been described by Massung *et al.* [30]. There is the Ap-ha variant, which is pathogenic for humans, dogs and horses, and the non-pathogenic variant Ap-V1. In North America, the main reservoir for Ap-ha variant, is the white-footed mouse *Peromyscus leucopus*. For the non-pathogenic type Ap-V1, the white-tailed deer (*Odocileus virginianus*) is considered to be a competent animal reservoir [30, 33].

Research performed by Massung *et al.* [30] indicate, that *Odocoileus virginianus* is considered to be an animal reservoir for the non-pathogenic type Ap-V1. Michalik *et al.* [32] suggest that analysis of sequences from fallow deer and red deer are similar to sequences of *A. phagocytophilum* pathogenic for humans in North America in 99.7 –100.0%. The role of Cervidae as a reservoir of the pathogenic type for HGE agent cannot be unambiguously determined. Unique genetic mechanism which leads to production of a wide range of surface proteins and distinctive pleomorphism is recognized within *A. phagocytophilum* [4, 26]. Distinct genetic variants of *A. phagocytophilum* may exist within the same populations and even simultaneously in the same animals. These variants may behave differently and interact in the mammalian hosts [43]. Diverse genetic variants of *A. phagocytophilum* have been observed among wild ruminants, in North America [30], Slovenia [36], Italy [8] and the Czech Republic [49].

The high prevalence of *A. phagocytophilum* natural infection of Cervidae indicates that this group of animals plays a significant role as an animal reservoir of HGE agent in Europe [29, 36, 37]. So far, serologic and molecular studies of natural infection with *A. phagocytophilum* have shown the especially high prevalence of infections of deer species in North America [6, 16, 27, 30, 46], Japan [25] Spain [12], Italy [3], Holland [19], Switzerland [28, 29], Austria (Po *et al.* 2004), Slovakia [7], Czech Republic [49], Great Britain [2] and Norway [44]. In Poland, the researches on *A. phagocytophilum* infections in wild mammals were conducted in north-western and eastern parts of the country [1, 32]. The infections were detected in red deer [32], roe deer [1], European bison and root vole [20, 21].

The main goal of our studies was to determine the presence of *A. phagocytophilum* DNA in the blood of animals from the Cervidae family living in the northeast of Poland, and establish their role as a HGE agent animal reservoir. The studies can be very helpful in defining the rate of risk of infection among humans living in the north-eastern part of Poland.

MATERIALS AND METHODS

Study sites. The studies were conducted in Białowieża Primeval Forest (N52°29'–52°57', E23°31'–24°21'), and in two forest divisions in the Mazurian Lakeland – Mikołajki (N53°48', E21°34'), and Strzałowo (N53°46', E21°27'). The blood samples of wild living cervids originatings from legally hunted animals, and additionally, blood samples were collected from animals bred in the Deer Farm of the Institute of Parasitology of the Polish Academy of Sciences in Kosewo Górne (N53°49', E21°23'). Material used in the study was gathered between 2004–2008. Blood specimens were collected into sterile, EDTA containing microtubes and stored until DNA isolation at +4ºC. Blood was collected from *Capreolus capreolus* (11♀ from Białowieża, 1♂ from Mikołajki, 17♂ from Strzałowo, 2♂ from Kosewo Górne), *Cervus elaphus* (13♀; 16♂ from Mikołajki, 10♀; 9♂ from Strzałowo, 25♀, 33♂ from Kosewo Górne), *Cervus nippon hortulorum* and *Dama dama* from Kosewo Górne, (19♀, 13♂ and 70♀; 60♂ respectively). To sum up, the blood samples from 106 red deer, 32 sika deer, 130 fallow deer and 31 roe deer were collected.

DNA extraction. DNA was isolated from 200 µl of each sample using a Genomic Mini AX blood kit (A&A Biotechnology, Gdynia) following the manufacturers protocol. DNA was eluted into a total volume of 100 µl of nuclease free water. Isolated DNA samples were stored at +4°C until further processing.

PCR diagnosis for *A. phagocytophilum*. To verify whether genomic DNA had been isolated successfully from each tissue sample, all the DNA templates were screened for the presence of bacterial DNA. Molecular detection of

A. phagocytophilum was based on nested PCR amplification of 16S rRNA species-specific gene fragment. Primers and PCR conditions were used as published previously (ge3a 5'CACATGCAAGTCGAACGGATTATTC3' and ge10r 5'TTCCGTTAAGAAGGATCTAATCTCC3', following ge9f 5'AACGGATTATTCTTTATAGCTTGCT3' and ge2 5'GGCAGTATTAAAAGCAGCTCCAGG3') [23, 31]. In the first reaction, 932 long fragments were amplified, and in the second round, the portion with a length of approximately 546 bp was obtained.

PCR reaction was performed in a total volume of 25 µl reaction mixture, contained 2 × DyNazymeII Master Mix PCR (Finzymes). As a positive control, the sequenced DNA of *A. phagocytophilum* was used. In the negative control, nuclease-free water was added to the PCR mix instead of the tested DNA. The final phase of PCR reaction included cooling of the samples to +4°C.

PCR products were visualized by electrophoresis on 1% agarose gel stained with GoldView Nucleic Acid Stain (Bejing SBS Genetech Co. Ltd.). The size of amplified fragments of an assumed 546 bp size was compared to a known DNA marker.

DNA Sequencing of PCR products. The sequencing reaction was performed at the Department of Molecular Biology (Faculty of Natural Science, Comenius University, Bratislava, Slovak Republic). The complementary strands of each sequenced products were manually assembled. Sequences were compared with GenBank entries by Blast N2.2.13. Sequence similarity among the sequences were calculated by EMBOSS Align, a pairwise alignment algorithm (http:www.ebi.ac.uk emboss align).

RESULTS

Prevalence of infection in cervides. *A. phagocytophilum* DNA was detected in the specimens of every deer species studied. The highest prevalence was reported in red deer (*Cervus elaphus*) – 50.9% (54 infected out of 106 investigated), roe deer (*Capreolus capreolus*) – 38.7% (12 out of 31) and sika deer (*Cervus nippon hortulorum*) – 34.4% (11 out of 32). The lowest rate of infection was detected in fallow deer (*Dama dama*). Out of the 130 tested animals, only 2 (1.5%) have had pathogen. The infection rate of farmed red deer was lower than those free living (Tab. 1).

Results of DNA sequencing nested PCR products. Sequence analysis of the 16S gene showed that the sequences obtained in this study shared 99% homology to *Anaplasma phagocytophilum*. The sequences were sent to the GenBank database, accesion numbers originally obtained in this study area: *Dama dama* (GQ450276), *Capreolus capreolus* (GQ450277), *Cervus elaphus* (GQ 450278).

DISCUSSION

Our results confirm the quite high prevalence of infection of wild ruminants with *Anaplasma phagocytophilum* in north-eastern Poland. The percentage of infected animals is variable, in comparison with results obtained in other parts of Poland, as well as in different countries.

The prevalence of infection of red deer *C. elaphus* in the Mazurian Lakeland (44.81 up to 58.62%) is higher than the prevalence in red deer recorded by Michalik *et al.* [32] in north-western Poland, where the overall prevalence was 10.2%. Moreover, the data from other countries is also different from Poland. In the Czech Republic and Slovenia the prevalence is higher than in the Mazurian Lakeland (86.0% and 87.5%, respectively) [36, 49]. In Slovakia and Austria, the percentage of infected red deer is relatively low (53.1% and 28.6%, respectively) [37]. It is interesting that the infection of farmed red deer was 10% lower than those free living. This may be caused by regular (twice a year) injections with ivermectin (Vetamectin) in order to remove helminths.

The same rule applies to the prevalence of infection of roe deer (*C. capreolus*) with *A. phagocytophilum*. In our study, the percentage of infected roe deer was 50.0–52.9%. According to the data given for north-western Poland by Adamska and Skotarczak [1], the roe deer is infected in 31.9%. In Slovenia and Slovakia, the prevalence of infection of roe deer is higher or similar (85.6% and 50.0%, respectively) [36, 42]. In Austria, Switzerland and Spain the prevalence is lower (43.0%, 18.4% and 18.0%, respectively) [13, 29, 41].

Michalik *et al.* [32] correlate the prevalence of infection of wild living animals from north-western Poland with the prevalence of *A. phagocytophilum* infection in female ticks *I. ricinus*. Their mean prevalence in north-western Poland is 22.7% (range 20–23.7%). Moreover, it is possible to extend this rule to the rest of the country, as well as to other regions of Europe. The higher prevalence of *A. phagocytophilum*

Table 1. Number of investigated animals and prevalence of infection with *Anaplasma phagocytophilum* in the studied areas.

Hosts	Prevalence (in %) of infected deer in the followed locality				
	Mikołajki	Strzałowo	Kosewo Górne	Białowieża	Total
C. elaphus	58.6% (17/29)	57.9% (11/19)	44.8% (26/58)	–	50.9% (54/106)
C. capreolus	0%* (0/1)	52.9% (9/17)	50%* (1/2)	18.18% (2/11)	38.7% (12/31)
C. nippon hortulorum		–	34.4% (11/32)	–	34.4% (11/32)
D. dama		–	1.5% (2/130)	–	1.5 % (2/130)

* – statistically insignificant

infections in north-eastern Poland correlates with the higher abundance of ticks [39]. The supposition confirms the high rate of European bison (*Bison bonasus*) infection which reaches 62.5% in Białowieża Primeval Forest [22].

The prevalence of *A. phagocytophilum* infection in fallow deer (*D. dama*) and sika deer (*C. nippon hortulorum*) – 1.5% and 34.4%, respectively – is relatively low. However, the animals were bred on farm; consequently, their exposition to ticks attacks is lower than free living animals. This may be the reason that the percentage of infected fallow deer in north-western Poland reaches up to 20.5% [32].

North-eastern Poland is considered an endemic region for many tick-borne zoonoses. For many years, this region has been the endemic area of TBE, borreliosis and anaplasmosis [38]. Wild living ruminants, being infected in more than 50.0% with *A. phagocytophilum*, are the important components in the structure of the HGE zoonotic foci in the natural environment.

Acknowledgement

This work was supported by the MNISW project N308 017 31/1488, VEGA 2/6163/26, No. APVV-51-009205, APVV 0108-06 and SAIA National Scholarship Programme.

REFERENCES

1. Adamska M, Skotarczak B: Wild game as a reservoir of *Anaplasma phagocytophilum* in north-western Poland. *Wiad Parazytol* 2007, **53**, 103–107.

2. Alberdi MP, Walker AR, Urquhart KA: Field evidence that roe deer (*Capreolus capreolus*) are a natural host for *Ehrlichia phagocytophila*. *Epidemiol Infect* 2000, **124**, 315–323.

3. Alberti A, Zobba R, Chessa B, Addis MF, Sparagano O, Pinna Parpaglia ML, Cubeddu T, Pintori G, Pittau M: Equine and canine *Anaplasma phagocytophilum* strains isolated on the island of Sardinia (Italy) are phylogenetically related to pathogenic strains from the United States. *Appl Environ Microbiol* 2005, **71**, 6418–6422.

4. Barbet AF, Lundgren AM, Alleman AR, Stuen S, Björsdorff A, Brown RN, Drazenovich NL, Foley JE: Structure of the expression site reveals global diversity in MSP2 (P44) variants in *Anaplasma phagocytophilum*. *Infect Immun* 2006, **74**, 6429–6437.

5. Bednorz E: Changes in snow cover occurrence in northern Germany in winters 1950/51–1999/00. **In:** Piotrowicz K, Twardosz R (Eds): *Wahania klimatu w różnych skalach przestrzennych i czasowych*, 215–223, IGiGP UJ, Kraków 2007.

6. Belongia EA, Reed KD, Mitchell PD, Mitchell PD, Kolbert CP, Persing DH, Gill JS, Kazmierczak JJ: Prevalence of granulocytic *Ehrlichia* infection among white-tailed deer in Wisconsin. *J Clin Microbiol* 1997, **35**, 1465–1468.

7. Bray DP, Bown KJ, Stockley P, Hurst JL, Benett M: Haemoparasites of common shrews (*Sorex araneus*) in Northwest England. *Parasitology* 2007, **134**, 819–826.

8. Carpi G, Bertolotti L, Pecchioli E, Cagnacci F, Rizzoli A: *Anaplasma phagocytophilum* groEL gene heterogenity in *Ixodes ricinus* larvae feeding on roe deer in Northeastern Italy. *Vector Borne Zoonotic Dis* 2000, **9**, 179–184.

9. Chen SM, JS, Bakken JS, Walker DH: Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *J Clin Microbiol* 1994, **32**, 589–595.

10. Czarkowski MP, Cielebąk E, Stępień E, Kondej B: *Choroby zakaźne i zatrucia w Polsce w 2000 roku*. PZH & GIS, Warszawa 2001.

11. Czarkowski MP, Cielebąk E, Dacka P, Kondej B: *Choroby zakaźne i zatrucia w Polsce w 2007 roku*. PZH & GIS, Warsaw 2008.

12. De La Fuente J, Naranjo V, Ruiz-Fons F, Höfle U, Fernández de Mera IG, Villanúa D, Almazán C, Torina A, Caracappa S, Kocan KM, Gortázar C: Potential vertebrate reservoir hosts and invertebrate vectors of *Anaplasma marginale* and *A. phagocytophilum* in central Spain. *Vector Borne Zoonotic Dis* 2005, **5**, 390–401.

13. De la Fuente J, Ruiz-Fons F, Naranjo V, Torina A, Rodriquez O, Gortázar C: Evidence of *Anaplasma* infections in European roe deer (*Capreolus capreolus*) from southern Spain. *Res Vet Sci* 2008, **84**, 382–386.

14. Dumler JS, Barbet AF, Bekker CPJ, Dasch GA, Palmer GH, Ray SC, Rikihisa Y, Rurangirwa FR: 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*. *Int J Syst Evol Microbiol* 2001, **51**, 2145–2165.

15. Falarz M: Variability and trends in the duration and depth of snow cover in Poland in the 20th century. *Int J Climatol* 2004, **24**, 1713–1727.

16. Foley JE, Barlough JE, Kimsey RB, Madigan JE, Derocke E, Poland A: *Ehrlichia* spp. in cervids from California. *J Wildl Dis* 1998, **34**, 731–737.

17. Gage KL, Burkot TR, Eisen RJ, Hayes EB: Climate and Vectorborne Diseases. *Am J Prev Med* 2008, **35**, 436–450.

18. Gordon WS, Brownlee A, Wilson DR, MacLeod J: Tick-borne fever (a hitherto undescribed disease of sheep). *J Comp Pathol* 1932, **45**, 301–212.

19. Groen J, Koraka P, Nur YA, Avsic-Zupanc T, Goessens WH, Ott A, Osterhaus AD: Serologic evidence of ehrlichiosis among humans and wild animals in The Netherlands. *Eur J Clin Microbiol Infect Dis* 2002, **21**, 46–49.

20. Grzeszczuk A, Karbowiak G, Ziarko S, Kovalchuk O: The Root-vole *Microtus oeconomus* (Pallas, 1776): a new potential reservoir of *Anaplasma phagocytophilum*. *Vector Borne Zoonotic Dis* 2006, **6**, 240–243.

21. Grzeszczuk A, Stańczak J, Kubica-Biernat B: Serogical and molecular evidence of human granulocytic ehrlichiosis focus in Białowieża Primeval Forest, northeastern Poland. *Eur J Clin Microbiol Infect Dis* 2002, **21**, 6–11.

22. Grzeszczuk A, Ziarko S, Prokopowicz D, Radziwoń PM: Zakażenie żubrów z Puszczy Białowieskiej bakteriami *Anaplasma phagocytophilum*. *Med Wet* 2004, **60**, 600–601.

23. Humair PF, Douet V, Morán Cadenas F, Schouls LM, Van De Pol I, Gern L: Molecular identification of bloodmeal source in *Ixodes ricinus* ticks using 12S rDNA as a genetic marker. *J Med Entomol* 2007, **44**, 869–880.

24. Karbowiak G, Víchová B, Majláthová V, Hapunik J, Pet'ko B: *Anaplasma phagocytophilum* infection of red foxes (*Vulpes vulpes*). *Ann Agric Environ Med* 2009, **16**, 299–300.

25. Kawahara M, Rikihisa Y, Lin Q, Isogai E, Tahara K, Itagaki A, Hiramitsu Y, Tajima T: Novel genetic variants of *Anaplasma phagocytophilum*, *Anaplasma bovis*, *Anaplasma centrale* and a novel *Ehrlichia* sp. in wild deer and ticks on two major islands in Japan. *Appl Environ Microbiol* 2006, **72**, 1102–1109.

26. Lin Q, Rikihisa Y, Felek S, Wang X, RF, Woldehiwet Z: *Anaplasma phagocytophilum* has a functional msp2 gene that is distinct from p44. *Infect Immun* 2004, **72**, 3883–3889.

27. Little S, Stalljnecht DE, Lockhart JM, Dawson JE, Davidson WR: Natural coinfection of a white-tailed deer (*Odocoileus virginianus*) population with three *Ehrlichia* spp. *J Parasitol* 1998, **84**, 897–901.

28. Liz JS, Andersen L, Sumner JW, RF, Gern L, Rutti B, Brossard M: PCR detection of granulocytic Ehrlichiae in *Ixodes ricinus* ticks and wild small mammals in western Switzerland. *J Clin Microbiol* 2000, **38**, 1002–1007.

29. Liz JS, Sumner JW, Pfister K, Brossard M: PCR detection and serological evidence of granulocytic *Ehrlichia* infection in roe deer (*C. capreolus*) and chamois (*Rupicapria rupicapria*). *J Clin Microbiol* 2002, **40**, 892–897.

30. Massung RF, Courtney JW, Hiratzka SL, Pitzer VE, Smith G, Dryden RL: *Anaplasma phagocytophilum* in white-tailed deer. *Emerg Infect Dis* 2005, **11**, 1604–1606.

31. Massung RF, Slater KG: Comparison of PCR assays for detection of the agent of human granulocytic ehrlichiosis, *Anaplasma phagocytophilum*. *J Clin Microbiol* 2003, **41**, 717–722.

32. Michalik J, Stańczak J, Racewicz M, Cieniuch S, Sikora B, Szubert-Kruszyńska A, Grochowalska R: Molecular evidence of *Anaplasma phagocytophilum* infection in wild cervids and feeding *Ixodes ricinus* ticks from west-central Poland. **In:** *5th International Conference on Rickettsiae and Rickettsial Diseases, 18-20 May 2008*: 70. Marseille 2008.

33. Morissette E, Massung RF, Foley JE, Alleman AR, Foley P, Barbet AF: Diversity of *Anaplasma phagocytophilum* strains, USA. *Emerg Infect Dis* 2000, **15**, 928–931.

34. Nováková M, Víchová B, Majláthová V, Lesňáková A, Pochybová M, Peťko B: First case of human granulocytic anaplasmosis from Slovakia. *Ann Agric Environ Med* 2010, **17**, 173–175.

35. Ogden NH, Bown K, Horrocks BK, Woldehiwet Z, Bennett M: Granulocytic *Ehrlichia* infection in ixodid ticks and mammals in woodlands and uplands of the U.K. *Med Vet Entomol* 1998, **12**, 423–429.

36. Petrovec M, Bidovec A, Sumner JW, Nicholson WL, Childs JE, Avšič-Županc T: Infection with *Anaplasma phagocytophila* in cervids from Slovenia: Evidence of two genotypic lineages. *Wien Klin Wochenschr* 2002, **114/13**, 641–647.

37. Polin H, Hufnagl P, Haunschmid R, Gruber F, Ladurner G: Molecular evidence of *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks and wild animals in Austria. *J Clin Microbiol* 2004, **42**, 2285–2286.

38. Prokopowicz D: *Choroby przenoszone przez kleszcze*. Wydawnictwa Fundacji Büchnera, Warsaw 1995.

39. Siuda K: The review of data on the distribution of Ixodida (Acari) in Poland. In: Kropczyńska D, Boczek J, Tomczyk A (Eds): *The acari: Physiological and ecological aspects of acari-host relationships*, 273-280, Oficyna Dabor, Warsaw 1995.

40. Smetanová K, Schwarzová K, Kocianová E: Detection of *Anaplasma phagocytophilum*, *Coxiella burnetii*, *Rickettsia* spp., and *Borrelia burgdorferi* s.l. in ticks, and wild-living animals in western and middle Slovakia. *Ann NY Acad Sci* 2006, **1078**, 312–315.

41. Stańczak J, Racewicz M, Kruminis-Łozowska W, Kubica-Biernat B: Coinfection of *Ixodes ricinus* (Acari: Ixodidae) in northern Poland with the agents of Lyme Boreliosis (LB) and human granulocytic ehrlichiosis (HGE). *Int J Med Microbiol* 2002, **291**, 198–2001.

42. Stefanidesova K, Kocianova E, Boldis V, Kostanova Z, Kanka P, Nemethova D, Spitalska E: Evidence of *Anaplasma phagocytophilum* and *Rickettsia helvetica* infection in free-ranging ungulates in central Slovakia. *Eur J Wildl Res* 2008, **54**, 519–524.

43. Stuen S: *Anaplasma phagocytophilum*- the most widespread tick-borne infection in animals in Europe. *Vet Res Commun* 2007, **31 (Suppl. 1)**, 79–84.

44. Stuen S, Akerstedt J, Bergstrom K, Handeland K: Antibodies to granulocytic *Ehrlichia* in moose, red deer, and roe deer in Norway. *J Wildl Dis* 2002, **38**, 1–6.

45. Tylewska-Wierzbanowska S, Chmielewski T, Kondrusik M, Hermanowska-Szpakowicz T, Sawicki W, Sułek K: First cases of acute human granulocytic ehrlichiosis in Poland. *Eur J Clin Microbiol Inf* 2001, **20**, 196–198.

46. Walls JJ, Asanovich KM, Bakken JS, Dumler JS: Serologic evidence of a natural infection of white-tailed deer with the agent of human granulocytic ehrlichiosis in Wisconsin and Maryland. *Clin Diagn Lab Immunol* 1998, **5**, 762–765.

47. Welc-Falęciak R, Hildebrandt A, Siński E: Co-infection with *Borrelia* species and other tick-borne pathogens in humans: two cases from Poland. *Ann Agric Environ Med* 2010, **17**, 309–313.

48. Woldehiwet Z: *Anaplasma phagocytophilum* in Ruminants in Europe. *Ann NY Acad Sci* 2006, **1078**, 446–460.

49. Zeman P, Pecha M: Segregation of genetic variants of *A. phagocytophilum* circulating among wild ruminants within a Bohemia forest (Czech Republic). *Int J Med Microbiol* 2008, **298**, 203–210.

50. Zwoliński J, Chmielewska-Badora J, Wójcik-Fatla A, Cisak E, Buczek A, Dutkiewicz J: Anaplazmoza granulocytarna jako nowy problem zdrowia publicznego. *Zdr Publ* 2007, **117**, 213–219.