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SHORT COMMUNICATION

# Evidence of *Anaplasma phagocytophilum* and *Rickettsia helvetica* infection in free-ranging ungulates in central Slovakia

**Katarina Stefanidesova · Elena Kocianova · Vojtech Boldis · Zina Kostanova · Pavel Kanka · Danka Nemethova · Eva Spitalska**

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**Abstract** The purpose of this study was to investigate the role of wild animals for *Anaplasma phagocytophilum*, other ehrlichiae/anaplasmae, *Rickettsia helvetica* and other rickettsiae and whether different genetic variants of *A. phagocytophilum* in central Slovakia exist. A total of 109 spleen samples from 49 red deer (*Cervus elaphus*), 30 roe deer (*Capreolus capreolus*), 28 wild boar (*Sus scrofa*) and two mouflon (*Ovis musimon*) were collected from June 2005 to December 2006. Polymerase chain reaction (PCR) amplification of the16S rRNA gene was used for detection of ehrlichiae/anaplasmae. A nested PCR targeting part (392 bp) of *groESL* gene was applied for the specific detection of *A. phagocytophilum*. Fragments of the *gltA* and *ompA* genes (381 bp and 632 bp, respectively) were amplified to detect rickettsiae, followed by sequencing. *A. phagocytophilum* and *R. helvetica* were detected in wild animals. The prevalence of *A. phagocytophilum* was 50.0± 18.2% in roe deer and 53.1±14.1% in red deer. None of the 28 wild boar was PCR positive for ehrlichiae/anaplasmae. *A. phagocytophilum* was detected in one mouflon. *R. helvetica* was found in one roe deer. Our study suggests a role of cervids as a natural reservoir of *A. phagocytophilum* in Slovakia. However, the role of cervids and wild boars in the circulation of *R. helvetica* remains unknown. The analysis of sequence variation in the *msp4* coding region of *A. phagocytophilum* showed the presence of different variants previously described in ruminants.

**Keywords** *Anaplasma* spp. · *Rickettsia* spp. · Tick-borne diseases · Slovakia · Wildlife

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K. Stefanidesova · E. Kocianova · V. Boldis · E. Spitalska (✉)
Institute of Virology, Slovak Academy of Sciences,
Dubravska cesta 9,
845 05 Bratislava, Slovakia
e-mail: eva.spitalska@savba.sk

Z. Kostanova
Regional Office of Public Health in Ziar nad Hronom,
Sladkovicova 484/9,
965 24 Ziar nad Hronom, Slovakia

P. Kanka
Regional Veterinary and Food Administration,
SNP 612/120,
965 24 Ziar nad Hronom, Slovakia

D. Nemethova
Research Centre for Environmental Chemistry and Ecotoxicology,
Faculty of Science, Masaryk University,
Kamenice 126/3,
634 00 Brno, Czech Republic

## Introduction

Members of the *Anaplasmataceae* and *Rickettsiaceae* families are widespread in natural foci throughout central Europe (Parola et al. 2005). They are obligate intracellular gram-negative bacteria. *Ehrlichia* and *Anaplasma* spp. replicate in the cytoplasmic vacuole of their host cells whereas *Rickettsia* spp. reside free in the cytosol of infected eukaryotic cells. Some of these organisms are well-known as human and veterinary pathogens. The vectors are ticks from the Ixodidae family.

*Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophila*, *E. equi* and human granulocytic ehrlichiosis—HGE agent) with affinity to granulocytic cells is the causative agent of human granulocytic anaplasmosis

(HGA), previously known as HGE, tick-borne fever of ruminants, equine and canine granulocytic anaplasmosis (Dumler et al. 2001). The life cycle of *A. phagocytophilum* in central Europe involves *Ixodes ricinus* ticks as vectors and wild or domestic animals as hosts. *A. phagocytophilum* has a wide geographic distribution. The human granulocytic anaplasmosis was firstly described in the United States (Chen et al. 1994). In Europe, the first confirmed case of HGE was recognised in Slovenia in 1996 (Petrovec et al. 1997). Since then, more cases of HGA, seroprevalences in tick-exposed populations as well as in healthy individuals, and presence of *A. phagocytophilum* in ticks have been reported from many European countries (Blanco and Oteo 2002). HGA is an acute non-specific systemic febrile illness that may be accompanied with high fever (>39°C), myalgia, headache, chills, malaise, arthralgia, anorexia, nausea and non-productive cough (Thompson et al. 2001). Tick-borne fever in animals (goats, sheep, cattle, horses, dogs) varies from undetectable illness to a severe febrile disease associated with depression, anorexia, leukopenia, thrombocytopenia, haemorrhage, abortions and opportunistic infections (Dumler et al. 2001). High prevalence of *A. phagocytophilum* in cervids suggests their role as natural reservoirs for this bacterium in Europe (Petrovec et al. 2002; Liz et al. 2002; Polin et al. 2004). Previous studies have confirmed the occurrence of *A. phagocytophilum* and related microorganisms in *I. ricinus* ticks and in small terrestrial mammals from southwestern, eastern and central Slovakia as well as in roe deer, red deer and wild boar from central Slovakia (Spitalska and Kocianova 2002; Derdakova et al. 2003; Smetanova et al. 2006). Genetic variability among *A. phagocytophilum* was observed. De la Fuente et al. (2005a) characterised some genetic variants of *A. phagocytophilum* obtained from different host species in the United States and Europe. The presence of different variant types in Slovakia has not been studied yet.

Rickettsiae are associated with arthropods, which can transmit these microorganisms to vertebrates via salivary secretions or faeces. Rickettsiae are distributed worldwide and many of them are well-known as agents of human and veterinary diseases. These zoonoses are among the oldest known vector-borne diseases (Raoult and Roux 1997; Parola et al. 2005). *Rickettsia helvetica* was first isolated from *I. ricinus* ticks in Switzerland in 1979 (Beati et al. 1993). This tick represents a potential vector and natural reservoir for *R. helvetica* in Europe. *R. helvetica* was implicated in perimyocarditis and sudden cardiac failure in two patients in Sweden (Nilsson et al. 1999). Seroconversion to *R. helvetica* was described for a French patient with an unexplained febrile illness (Fournier et al. 2000). The pathogenicity of *R. helvetica* is not clearly confirmed, it occurs not only in Europe, but also in Asia (Fournier et al. 2004).

In the present study, the role of roe deer, red deer, wild boar and mouflon as reservoirs for *A. phagocytophilum*, other members of *Anaplasmataceae* family, *R. helvetica* and other *Rickettsia* spp. in Slovakia and variants of *A. phagocytophilum* circulating in wild animals were investigated.

## Materials and methods

### Area of study

The studied area comprises 37 localities belonging to three districts (Ziar nad Hronom, Zarnovica and Banska Stiavnica) in central Slovakia (Fig. 1). Localities are either woodland areas (Carpathian deciduous and mixed forests) or pasture and meadow habitats. Climate varies from moderately warm to cold montane.

### Collection of samples

A total of 109 spleen samples, 30 from roe deer (*Capreolus capreolus*), 49 from red deer (*C. elaphus*), 28 from wild boar (*Sus scrofa*) and two from mouflon (*Ovis musimon*), were collected in cooperation with individual hunters from June 2005 to December 2006. Tested animals were from

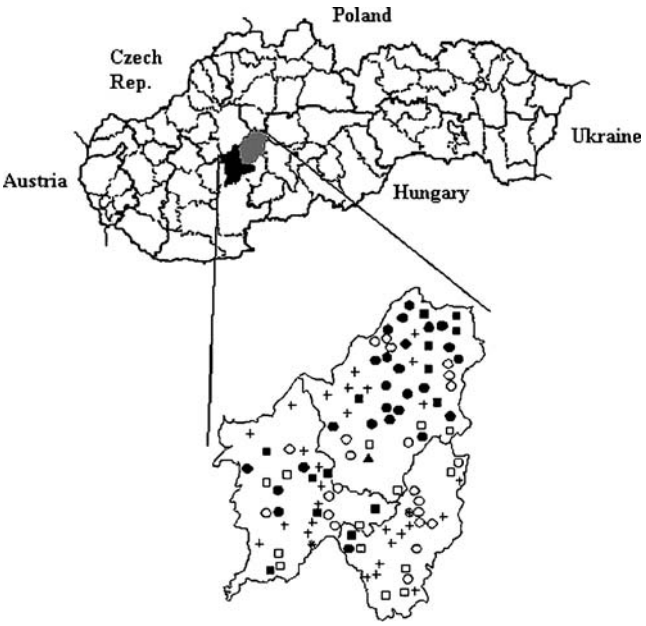


**Fig. 1** Map of hunting sites. *Empty square* Hunting site of roe deer. *Filled square* Hunting site of roe deer positive for *A. phagocytophilum*. *Empty circle* Hunting site of red deer. *Filled circle* Hunting site of red deer positive for *A. phagocytophilum*. *Plus sign* Hunting site of wild boar. *Asterisk* Hunting site of mouflon. *Circled asterisk* Hunting site of mouflon positive for *A. phagocytophilum*. *Filled triangle* Hunting site of roe deer positive for *R. helvetica*

**Table 1** Positivity of wild animals for *Anaplasma phagocytophilum* (A. ph) and *Rickettsia helvetica* (R. he) in monitored districts of central Slovakia

| | | Ziar nad Hronom | Zarnovica | Banska Stiavnica | Total |
|---|---|---|---|---|---|
| Roe deer | A. ph | 8/37.5±35.9% | 16/68.8±23.5% | 6/16.7±32.7% | 30/50.0±18.2% |
| | R. he | 8/12.5±24.5% | 16/0% | 6/0% | 30/3.3±6.5% |
| Red deer | A. ph | 23/69.6±19.2% | 17/52.9±24.5% | 9/11.1±21.8% | 49/53.1±14.1% |
| | R. he | 23/0% | 17/0% | 9/0% | 49/0% |
| Total | A. ph | 31/61.3±17.4% | 33/60.6±16.9% | 15/13.3±17.8% | 79/51.9±11.1% |
| | R. he | 31/3.2±6.3% | 33/0% | 15/0% | 79/1.3±2.5% |
| Wild boar | A. ph | 5/0% | 13/0% | 10/0% | 28/0% |
| | R. he | 5/0% | 13/0% | 10/0% | 28/0% |
| Mouflon | A. ph | | 1/0% | 1/100% | 2/50.0±98.0% |
| | R. he | | 1/0% | 1/0% | 2/0% |
| Total | A. ph | 36/52.8±16.5% | 47/42.6±14.3% | 26/11.5±12.5% | 109/38.5±9.2% |
| | R. he | 36/2.8±5.4% | 47/0% | 26/0% | 109/0.9±1.8% |

The prevalence is expressed in following form: *N*/95% confidence interval.

both sexes; nine roe deer, 24 red deer, 15 wild boar and one mouflon were females; and 21 roe deer, 25 red deer, 13 wild boar and one mouflon were males.

The monitored localities are common habitats for red deer, roe deer, wild boar and mouflon. Numbers of hunted animals in all monitored districts are shown in Table 1. Parts of spleens of hunter-killed animals were immediately transferred into tubes with 70% ethanol and maintained at 4°C until examination.

## DNA isolation, PCR amplification and sequencing

Spleen samples from wild animals were screened by polymerase chain reaction (PCR) and DNA sequencing methods for the presence of tick-borne pathogens—members of the *Anaplasmataceae* and *Rickettsiaceae* families and for study of variants of *A. phagocytophilum*. Isolation of DNA from spleen samples was performed using DNeasy Tissue kit (Qiagen, Germany) according to recommendations of the manufacturer. DNA amplification was performed in a PTC-200 Peltier thermal cycler (MJ-Research, USA). Twenty-five μl of PCR reaction mixture contained 12.5 μl of 2× PCR MasterMix (Fermentas, Germany), 10 pmol of each primer, 1 ng of DNA and nuclease-free water (Fermentas, Germany) up to the final volume. DNA of *R. slovaca*, *R. helvetica*, *A. phagocytophilum* and *Candidatus* Neoehrlichia mikurensis, were used as positive controls in PCR assays. Nuclease-free water was used as negative control. Used primers are displayed in Table 2. PCR products were separated on 1.5% agarose gels stained with ethidium bromide and visualised under UV light.

The PCR products were purified before sequencing by using a QIAquick Spin PCR Purification Kit (Qiagen, Austria) as described by the manufacturer. DNA sequencing was conducted by Macrogen Inc. (Seoul, South Korea; www.macrogen.com) under BigDyeTM terminator cycling conditions. Sequences were analysed using an Automatic Sequencer 3730xl followed by analysis with the ABI sequence-analysis software. Obtained nucleotide sequences were compared with those available in the EMBL Nucleotide Databases using available tools (www.ebi.ac.uk).

## Statistical analysis

With the aim to compare the prevalence of *A. phagocytophilum* in red and roe deer across districts and between males and females of deer $\chi^2$ goodness-of-fit was used. Data analysis was performed using the software Statistica 7.1.

**Table 2** Oligonucleotide primers used in PCR assays

| Oligonucleotide primers | Target organisms | Target gene | PCR product size (bp) | Reference |
|---|---|---|---|---|
| 16S8FE, GA1B | Eubacteria | 16S rRNA | 470 | Bekker et al. 2002 |
| EHR521, EHR790 | *Anaplasma/Ehrlichia* spp. | 16S rRNA | 298 | Kolbert 1996; Massung and Slater 2003 |
| HS43, HS45, GEHS1, EHS6 | *A. phagocytophilum* | *groESL* nested | 392 | Bjoersdorff et al. 2002 |
| RpCS.877p, RpCS.1258n | *Rickettsia* spp. | *gtlA* | 381 | Regnery et al. 1991 |
| Rr190.70p, Rr190-701 | *Rickettsia* spp. | *rompA* | 632 | Regnery et al. 1991; Roux et al. 1996 |
| MSP4AP5, MSP4AP3 | *A. phagocytophilum* | *msp4* | 849 | de la Fuente et al. 2005a |

**Table 3** Percentage identity among nucleotide sequences between *Anaplasma* species identified in our study (Acc. Nos. EU180058 to EU180066) and *Anaplasma* species as previously described (marked Ovine 5, Horse 31, Bison 7, Bison 12, Elsa, Roe deer 1539/25, Acc. Nos. AY706391, AY706390, AY706387, AY706388, AY530198, AY829457, respectively), (de la Fuente et al. 2005a)

| | *Anaplasma* species identified in this study | | | | | | | | *Anaplasma* species as previously described | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EU180058 | EU180059 | EU180060 | EU180061 | EU180062 | EU180063 | EU180064 | EU180065 | Ovine 5 | Horse 31 | Bison 7 | Bison 12 | Elsa | Roe deer 1539/25 |
| EU180066 | 93.8 | 94.1 | 93.2 | 90.9 | 95.4 | 90.5 | 94.4 | 93.9 | 98.2 | 96.5 | 97.1 | 96.2 | 97.1 | 99.4 |
| EU180058 | | 98.2 | 99.3 | 95.3 | 97.6 | 94.7 | 95.8 | 95.4 | 99.3 | 95.7 | 96.4 | 95.7 | 96.4 | 98.3 |
| EU180059 | | | 97.5 | 96.8 | 97.5 | 95.6 | 96.5 | 95.8 | 99.0 | 97.1 | 97.5 | 97.1 | 97.5 | 98.5 |
| EU180060 | | | | 95.4 | 96.9 | 94.8 | 96.0 | 94.7 | 99.2 | 95.5 | 96.2 | 95.5 | 96.2 | 98.2 |
| EU180061 | | | | | 94.3 | 96.3 | 96.4 | 96.5 | 96.4 | 98.9 | 98.5 | 98.9 | 98.5 | 96.1 |
| EU180062 | | | | | | 93.2 | 96.8 | 96.9 | 99.4 | 96.3 | 96.4 | 96.0 | 96.4 | 98.3 |
| EU180063 | | | | | | | 95.5 | 94.0 | 97.3 | 97.0 | 97.4 | 97.0 | 97.4 | 96.8 |
| EU180064 | | | | | | | | 97.6 | 97.9 | 97.6 | 98.3 | 97.9 | 98.3 | 97.5 |
| EU180065 | | | | | | | | | 96.6 | 98.3 | 98.7 | 98.3 | 98.7 | 97.0 |

## Results

Spleen samples of 109 wild animals were analysed for the presence of ehrlichiae/anaplasmae and rickettsiae. Using two sets of general primers (GA1B and 16S8FE, Ehr521 and Ehr790), ehrlichiae/anaplasmae were found in 15 roe deer, 26 red deer and in one female mouflon. However, all wild boar were negative for ehrlichiae/anaplasmae. By the nested PCR, *A. phagocytophilum* was confirmed in all positive samples; prevalence of this agent was 50.0±18.2% in roe deer and 53.1±14.1% in red deer (Table 1). The difference was not statistically significant ($\chi^2$=0.070, *df*=1, *P*=0.792).

The difference between males and females was not statistically significant, *A. phagocytophilum* was detected in 4/44.5±34.4% females and 11/52.4±21.9% males of roe deer ($\chi^2$=0.159, *df*=1, *P*=0.690) and in 11/45.8±20.4% females and 15/60.0±19.6% males of red deer ($\chi^2$=0.987, *df*=1, *P*=0.321).

The overall prevalence of *A. phagocytophilum* in deer was 51.9±11.1%. Other species of the members of *Anaplasmataceae* family were not detected.

The highest positivity of deer infected with *A. phagocytophilum* was in the Ziar nad Hronom district (61.3±17.4%) while the positivity in the Zarnovica and the Banska Stiavnica districts were 60.6±16.9% and 13.3±17.8%, respectively. The prevalence of *A. phagocytophilum* differed significantly among districts ($\chi^2$=11.034, *df*=2, *P*=0.004); being significantly lower in Banska Stiavnica than in the Ziar nad Hronom and Zarnovica districts. This situation was caused by different prevalence of *A. phagocytophilum* in red deer across districts ($\chi^2$=8.875, *df*=2, *P*=0.012). Prevalence of *A. phagocytophilum* in roe deer did not differ significantly among districts ($\chi^2$=5.417, *df*=2, *P*=0.067). The positivity of wild animals in these three districts is summarised in Table 1.

To confirm the presence of *A. phagocytophilum*, sequencing was performed on three randomly selected DNA samples isolated from the spleen of two roe deer and one red deer and an amplified part of 16S rRNA using GA1B and 16S8FE primers. These three sequences were 99.2% similar to each other and were homologous (100%, 100% and 99.5%) to *A. phagocytophilum* under accession number AY055469.

The analysis of sequence variations in the *msp4* coding region of *A. phagocytophilum* showed some heterogeneity. Table 3 shows percent identity among nucleotide sequences between *Anaplasma* species identified in three roe and six red deer in our study (the GenBank accession numbers are EU180058 to EU180066) and *Anaplasma* species described before (de la Fuente et al. 2005a).

Rickettsial DNA was detected in one male roe deer (Table 1), of which only the *gltA* gene was amplified (the

GenBank accession number is EU183407). In this roe deer, mixed infection with ehrlichiae/anaplasmae was not recorded. Sequencing showed 99.2% homology to *R. helvetica* (acc. Nos. DQ821857 and AM418450, which are 100% identical).

## Discussion

Several studies regarding the prevalence of *A. phagocytophilum* in deer have been performed in some European countries but not in Slovakia. The prevalence was assessed using various PCR protocols and serologic methods. Previous results obtained by PCR showed that the highest prevalence in roe deer (85.6%) and red deer (87.5%) were reported from Slovenia (Petrovec et al. 2002). A prevalence of 42.6% was detected in roe deer in Denmark (Skarphedinsson et al. 2005). Lower prevalences were recorded in roe deer in United Kingdom (29%) and in Switzerland (18.4%). In Austria, 28.6% red deer and 12.5% roe deer were *A. phagocytophilum*-positive, while it was 13.3% in red deer from the Czech Republic (Alberdi et al. 2000; Liz et al. 2002; Polin et al. 2004; Hulinska et al. 2004). Estimated prevalence of *A. phagocytophilum* in our study was lower than in Slovenia, similar to Denmark and higher than in the UK, Switzerland, Austria and Czech Republic.

Roe and red deer are widely distributed in woodland areas of Slovakia and they are commonly infested with adult ticks mainly from the *I. ricinus* species (unpublished). The statistically significant difference of prevalence of *A. phagocytophilum* in monitored localities of our study is not clear at this moment. However, it is not quite surprising. The higher prevalence of *A. phagocytophilum* in *I. ricinus* ticks has been observed in the localities near Ziar nad Hronom for three consecutive years (our unpublished results). Nevertheless, the role of the age of animals, the habitat or the climatic conditions needs to be clarified in futures studies.

Related to the high prevalence of *A. phagocytophilum* in cervids, the mentioned studies in some European countries as well as our own study suggest that cervids could be competent reservoirs for this pathogen.

The analysis of sequence variation in the *msp4* coding region of *A. phagocytophilum* showed heterogeneity among ruminant and non-ruminant strains obtained from the United States and some European countries, i.e. Switzerland, Italy, Germany, Poland, Norway and the UK (de la Fuente et al. 2005a). Variants of *A. phagocytophilum* in wild deer in central Slovakia were identical with those already published (Table 3). However, it remains unknown if we are dealing with pathogenic, mild or non-pathogenic species as there was no report about clinical signs in deer. But based on the results of Petrovec et al. (2002), these genetic variants in deer should not be pathogenic for humans.

Studies on the presence of the members of *Anaplasmataceae* family in the wild boar from Polin et al. (2004) and de la Fuente et al. (2005b) recorded no evidence of infection with *A. phagocytophilum*. On the other hand, Hulinska et al. (2004) found three out of 69 (4.35%) wild boar and Petrovec et al. (2003) found nine out of 63 (14.3%) wild boar to be *A. phagocytophilum*-positive. Petrovec et al. (2003) suggested that *S. scrofa* could serve as a competent reservoir of a variant of *A. phagocytophilum* pathogenic to humans and dogs in Europe.

The results from Hulinska et al. (2004) showed that two out of 15 mouflon (13.33%) were positive for *A. phagocytophilum*, but as our study investigated a low number of samples in both surveys, we can not conclude whether mouflon participate in the circulation of *A. phagocytophilum* as reservoir animals.

*R. helvetica* has been identified in *I. ricinus* ticks in many European countries, including France, Germany, Sweden, Slovenia, Portugal, Italy and Bulgaria (Parola et al. 2005). Its association with deer has not been studied yet. Up to our knowledge, only deer in a Danish study were tested for *R. helvetica* but this bacterium was not detected (Skarphedinsson et al. 2005). The role of deer and wild boar in its circulation is uncertain and requires further studies.

Our results demonstrate the existence and the high prevalence (51.9±11.1%) of *A. phagocytophilum* in roe deer and red deer in central Slovakia and suggest their role in the circulation of this bacterium in the natural cycle as its competent reservoirs. Sequence analysis of selected samples revealed that different genetic variants of *A. phagocytophilum* are present in wild living game animals. The role of wild boar as competent reservoirs was not confirmed. Our study could not show the role the wild animals played in the epidemiology of *R. helvetica*.

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The experiments presented in this paper comply with the current laws in Slovakia.

## References


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

Beati L, Peter O, Burgdorfer W, Aeschlimann A, Raoult D (1993) Confirmation that *Rickettsia helvetica* sp. nov. is a distinct species of the spotted fever group of rickettsiae. Int J Syst Bacteriol 43:521–526

Bekker CPJ, de Vos S, Taoufik A, Sparagano OAE, Jongejan F (2002) Simultaneous detection of *Anaplasma* and *Ehrlichia* species in ruminants and detection of *Ehrlichia ruminantium* in *Amblyomma variegatum* ticks by reverse line blot hybridization. Vet Microbiol 89:223–238

Bjoersdorff A, Bagert B, Massung F, Gusa A, Eliasson I (2002) Isolation and characterization of two European strains of *Ehrlichia phagocytophila* of equine origin. Clin Diagn Lab Immunol 9:341–343

Blanco JR, Oteo JA (2002) Human granulocytic ehrlichiosis in Europe. Clin Microbiol Infect 8:763–772

Chen SM, Dumler JS, Bakken JS, Walker DH (1994) Identification of a granulocytotropic ehrlichia species as the etiologic agent of human diseases. J Clin Microbiol 32:589–595

de la Fuente J, Massung RF, Wong SJ, Chu FK, Lutz H, Meli M, von Loewenich FD, Grzeszcuk A, Torina A, Caracappa S, Mangold AJ, Naranjo V, Stuen S, Kocan KM (2005a) Sequence analysis of the *msp4* gene of *Anaplasma phagocytophilum* strains. J Clin Microbiol 43:1309–1317

de la Fuente J, Naranjo V, Ruiz-Fons F, Hofle U, de Mera IGF, Villanua D, Almazan C, Torina A, Caracappa S, Kocan KM, Gortazar C (2005b) Potential vertebrate reservoir hosts and invertebrate vectors of *Anaplasma marginale* and *A. phagocytophilum* in central Spain. Vector-borne Zoon Dis 5:390–401

Derdakova M, Halanova M, Stanko M, Stefancikova A, Cislakova L, Petko B (2003) Molecular evidence for *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks from eastern Slovakia. Ann Agric Environ Med 10:269–271

Dumler JS, Barbet AF, Bekker PJ, Dasch GA, Palmer GH, Ray SC, Rikihisa Y, Rurangirwa FR (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*. Int J System Evol Microbiol 51:2145–2165

Fournier PE, Grunnenberger F, Jaulhac B, Gastinger G, Raoult D (2000) Evidence of *Rickettsia helvetica* infection in humans, eastern France. Emerg Infect Dis 6:389–392

Fournier PE, Allombert C, Supputamonkol Y, Caruso G, Brouqui P, Raoult D (2004) An eruptive fever associated with antibodies to *Rickettsia helvetica* in Europe and Thailand. J Clin Microbiol 42:816–818

Hulinska D, Langrova K, Pejcoch M, Pavlasek I (2004) Detection of *Anaplasma phagocytophilum* in animals by real-time polymerase chain reaction. APMIS 112:239–247

Kolbert C (1996) Detection of the agent of human granulocytic ehrlichiosis by PCR. In: Persing D (ed) PCR Protocols for Emerging Infectious Diseases. ASM, Washington DC, pp 106–111

Liz JS, Sumner JW, Pfister K, Brossard M (2002) PCR detection and serological evidence of granulocytic ehrlichial infection in roe deer (*Capreolus capreolus*) and chamois (*Rupicapra rupicapra*). J Clin Microbiol 40:892–897

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

Nilsson K, Lindquist O, Pahlson C (1999) Association of *Rickettsia helvetica* with chronic perimyocarditis in sudden cardiac death. Lancet 354:1169–1173

Parola P, Paddock CD, Raoult D (2005) Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev 18:719–756

Petrovec M, Lotric Furlan S, Avsic-Zupanc T, Strle F, Brouqui P, Roux V, Dumler JS (1997) Human disease in Europe caused by a granulocytic *Ehrlichia* species. J Clin Microbiol 35:1556–1559

Petrovec M, Bidovec A, Sumner JW, Nicholson WL, Childs JE, Avsic-Zupanc T (2002) Infection with *Anaplasma phagocytophila* in cervids from Slovenia: evidence of two genotypic lineages. Wien Klin Wochenschr 114:641–647

Petrovec M, Sixl W, Schweiger R, Mikulasek S, Elke L, Wust G, Marth E, Strasek K, Stunzner D, Avsic-Zupanc T (2003) Infections of wild animals with *Anaplasma phagocytophila* in Austria and the Czech Republic. Ann N Y Acad Sci 990:103–106

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

Raoult D, Roux V (1997) Rickettsiosis as paradigms of new or emerging infectious diseases. Clin Microbiol Rev 10:694–719

Regnery RL, Spruill CL, Plikaytis BD (1991) Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J Bacteriol 173:1576–1589

Roux V, Fournier PE, Raoult D (1996) Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA. J Clin Microbiol 34:2058–2065

Skarphedinsson S, Jensen PM, Kristiansen K (2005) Survey of tickborne infections in Denmark. Emerg Infect Dis 11:1055–1061

Smetanova K, Schwarzova K, Kocianova E (2006) 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 N Y Acad Sci 1078:312–315

Spitalska E, Kocianova E (2002) Agents of *Ehrlichia phagocytophila* group and other microorganisms co-infecting ticks in southwestern Slovakia. Acta Virol 46:49–50

Thompson C, Spielman A, Krause PJ (2001) Coinfecting deer-associated zoonoses: Lyme disease, babesiosis, and ehrlichiosis. Clin Infect Dis 33:676–685