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Wild game as a reservoir of *Anaplasma phagocytophilum* in north-western Poland

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ABSTRACT. **Background**. The aim of the study was to determine the role of game animals as reservoirs of *Anaplasma phagocytophilum*, a bacteria species transmitted by *Ixodes ricinus* ticks, from north-western Poland (Zachodniopomorskie vovoidship). The area under question is endemic for *A. phagocytophilum*. **Material and methods**. Blood and spleen samples were taken from 72 roe deer between April and December 2003. Animals culled during winter did not harbour ticks, on the other hand 155 individuals of *Ixodes ricinus* were collected from 35 of 43 animals taken during spring. We tested all samples for *A. phagocytophilum* by PCR amplification of the *msp2* gene. An individual was considered infected if pathogens were detected in at least one isolate (blood or a tissue sample). **Results**. DNA from *A. phagocytophilum* was not found in isolates from ticks collected from the animals. The general level of infection for the roe deer was 31.94% (23/72). DNA of *A. phagocytophilum* was most commonly detected in blood samples; only in three cases was anaplasma DNA detected in spleen and not in blood. Ruminants seem to be the most competent reservoir for *A. phagocytophilum* in north-western Poland.

Key words: *Anaplasma phagocytophilum*, PCR, roe deer, ticks.

Introduction

Anaplasma phagocytophilum is a Gram negative bacteria of the family *Rickettsiaceae* and an obligatory intracellular parasites. It is the ethiological factor of granulocytic anaplasmosis, also called tick-borne fever because it is a disease transmitted by ticks. It occurs not only in humans, but also in cattle, sheep, horses, dogs and cats [1–6].

Anaplasma phagocytophilum was classified early as *Ehrlichia*, and different strains isolated from people, cattle and horses were considered separate species and named HGE factor, *Ehrlichia phagocytophila* and *E. equi*, respectively [7]. This pathogen is broadly distributed in Europe and North America. Numerous American studies have shown that the vectors of *A. phagocytophilum* in this part of the world are the ticks *I. scapularis* and *I. pacificus*, and the reservoirs — forest species of rodents and ruminants [8–11]. Birds have also been considered potential reservoirs [12]. In Europe, studies have shown that the main vector of this parasite is the common tick *Ixodes ricinus* [13–19], however, only a limited amount of information exists on the reservoirs of this pathogen. Studies carried out in Great Britain by Bown et al. [20] have shown that *A. phagocytophilum* infests the bank vole (*Clethrionomys glareolus*) and field mouse (*Apodemus sylvaticus*). In Switzerland, Liz et al. [21] have detected *A. phagocytophilum* in four rodent species: *A. sylvaticus*, *A. flavicollis* (yellow-necked mouse), *Pitymys subterraneus* (pine vole) and *C. glareolus*, and in the insectivorous common shrew, *Sorex araneus*. These species are probably natural reservoirs of *A. phagocytophilum* in Switzerland. Work in Great Britain [22] has shown that the roe deer (*Capreolus capreolus*) is an important natural reservoir of *A. phagocytophilum*. In Norway, Stuen et al. [1] have found antibodies against *A. phagocytophilum* in roe deer, red deer

(*Cervus elaphus*) and moose (*Alces alces*). The percentage of animals in which the amount of antibodies suggested previous infection was 96, 55 and 43%, respectively. Petrovec et al. [23] carried out similar studies in Slovenia. The level of antibodies against *A. phagocytophilum* revealed that 35% of the red deer and 94% of the roe deer populations suffered from infection. The DNA of *A. phagocytophilum* was detected in 86% of individuals of both species. Moreover, Stuen et al. [24] have found DNA of *A. phagocytophilum* in tissues of an 8-week old roe deer from a forested area of Norway. Hulinska et al. [25] detected this parasite in the tissues of roe deer and wild boar.

The aim of this study was to assess the role of wild ruminants (roe deer and red deer) and also wild boar as reservoirs of *A. phagocytophilum*. This will allow a more thorough description of the ecology of this pathogen and the epidemiology of anaplasmosis. The mammals used in the our study originated from north-western Poland (Zachodniopomorskie voivodship).

Material and methods

Sample collection. The animals originated from forested areas near Szczecin, endemic for *A. phagocytophilum*, and were shot by the hunters between April and December 2003. Fourty three roe deer were taken during spring (April and May 2003), 35 of these were infested by ticks. In all, 155 individuals of *Ixodes ricinus* were collected (132 females and 23 males). All female ticks but no males had already taken blood. Twenty nine mammals were culled during the autumn-winter seasons (November and December 2003). These individuals did not harbour ticks. Blood (1 cm^3 volume) and spleen (about 2 cm^3) samples were taken from all 72 roe deer within the hour after their death. Blood was transferred to tubes containing 100 (l Na-EDTA.

DNA extraction. DNA from blood, tissue samples and ticks was isolated using the MasterPure™ DNA Purification Kit (Epicentre, USA) according to the manufacturer's instructions. For extraction, 200 µl of blood and 5 mg of tissue (homogenized in 100 µl of PBS) were used.

PCR amplification. Primers msp2-3f: CCAGCGTTTAGCAAGATAAGAG and msp2-3r: GCCCAGTAACAACATCATAAGC were used in PCR reactions in order to detect *A. phagocytophilum* DNA in the isolates. This primer set amplifies a 334 bp fragment of the *msp2* gene [26]. DNA of *A. phagocytophilum* cultivated in HL60 cell line, obtained from *A. phagocytophilum* IFA slide (Focus Diagnostics, USA), was used as a positive control [19]. The thermal profile of the PCR reactions was as follows: initial denaturation at 94°C — 2 minutes; 8 cycles: denaturation at 94°C — 30 seconds, primer annealing at 62°C–56°C (decrease in temperature every 2 cycles) — 45 seconds, extension at 72°C — 30 seconds; 28 cycles: denaturation at 94°C — 30 seconds, primer annealing at 54°C — 45 seconds, extension at 72°C — 30 seconds, and final extension at 72°C — 5 minutes. Taq DNA polymerase and PCR buffer used in the reactions were manufactured by Qiagen (Germany). Nucleotides and primers were manufactured by Polgen (Poland). PCR products were electroforesed in 2,5 % agarose gels and stained with ethidium bromide.

Results

An individual was considered infected if pathogens were detected in at least one isolate (blood or spleen sample). Pathogen infection in roe deer culled during the spring reached 23.25% — 10 infected individuals out of 43 (Table 1). The level of infection in animals taken during the autumn-winter seasons was 44.82% — infection was detected in 13 out of 29 deer (Table 1). Animals sampled during

Table 1. Presence of *A. phagocytophilum* DNA in tissues from examined animals

Tissues in which pathogen DNA was detected	Number of animals		
	Spring	Autumn-Winter	Total
Blood	6/43 (13.95%)	8/29 (27.58%)	14/72 (19.44%)
Blood and spleen	2/43 (4.65%)	4/29 (13.79%)	6/72 (8.33%)
Spleen	2/43 (4.65%)	1/29 (3.45%)	3/72 (4.16%)
Total	10/43 (23.25%)	13/29 (44.82%)	23/72 (31.94%)

the autumn and winter season were not infested by ticks. The general level of infection was 31.94% (23/72). Most often a positive PCR result came from blood isolates (in 14 individuals). The presence of *A. phagocytophilum* DNA in both blood and spleen was recorded in 6 individuals. Pathogen DNA was detected in spleen (but not in the blood) in 3 animals (Table 1). *A. phagocytophilum* DNA was not detected in *I. ricinus* collected from the mammals.

Discussion

The threat of tick-borne diseases to the human population has dramatically risen in recent years because current climatic changes have promoted tick reproduction and forest-based recreational activities have increased in popularity. One tick-borne zoonosis is human granulocytic anaplasmosis, caused by *A. phagocytophilum*. The vector of this pathogen in Europe is the common tick, *I. ricinus*. Investigations into the reservoirs of this pathogen allow a more thorough description of its ecology and can also be the basis of risk assessment for people residing in tick infested areas. The risk of human infection is influenced not only by the presence of ticks, but also indirectly by the presence of host animals maintaining the pathogen in the ecosystem. There have also been records of infections from people who did not notice ticks on their bodies, but admitted having been in contact with infected blood of game animals [22]. Foresters, hunters and people that deal with the meat of wild game on a regular basis (such as shopkeepers) are therefore especially at risk from tick-borne diseases, not only because of their increased exposure to ticks, but also because of contact with infected tissues such as the blood of wild mammals.

Ruminants seem to be the most competent reservoirs for *A. phagocytophilum*. Even though many studies have documented infection in various species of rodents, the latter are probably not capable of maintaining the pathogen in the environment because of the low efficiency of transmission [27]. Studies in Great Britain by Bown et al. [20] have disclosed a distinct seasonality of infection in *C. glareolus* and *A. sylvaticus*. *A. phagocytophilum* was not detected in rodents trapped between January and April, while the highest level of infection was recorded during late summer and autumn. These results suggest a short-term bacteremia in rodent hosts (estimated at only a few weeks). Relatively little is known of the bird reservoirs of *A. phagocytophilum*. Alekseev et al. [28] detected *A. phagocytophilum* in *I. ricinus* from 5 species of passerines caught near Kaliningrad; similar studies were conducted in Sweden by Bjöersdorff et al. [29]. Our latest investigations on the rodent *A. flavicollis* and 9 species of birds in Wielkopolski National Park have shown that *A. phagocytophilum* is absent in the blood of these animals, although it was found in ticks collected from rodents, birds and vegetation in the same area (Skotarczak et al., in preparation). The ticks must have become infected by a different group of host species, possibly from ruminants.

The level of *A. phagocytophilum* infection in roe deer from forested habitat near Szczecin (31.94%) suggests that these animals are a natural reservoir of this pathogen, which has already been shown by other authors [1, 22, 23, 25]. As in rodents [20] a clear seasonality of infection was found. During the autumn/winter seasons, infection was nearly twice as high (44.82%) as during spring (23.25%). It seems that in contrast to rodents, bacteremia in roe deer is long-term and these hosts are capable of maintaining *A. phagocytophilum* in the environment. However, in order to fully understand the seasonal dynamics of infection in roe deer, it is necessary to study the species throughout one or several years.

DNA of *A.phagocytophilum* was most commonly detected in blood samples; only in three cases was anaplasma DNA detected in tissues and not in blood. This may be the result of a late phase of infection during which the bacteremia disappears because of pathogen migration into the spleen of hosts.

In roe deer, *A. phagocytophilum* DNA was found most often in blood. This is in contrast to rodent studies, in which the bacteria were present in the spleen for a longer period of time than in the blood. Results from spleen samples in rodents have also shown a twofold higher level of infection when compared to blood samples, which was probably caused by a short-term bacteremia [21]. Roe deer can therefore infect feeding ticks for a much longer period of time than rodents, hence their more important role as vectors of *A. phagocytophilum*. The abscence of the pathogen in ticks collected from the animals is not surprising considering that only 4 blood samples from tick-infested roe deer were positive.

Moreover, only a small number of ticks were collected: 2 female ticks each from two of the deer,

4 female ticks from another animal and 4 females and 2 males (unfed) from the fourth deer. This very small tick sample explains why the DNA of *A. phagocytophilum* was not detected even though the ticks had taken a blood meal. Also, they might have been feeding for an insufficient period of time for infection to take place. The abscence of *A. phagocytophilum* DNA in the remaining ticks could have been caused by the low level of infection seen consistently in individuals collected from vegetation at several sites in the Zachodniopomorskie vovoidship. This level has been maintained for several subsequent years [17, 18, 30, 31].

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