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PCR DETECTION OF GRANULOCYTIC *ANAPLASMA* AND *BABESIA* IN *IXODES RICINUS* TICKS AND BIRDS IN WEST-CENTRAL POLAND

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Abstract: The aim of the study was to establish the role of forest birds as reservoirs of *Anaplasma phagocytophilum* and *Babesia* spp. in Wielkopolski National Park. A total of 108 birds from 9 species were collected between May–September 2002. Blood samples were taken from 84 specimens and 442 individuals of the common tick, *Ixodes ricinus*, were collected from the birds. The 73 additional ticks were collected from vegetation. PCR amplification of a fragment of the *epank 1* gene and 18S rRNA gene was used for detection of *A. phagocytophilum* and *Babesia* spp. DNA, respectively.
Pathogen DNA was not detected in any of the blood samples or ticks collected from birds. On the other hand, 3 ticks collected from vegetation (4.1% of all examined specimens) were positive for *A. phagocytophilum* DNA. In spite of the high level of infestation of birds by *I. ricinus*, it is clear that they do not constitute a competent reservoir of *A. phagocytophilum* and *Babesia* in WNP. Additionally, *I. ricinus* is not a significant vector in this area.

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INTRODUCTION

A new systematic classification of ehrlichia has been implemented recently, based on genetic analysis of 16S rRNA, *groESL* and surface proteins. The former *Ehrlichia phagocytophila*, *Ehrlichia equi* and the human granulocytic ehrlichiosis agent are currently assigned to the same species *Anaplasma phagocytophilum* because of their genetic similarity [7]. This pathogen is widely distributed in Europe and North America. Its reservoirs are forest dwelling rodents and ruminants [1, 4]; birds have also been considered as potentially maintaining *A. phagocytophilum* in the environment [2, 3]. Numerous studies have shown that the common tick, *Ixodes ricinus*, is the main vector of anaplasma in Europe [5, 6, 10, 19]. This tick also transmits other pathogens such as *Babesia* [11, 15, 16].

Our previous studies in a forest habitat of the Wielkopolski National Park (WNP) have shown that yellow-necked mice (*Apodemus flavicollis*) are a competent reservoir of *Borrelia burgdorferi* sensu lato, and also that feeding *I. ricinus* ticks are competent vectors for *B. burgdorferi* s.l. and *A. phagocytophilum* [12, 17]. In the present study, the role of forest birds as reservoirs of *A. phagocytophilum* and *Babesia* spp. is explored in WNP.

MATERIAL AND METHODS

Birds were caught in ornithological nets (permit no. DLOPiKog.4201/154/00). The nets were checked at least every 2 hours from sunrise to sunset during four consecutive days between May–September 2002. The captured specimens were put in cloth bags and transported

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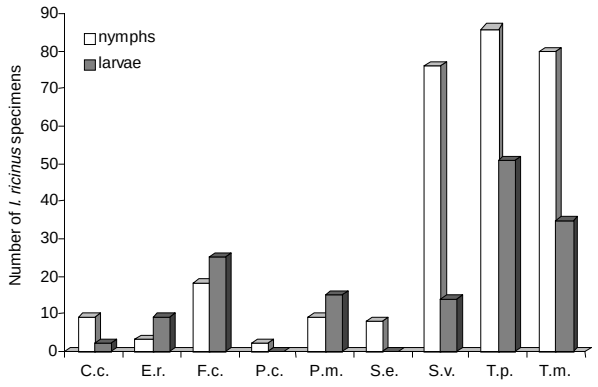
to a field station. Ticks were removed from infested birds and blood (about 2 µl) was drawn by hypodermic needle and pipette from the shoulder vein. Birds were kept alive through the entire process. A total of 108 birds from 9 species were captured. Blood samples were drawn from 84 of these. 442 specimens of *I. ricinus* ticks (291 nymphs and 151 larvae) were collected from the birds, and an additional 73 ticks (33 imago and 8 pools consist of 5 nymphs) were collected from vegetation. The DNA was extracted from ticks with the method described by Guy and Stanek (1991). The DNA from bird blood was isolated using the Master Pure^{TM} DNA Purification Kit (Epicentre, USA) and stored at –70ºC.

Detection of *A. phagocytophilum* DNA. For the *epank 1* gene, a fragment of 444 bp was amplified by using primers LA1 and LA6 [21].

Detection of *Babesia* DNA. A fragment of the gene encoding the nuclear small-subunit ribosomal RNA (SS-rDNA) was used as a target with primers 1FOR(5'-TGTCTTAAAGATTAAGCCATGCATGT-3') and 1REV (5'-TTGTGA ACC TTATCACTTAAAGGAAG-3') with an expected product size of 1650 bp. These primers were constructed by our group. The time/temperature profiles of the PCR's were the same as described earlier [19]. PCR products were separated by electrophoresis in 2% agarose (ICN, USA) and stained with ethidium bromide. For molecular size assessment, a mass marker was used (MW501, Polgen, Łódź).

RESULTS

The 108 captured birds were assigned to the following species (number of specimens in parentheses): *Coccothraustes coccothraustes* (6), *Erithacus rubecula* (6), *Fringilla coelebs* (10), *Parus caeruleus* (1), *Parus major* (11), *Sitta europaea* (6), *Sturnus vulgaris* (20), *Turdus philomelos* (24), *T. merula* (24). The largest number of ticks was collected from *T. philomelos* – 86 nymphs and 51 larvae, and also *T. merula* – 80 nymphs and 35 larvae.



Birds species: C.c. - *Coccothraustes coccothraustes*, E.r. - *Erithacus rubecula*, F.c. - *Fringilla coelebs*, P.c. - *Parus caeruleus*, P.m. - *Parus major*, S.e. - *Sitta europaea*, S.v. - *Sturnus vulgaris*, T.p. - *Turdus philomelos*, T.m. - *Turdus merula*

Figure 1. Number of ticks *I. ricinus* collected from birds from Wielkopolski National Park (May–September 2002).

25 larvae were collected from *F. coelebs* (chaffinch). The smallest number of ticks was collected from *P. caeruleus* (blue tit) - 2 and *S. europaea* (nuthatch) – 8. (Fig. 1). No larvae were present on the blue tit and nuthatch. A total of 73 ticks I. *ricinus* were taken from vegetation, the largest number in June (41.1%), the smallest in September (9.6%). During July, no ticks were collected from the vegetation, which may have been the result of high air temperature and low humidity. From 73 individuals of *I. ricinus* were fixed in 41 isolates (33 imago and 8 pools consist of 5 nymphs). Pathogen DNA was not detected in any of the blood samples or ticks collected from birds. On the other hand, 3 females of ticks collected from vegetation (4.1% of all examined specimens, 9.1% of imago) were positive for *A. phagocytophilum* DNA.

DISCUSSION

In Poland, only a few areas have been screened for the level of infection of *I. ricinus* by *A. phagocytophilum*. Studies from the northwest and northeast have shown that the mean level of infection varies between 1.4% in the Zachodniopomorskie Province [18] to 16% in Białowieża forest [9] and 19.2% in Pomorskie Province [20]. Areas inhabited by ticks also support many species of animals potentially serving as reservoirs for *A. phagocytophilum* or the *Babesia* species. The zoonotic reservoir of *A. phagocytophilum* is still being explored; it is assumed that small mammals, birds and game animals are reasonable candidates [1]. This study, screening blood collected from birds for *A. phagocytophilum*, has shown that birds are not reservoirs of this pathogen in WNP. A previous study has also ruled out rodents [17]. Game animals are probably the most important in this respect.

Bird reservoirs of *A. phagocytophilum* have so far been little studied. Alekseev *et al.* [2] studied infection in ticks collected from 8 species of migrating passerine birds. These birds were captured in the Kaliningrad district during spring and autumn 2000. A total of 1606 birds were captured but ticks were removed from only 110 (6.8%). Pathogen DNA was detected in 14% of the examined ticks. Alekseev *et al.* [2] suggest that the human granulocytic agent was acquired by co-feeding, not from an infected bird.

Björsdorff *et al.* [3] conducted a similar study in Sweden. From 3054 passerine birds, only 73 (from 18 species) were infested by ticks, from which 165 individuals of *I. ricinus* were collected. Anaplasma DNA was detected in 8% of nymphs. A study establishing the significance of pheasant, *Phasianus colchicus*, as a zoonotic reservoir was conducted in England [8, 13]. Pheasants were host to *Borrelia* but were not a competent reservoir of *Anaplasma* because this pathogen was not present in any of the studied birds.

From literature published to date, it seems that birds from several families can be hosts of many species of *Babesia*. According to a review by Peirce [14], the most common pathogens of birds are: *B. ardeae*, *B. avium*,

B. balearicae, *B. bennetti*, *B. emberizica*, *B. frugilegica*, *B. henryi*, *B. kazachstanica*, *B. krylovi*, *B. moshkovskii*, *B. mujunkumica*, *B. peircei*, *B. polea*, *B. rustica*, *B. shortti* and *B. socius*. The PCR primers used in the present study allow for the detection of most of these pathogens, however, a negative result was obtained in all samples. This may be caused by the small number of birds collected from each species. These pathogens seem to be highly species-specific in their choice of host.

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