 

# Genetic diversity of *Babesia* spp. in adult *Dermacentor* reticulatus ticks in urban and natural biotopes of north-eastern Poland

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The intraerythrocytic protozoans of the *Babesia* genus are etiological agent of the important tick-borne disease in humans and animals. The aim of the study was to determine the prevalence of the *Babesia* species and evaluate their molecular diversity in adult *D. reticulatus* ticks collected in urban and natural biotopes of north-eastern Poland.

Questing adult *D. reticulatus* ticks (n = 482, 302 females, 180 males) were collected between March–June in 2016–2017 in five sites located in urbanized areas (n=395), within the administrative borders of the city of Olsztyn and eight sites in natural biotopes of the central part of the Warmia and Mazury region (n=87). The presence of *Babesia* spp. in tick genomic DNA samples was confirmed by the nested PCR method using two sets of primers specific to 18S rRNA gene (CRYPTO R/CRYPTO F and BabGR2/BabGF2) (Mierzejewska *et al.,* 2015). The identification *Babesia* species were based on sequencing of inner PCR products (Macrogen Europe, the Netherlands). The obtained nucleotide sequences were edited in BioEdit software and compared with data registered in the GenBank database (http://www.ncbi.nih.gov/Genbank/index.html) using the BLAST-NCBI program. A chi-square test was used to compare the infection prevalence of *Babesia* spp. between sex of ticks, study areas and years of study.

In north-eastern Poland the overall infection rate of *Babesia* spp. in *D. reticulatus* ticks was 3.9% (19/463). The infection rate did not differ between females (4.6%) and males (2.9%) and year of study (2016–2017), 2.8% and 4.8%, respectively. *Babesia* prevalence was significantly higher in ticks collected in natural biotopes (9.2%) than in urban areas (2.8%) ($\chi 2$=7.738, df=1; p=0.005).

Sequence analysis of partial 18S rRNA gene indicated the presence of *B. canis* and *B. microti*. *B. canis* was identified in 63.2% (n=12) positive isolates from ticks collected in both natural (n=8) and urban (n=4) biotopes. *B. microti* were found in 36.8% (n=7) positive samples obtained only from *D. reticulatus* ticks collected in urban area.

In partial sequences of *B. canis* 18S rRNA gene at the position corresponding to the 609–610 nucleotides of complete gene (GenBank: AY072926) the substitutions (GA/AG) were observed. In 66.7% (8/12) of B. canis isolates GA dinucleotide (genotype I) was detected. Among them two polymorphic variants (T↔C) were identified. 25% (3/12) *B. canis* isolates were monomorphic and represented group with GA/AG nucleotides double pick in these position (genotype II). AG

nucleotide combination (genotype III) was presented in one *B. canis* positive sample (8.3%, 1/12). All isolates identified as *B. microti* showed 99–100% identity with the genetic variants included to nonpathogenic *B. microti* Munich type (GenBank: AB071177, AY789075).

In conclusion, prevalence and species diversity of *Babesia* in *D. reticulatus* ticks in the Warmia-Mazury region is generally low. Populations of ticks from urban areas have greater species diversity of *Babesia* in compared to natural biotope. The presence of *B. canis* and the non-pathogenic Munich strain of *B. microti* indicates that *D. reticulatus* ticks play more significant role as a vector of tick-borne diseases for animals than humans in north-eastern Poland.