Detection of *Borrelia burgdorferi* sensu lato, *Anaplasma* phagocytophilum and *Babesia microti* in *Ixodes ricinus* collected on selected recreational areas of Kraków-Częstochowa Upland

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Borrelia burgdorferi sensu lato, Anaplasma phagocytophilum and Babesia microti are the etiological agents of such diseases as Lyme disease, human granulocytic anaplasmosis and human babesiosis. In Poland and Europe, the main vector of these pathogens is *Ixodes ricinus*. This tick species occurs mainly in forests, meadows and fields, and so poses a real threat to health of humans and animals.

The aim of study was to identify *B. burgdorferi* s. l., *A. phagocytophilum* and *B. microti* in ticks collected on the selected areas of the Kraków-Częstochowa Upland. The examined ticks were obtained from three popular recreational areas of the Polish Highlands: Rabsztyn, Ogrodzieniec and Mirów. In total, DNA was isolated form 153 ticks (61 females, 39 males, 53 nymphs) by the ammonia method. Pathogens in ticks were identified by PCR and nested PCR. *B. burgdorferi* s. l. was detected using primers specific to the flagelline gene, *A. phagocytophilum* for the 16S rDNA gene and *B. microti* with primers for 18S rRNA. The PCR and nested PCR products were separated electrophoretically in 2% ethidium bromide stained agarose gels. The expected products sizes were as follows: 482 base pairs [bp] for *B. burgdorferi* s. l., 932 bp and 546 bp for *A. phagocytophilum* and 238 bp and 154 bp for *B. microti*.

Generally, A. phagocytophilum and B. microti were found in 1.96% and 28.10% of the examined ticks, respectively. The rickettsia was detected in 4.88% of ticks collected in Rabsztyn and in 1.37% of ticks collected in Mirów. The protozoan B. microti was found in 41.46% of ticks collected in Rabsztyn, 20.51% of ticks collected in Ogrodzieniec and in 24,66% of ticks collected in Mirów. A. phagocytophilum was detected only in adult ticks (3% of the examined adults) and B. microti mainly in nymphs (35.85% of examined nymphal stages). B. burgdorferi s. l. was not found in the studied material.

Our findings indicate a high risk of exposure of humans to *B. microti* and a low risk to *A. phagocytophilum* in the studied areas. Tourists and other people who spend their free time in these areas of the Kraków-Częstochowa Upland should be careful and take appropriate precautions against ticks.