

Study on prevalence of *Toxoplasma gondii* infection in free-living animals in Poland

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Free-living animals are important reservoir of *Toxoplasma gondii*, however data concerning this issue in Poland are limited. Identification and genetic characteristics of *T. gondii* isolated from these animals can help to understand the ways of circulating of the parasite in the environment.

The aim of the study was the assessment of prevalence of *Toxoplasma* infection in selected species of free-living animals in Poland using PCR examination. Samples of tissues (muscles, diaphragm) from 336 animals (foxes, birds, martens, badgers, polecats, raccoons, minks, raccoon dogs, squirrels, mice and snakes) originated from different regions of Poland. After samples digestion, DNA was isolated using QIAamp DNA Mini Kit (Qiagen). Next, nested PCR with the use of B1 gene was performed. For selected B1 positive samples, multiplex PCR was performed using genetic markers SAG1, SAG2 (5'-SAG2 and 3'-SAG2), SAG3, BTUB and GRA6, according to Dubey et al. (2006) method. Amplicons were sequenced and analyzed using Bioedit and MEGA 7 software, and compared with the NCBI database using Blast.

In total, in 44 of 336 examined animals DNA of *T. gondii* was detected (13.1%). The highest percentages of positive results in PCR was obtained for martens (40.9%) and badgers (38.5%), lower for birds (27.3%) and the lowest for foxes (7.4%). Multilocus sequence typing of selected B1 positive samples found type II and III.

The results of study indicate on frequent *Toxoplasma* infection among free living animals in Poland, especially martens and badgers, what can be an indirect indicator for the toxoplasmosis epidemiology assessment. The multilocus sequence analysis showed the dominance of *T. gondii* clonal type II and III.