

## Molecular detection of *Anaplasma phagocytophilum* in carnivores in north-east Poland

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*Anaplasma phagocytophilum* is an obligate parasitic intracellular bacterium. It is the causative agent of granulocytic anaplasmosis, with effects on human and animal health. In Europe, the pathogen is mainly transmitted among a wide range of vertebrate hosts by blood-sucking arthropods. The aim of this study was to determine the presence of *A. phagocytophilum* in wild carnivores, viz. raccoon dogs (*Nyctereutes procyonoides*), badgers (*Meles meles*), foxes (*Vulpes vulpes*), martens (*Martes* sp.) and European polecats (*Mustela putorius*), using molecular methods in north-eastern Poland.

A total of 174 samples were collected from the spleens of test subjects. The largest group were extensively studied raccoon dogs (68 pers.), foxes (29 pers.), badgers (49 pers.), martens (24 pers.) and polecats (4 pers.). DNA was isolated by Genomic AX Tissue Mini kit (A & A Biotechnology, Gdynia). In the present study, 174 spleen samples were collected from adult, wild carnivores hunted in the years between 2013–2016. A short fragment (383 bp) of the 16S ribosomal RNA gene, partial sequence was used as a marker to identify *A. phagocytophilum* in spleen samples collected from carnivores using nested PCR.

In our study the prevalence for *A. phagocytophilum* in wild carnivores was 31.61% (55/174). Among the respondents, the highest producing animals extensiveness of infection was at martens and amounted to 41.70%, with raccoon dog was 35.30% and 34.48% in foxes. In the case of badgers extensiveness of infection was 18.70%. *A. phagocytophilum* was detected in two of four investigated European polecats. During the tests, it was observed that there is a large difference between the first and the second reaction in nested PCR. After first reaction our prevalence was only 2.00% in all tested animal. After the second stage, the extensity was higher and amounted to 31.60%. Seven sequences of *A. phagocytophilum* were collected from two raccoon dogs, two badgers, one marten, one red fox and one European polecat (MH328205–MH328211).

Our findings represent the first detection of *A. phagocytophilum* in badgers, raccoon dogs, martens and polecats from Poland. The nested PCR used in the present study was more sensitive to detection of *A. phagocytophilum* than standard PCR for spleen tissue samples.