**ANAPLASMA PHAGOCYTOPHILUM INFECTION OF RED FOXES (VULPES VULPES)**

Grzegorz Karbowiak¹, Bronislava Víchová², Viktória Majláthová², Joanna Hapunik¹, Branislav Peťko²

¹W. Stefański Institute of Parasitology of the Polish Academy of Sciences, Warsaw, Poland
²Parasitological Institute of the Slovak Academy of Sciences, Košice, Slovakia


Abstract: The investigation of Anaplasma phagocytophilum infection covered 111 red foxes from the Mazovian Province. Determination was based on PCR amplification of 16S rRNA gene and the portion of msp4 gene coding MSP4 major surface protein. The presence of A. phagocytophilum was found in 2.7% of foxes.

Address for correspondence: Dr. Grzegorz Karbowiak, W. Stefański Institute of Parasitology of the Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw, Poland.
E-mail: grzgrz@twarda.pan.pl

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INTRODUCTION

Wild canids can play an important role in the transmission and maintenance of metazoan parasites in natural foci [3, 8]. However, little is known about the blood parasites of foxes and other wild living carnivores. In our study, we focused on Anaplasma phagocytophilum infection of red foxes (Vulpes vulpes) in the north-eastern part of Poland. The main vector of A. phagocytophilum in Poland is Ixodes ricinus tick [1, 13], and the animal reservoirs are small rodents and ruminants [2, 4, 5]. However, other mammals able to serve as animal reservoir are still being found.

MATERIALS AND METHODS

The diagnostic was made using conventional as well as nested PCR methods. The reactions were conducted on spleen samples from 111 red foxes, obtained from the Sanitary and Epidemiological Station in Ostrołęka, Mazovian Province, Poland. DNA was isolated and purified using Genomic Mini AX tissue (A&A Biotechnology), according to the manufacturer’s protocol. Molecular searching for A. phagocytophilum was based on nested PCR amplification of approximately 640 bp long fragment of 16S rRNA gene [7], and the portion of msp4 gene coding MSP4 major surface protein with a length of 849 bp [2, 7].

PCR amplifications were performed in a total volume of 25 μL of reaction mixture from the MasterTaq DNA polymerase kit (Eppendorf AG, Hamburg, Germany) containing 10.4 μL deionized water, 5 μL 5× TaqMaster PCR Enhancer, 2.5 μL 10× Taq buffer (with 15 mmol/L Mg2+), 1.5 μL 25-mmol/L solution of Mg (OAc)2, 0.1 μL Taq DNA polymerase (5 U/μL), 0.5 μL deoxynucleoside triphosphate (dNTP) mix (10 mmol/L) (Fermentas, Vilnius, Lithuania), and 1.25 μL of each primer (10 pmol/μL) (Invitrogen, Paisley, Scotland). The PCR assays were carried out using primers (EC9,EC12A and SSAP2f, SSAP2r) for the nested PCR amplification of 16S rRNA and primers (MSP4AP5, MSP4AP3) for conventional PCR amplification of msp4 gene, as published previously [2, 7]. As a template, 2.5 μL of isolated DNA was used. As a negative control, nuclease free water was added to a PCR mix instead of DNA. Sequenced A. phagocytophilum DNA isolated from A. phagocytophilum – positive deer blood sample was used as a positive control. Amplification was performed using a nested PCR protocol [2, 7].

PCR products were electrophoresed on 1% agarose gel, stained with GoldView Nucleic Acid Stain (Beijing SBS Genetech Co. Ltd.). The fragments amplified, 641 bp in size, were compared with 100 bp DNA molecular weight marker. PCR products were purified by using a QIAquick PCR purification kit (QIAGEN) for further sequencing.

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performed at the Department of Molecular Biology (Faculty of Natural Science, Comenius University, Bratislava, Slovak Republic). The complementary strands of sequenced products were manually adjusted and compared with GenBank entries by Blast N2.2.13. 16S rRNA gene nucleotide sequences of approximately 600 bp were submitted into the GenBank, and deposited under accession numbers GQ162213 and GQ162214, respectively.

RESULTS

The presence of *A. phagocytophilum* DNA was recorded in 3 out of 111 examined samples. The overall prevalence of bacterial infection represents 2.7%. Sequences obtained in our study shared a 99% similarity with partial sequences of *A. phagocytophilum* 16S rRNA gene deposited in the GenBank under accession numbersAY055469, AY527214, AY527213, respectively, as well as with *A. phagocytophilum* HZ strain’s complete genome deposited under CP000235.

DISCUSSION

Until now, only serological studies in Switzerland have suggested that red foxes might be infected with *Anaplasma* [11]. Later, *A. phagocytophilum* was detected using PCR method in ticks collected from red foxes in Hungary [12] and in many wild mammals in the Czech Republic, Slovenia and Spain [6, 9, 10]. Small rodents and the European bison *Bison bonasus* in Poland, as potential hosts of *A. phagocytophilum* have also been recognized [4, 5]. However, the presence of this pathogen in foxes and other wild carnivores in Poland has so far not been considered.

The prevalence of infection of red foxes in Poland is similar to the percentage noted in Switzerland. The prevalence of infection with *Anaplasma phagocytophilum* in animals by real-time polymerase chain reaction, *APMIS* 2004, 112, 239-247.

References


