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

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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.

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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 numbers AY055469, 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 [11] and higher than that recorded in the Czech Republic [6]. Data obtained represent the first records for red foxes in Poland, and can demonstrate the potential ability of foxes to serve as a reservoir of infection for domestic dogs and other wild canine species. It is significant that the prevalence of infection is lower than in vectors – the prevalence of infection in I.ricinus tick is ranges to 24% [1, 13].

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REFERENCES

1. Chmielewska-Badora J, Zwoliński J, Cisak E, Wójcik-Fatla A, Buczek A, Dutkiewicz J: Prevalence of *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks determined by polymerase chain reaction with two pairs of primers detecting 16S rRNA and ankA genes. *Ann Agric Environ Med* 2007, **14**, 281-285.

2. De la Fuente J, Naranjo V, Ruiz-Fons F, Höfle U, Fernández De Mera IG, Villanúa D, Almazán C, Torina A, Caracappa S, Kocan KM, Gortázar C: Potential vertebrate reservoir hosts and invertebrate vectors of *Anaplasma marginale* and *A. phagocytophilum* in Central Spain. V*ector-Borne Zoonotic Dis* 2005, **5**, 390-401.

3. Di Cerbo AR, Manfredi MT, Bregoli M, Ferro Milone N, Cova M: Wild carnivores as source of zoonotic helminths in north-eastern Italy. *Helminthologia* 2008, **45**, 13-19.

4. Grzeszczuk A, Karbowiak G, Ziarko S, Kovalchuk O: The Root-vole *Microtus oeconomus* (Pallas, 1776): a new potential reservoir of *Anaplasma phagocytophilum*. *Vector-Borne Zoonotic Dis* 2006, **6**, 240-243.

5. Grzeszczuk A, Ziarko S, Prokopowicz D, Radziwon PM: Zakażenie żubrów z Puszczy Białowieskiej bakteriami *Anaplasma phagocytophilum*. *Med Wet* 2004, **60**, 600-601.

6. Hulínská D, Langrová K, Pejcoch M, Pavlásek I: Detection of *Anaplasma phagocytophilum* in animals by real-time polymerase chain reaction. *APMIS* 2004, **112**, 239-247.

7. Kawahara M, Rikihisa Y, Lin Q, Isogai E, Tahara K, Itagaki A, Hiramitsu Y, Tajima T: Novel genetic variants of *Anaplasma phagocytophilum*, *Anaplasma bovis*, *Anaplasma centrale*, and a novel *Ehrlichia* sp. in wild deer and ticks on two major islands in Japan. *Appl Environ Microbiol* 2006, **72**, 1102-1109.

8. Miterpáková M, Hurníková Z, Antolová D, Dubinský P: Endoparasites of red fox (*Vulpes vulpes*) in the Slovak Republic with the emphasis on zoonotic species *Echinococcus multilocularis* and *Trichinella* spp. *Helminthologia* 2009, **46**, 73-79.

9. Oporto B, Gil H, Barral M, Hurtado A, Juste RA, Garcia-Perez AL: A survey on *Anaplasma phagocytophila* in wild small mammals and roe deer (*Capreolus capreolus*) in Northern Spain. *Ann NY Acad Sci* 2003, **990**, 98-102.

10. Petrovec M, Bidovec A, Sumner JW, Nicholson WL, Childs JE, Avšič-Županc T: Infection with *Anaplasma phagocytophila* in cervids from Slovenia: evidence of two genotypic lineages. *Wien Klin Wochenschr* 2002, **14/113**, 641-647.

11. Pusterla N, Deplazes P, Braun U, Lutz H: Serological evidence of infection with *Ehrlichia* spp. in red foxes (*Vulpes vulpes*) in Switzerland. *J Clin Microbiol* 1999, **37**, 1168-1169.

12. Sréter T, Sréter-Lancz Z, Széll Z, Kálmán D: *Anaplasma phagocytophilum*: an emerging tick-borne pathogen in Hungary and Central Eastern Europe. *Ann Trop Med Parasitol* 2004, **98**, 401-405.

13. Wójcik-Fatla A, Szymańska J, Wdowiak L, Buczek A, Dutkiewicz J: Coincidence of three pathogens (*Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia microti*) in *Ixodes ricinus* ticks in the Lublin macroregion. *Ann Agric Environ Med* 2009, **16**, 151-158.