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Short communication

The identification of *Anaplasma* spp. isolated from fallow deer (*Dama dama*) on a free-range farm in eastern Poland

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Abstract

The aim of the present study was to investigate the occurrence of *Anaplasma* spp. in group of 50 fallow deer (*Dama dama*) from free-range farm in eastern Poland and determine what species of *Anaplasma* could infect these animals based on PCR gene sequencing. The PCR technique revealed the presence of 16S RNA *Anaplasma* spp. genetic material in the blood of 7 out of 50 examined animals. The sequences of the PCR products obtained showed a 100% homology with each other and 100% homology with GU 183908 sequence of *A. phagocytophilum*, isolated in our earlier study from a horse with clinical form of anaplasmosis. Here, we report the first molecular evidence of *Anaplasma* spp. among naturally infected fallow deer in eastern Poland.

Key words: *Anaplasma phagocytophilum*, fallow deer, PCR, molecular sequencing

Introduction

Anaplasmosis is a febrile disease and an acute infection in humans, ruminants, equines, and canines (Winiarczyk et al. 2007), transmitted by Ixodid ticks. In eastern Poland Winiarczyk et al. (2007) and Adaszek and Winiarczyk (2007) demonstrated antibodies against *A. phagocytophilum* among serum samples from dogs, pigs, and cattle. The clinical disease in this region of the country was diagnosed in horses (Adaszek and Winiarczyk 2011), dogs and cat (Zygner et al.

2009, Adaszek et al. 2011). Wild deer are suspected reservoirs of *Anaplasma* and *Ehrlichia* species infection. There are only few reports about detection of *Anaplasma phagocytophilum* DNA in wild ruminants in Poland (Adamska and Skotarczak 2007).

The aim of the present study was to investigate the occurrence of *Anaplasma* spp. infections in fallow deer (*Dama dama*) and determine what species of *Anaplasma* could infect these animals based on PCR gene sequencing.

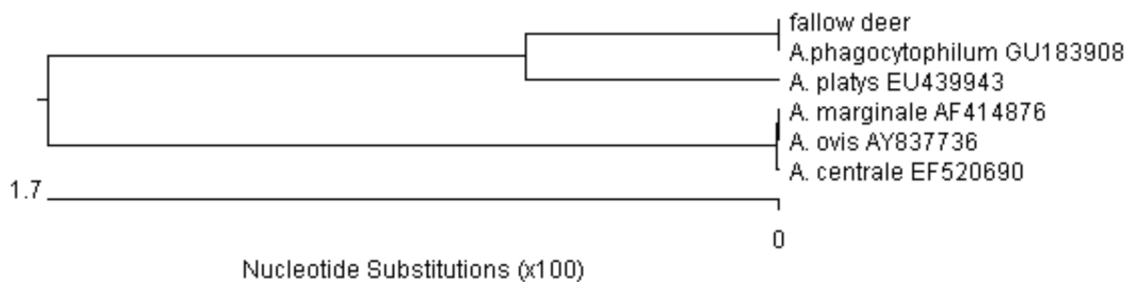


Fig. 1. Phylogenetic relationships between sequences of the isolates investigated in the present study (all isolates showed 100% sequence homology, therefore they were described as a one sequence- fallow deer) and sequences deposited in the GenBank.

Materials and Methods

The study covered 50 fallow deer from a free-range farm situated in eastern Poland (Polesie Lubelskie province). From each animal one EDTA-anticoagulated whole blood sample was collected for molecular examination of the presence of *Anaplasma* spp. genetic material.

A process of DNA extraction and *Ehrlichia/Anaplasma* 16S rRNA gene amplification was described in the earlier study (Adaszek and Winiarczyk 2011). PCR products obtained in the present study were purified using the QIAquick spin columns (Qiagen) and sequenced. DNA sequences were assembled and edited using SeqMan (DNASTar, Lasergene, Madison, USA), and ClustalV alignments to the published in GenBank european *Anaplasma* spp. 16S rRNA gene (*A. ovis* AY837736; *A. marginale* AF414876; *A. platys* EU439943; *A. centrale* EF520690; *A. phagocytophilum* GU183908).

Results and Discussion

Out of the 50 samples examined for *Anaplasma/Ehrlichia* spp., 7 showed amplification products with a size of 252-bp. Legible sequences of DNA were obtained for all 7 PCR products. All of them showed 100% homology of the studied sequence of 16S rRNA gene fragment. Using the application of Lasergene a comparison was made between the sequences of 16S rRNA gene fragment from our own studies, with sequences of other *Anaplasma* spp., that can infect deer and wild ungulates in Europe. The results of this analysis demonstrated a close similarity between tested isolates ranging from 96.4 to 100%. The highest homology (100%) was observed between the in the present study examined isolates and GU183908 *A. phagocytophilum* sequences from our earlier study (Adaszek and Winiarczyk 2011). These sequences were placed on one branch of a dendrogram (Fig. 1). Considering the remaining investigated isolates, the highest similarity (98.8%) characterized *Anaplasma* sequences obtained in the present study and sequences of *A. platys* EU439943. Other sequences from GenBank showed lower homology with isolates obtained in the present study, and therefore they were all placed on a separate branch of the phylogenetic tree. The most common rickettsia species

isolated globally from the blood of wild ruminants are: *A. phagocytophilum*, *A. platys*, *A. ovis*, *A. marginale*, and *A. centrale* (Munderloh et al. 2003). This study showed the presence of genetic material of *A. phagocytophilum* in 7 out of the 50 tested fallow deer. This is the first report of *A. phagocytophilum* in fallow deer (*Dama dama*) in eastern Poland, suggesting that they may act as reservoirs for anaplasmosis zoonotic pathogens. Along with the changing climatic conditions and the increasing risk of anaplasmosis in native animals, it seems advisable to introduce continuous monitoring of this infection among domestic, farm and wild animals.

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