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MOLECULAR DETECTION OF *ANAPLASMA PHAGOCYTOPHILUM* IN WILD BOAR (*SUS SCROFA*) FROM HUNEDOARA AND TIMIȘ COUNTIES - PRELIMINARY STUDY

DETECȚIA MOLECULARĂ A SPECIEI *ANAPLASMA PHAGOCYTOPHILUM* LA MISTREȚII (*SUS SCROFA*) DIN JUDEȚELE HUNEDOARA ȘI TIMIȘ - STUDIU PRELIMINAR

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ABSTRACT

Anaplasma spp., gram-negative bacteria with a specific cell wall structure, are tick-borne pathogens that cause disease in both humans and animals. *Anaplasma phagocytophilum* is an obligate intracellular parasite that causes granulocytic anaplasmosis in humans, horses, and dogs, respectively, granulocytic anaplasmosis or tickborne fever in ruminants. This study aimed to investigate the occurrence of *Anaplasma phagocytophilum* in hunted wild boars from Hunedoara and Timiș counties. A total of 29 blood samples were collected (Hunedoara =14 and Timiș =15) and examined using polymerase chain reaction (PCR) techniques and specific primers (LA6 and LA1) for *A. phagocytophilum*. One sample out of 29 was positive for *Anaplasma phagocytophilum* infection (3.44%). The results of the present study indicate the necessity to extend the evaluation of *Anaplasma phagocytophilum* occurrence in wild boar populations in order to establish the role of the reservoir host in disease transmission in the studied area.

Keywords: anaplasmosis, *Anaplasma phagocytophilum*, wild boar, PCR

REZUMAT

Anaplasma spp., o bacterie gram-negativă cu o structură specifică a peretelui celular, este un agent patogen transmis de căpușe care provoacă boli atât la om, cât și la animale. *Anaplasma phagocytophilum* este o bacterie intracelulară care provoacă anaplasmoza granulocitară la om, anaplasmoza granulocitară ecvină și canină și febra transmisă de căpușe la rumegătoare. Acest studiu a avut ca scop investigarea prezenței *Anaplasma phagocytophilum* la mistreții vânați din județele Hunedoara și Timiș. Un număr total de 29 de probe de sânge au fost colectate (Hunedoara =14 și Timiș =15) și examinate prin reacția în lanț a polimerazei (PCR). Au fost utilizați primeri specifici (LA6 și LA1) pentru *A. phagocytophilum*. Din 29 de probe, doar o singură probă a fost pozitivă pentru infecția cu *Anaplasma phagocytophilum* (3,44%). Rezultatele prezentului studiu indică necesitatea de a extinde evaluarea prezenței *Anaplasma phagocytophilum* în populațiile de mistreți pentru a stabili rolul gazdei rezervor în zona studiată.

Cuvinte cheie: anaplasmoză, *Anaplasma phagocytophilum,* mistreț, PCRai

Anaplasma spp., gram-negative bacteria with a specific cell wall structure, are vector-borne pathogens causing disease in both humans and animals. Swine anaplasmosis is an infectious disease caused by bacteria of the genus *Anaplasma*. The most common species involved is *Anaplasma phagocytophilum*. This bacterium is transmitted by ticks that feed on the blood of infected animals. After the tick has fed on/ingested infected blood, it can pass the bacteria to another healthy animal during another feeding. Symptoms of swine anaplasmosis include fever, loss of appetite, lethargy,conjunctivitis, and weight loss. In severe cases, the disease can lead to death (7, 11, 20).

Anaplasma infection has been detected in several mammalian species, including humans, in areas of the northern hemisphere where the presence of *Ixodes* ticks is endemic. *A. phagocytophilum*, as a bacterial species, appears to be generalist, infecting a wide range of animals. Several genetic variants of the bacterium have been characterised (29), and subpopulations within the species are currently being discussed.

A. phagocytophilum has, as implied by its name, a predilection for phagocytic cells and is one of the very few bacteria known to survive and replicate inside neutrophil granulocytes (5). When ticks feed, neutrophil-associated inflammatory responses are modulated by various stimuli deployed by components in tick saliva (2, 13, 14). Bacterial translocation within host cells is receptor-mediated and depends on transglutaminase activity (27).

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A. phagocytophilum is transmitted by hard ticks of the *I. persulcatus* complex. The main vector in Europe is *I. ricinus* (commonly known as the sheep tick or castor bean tick); in the eastern USA, it is *I. scapularis* (deer tick or blacklegged tick); in the western US, *I. pacificus* (western blacklegged tick); and in Asia, *I. persulcatus* (taiga tick) (36). Vector competence has been demonstrated for the American tick species *I. scapularis* (formerly *I. dammini*), *I. pacificus,* and *I. spinipalpis* (33). Transovarial transmission has not been proven in *Ixodes* species, but it has been demonstrated in *Dermacentor albipictus*, whose life cycle involves a single host animal, representing a distinct ecological spectrum (1). According to current knowledge, a vertebrate reservoir host is required in nature to maintain the endemic cycle.

Viable organisms of *A. phagocytophilum* have been isolated from several hosts, such as cattle, sheep, goats, dogs, horses, humans, deer (*Cervus elaphus*), roe deer (*Caperolus capreolus*), and white-tailed deer (*Odocoileus virginianus*) (9, 32, 30). However, several prerequisites must be met for a reservoir host to be competent for a transstadially transmitted pathogen. A vector tick must feed off an infected reservoir host and it must take up a critical number of infectious agents. It must allow the pathogen to multiply and survive for a period of time and it must allow the pathogen to reach other feeding ticks (18). Over the last decade, in the western region of the country, the continuous expansion of vector-borne pathogens has been molecularly demonstrated in several hosts including red foxes for *Hepatozoon canis* (16), dogs for *Babesia canis* (17), *Ehrlichia canis* (23), or *Dirofilaria* spp. and *Acanthocheilonema reconditum* (12). Thus, continuous monitoring of the occurrence of the main vector-borne pathogens in different hosts can constitute a useful tool in the implementation of effective control strategies. Therefore, this study aimed to investigate the occurrence of *Anaplasma phagocytophilum* in hunted wild boars from Hunedoara and Timiș counties.

MATERIALS AND METHODS

A total of 29 blood samples were collected (Hunedoara =14 and Timiș =15) and examined by polymerase chain reaction (PCR). Specific primers (LA6 and LA1) for *A. phagocytophilum* were used.

The DNA was extracted using the ISOLATE II Genomic DNA (BIOLINE®) kit. The polymerase chain reaction (PCR) was conducted in 25 µl reactions using DNA, Master Mix MyTaqTM Red Mix (BIOLINE®) and the specific primers that amplify a fragment of *A. phagocytophilum* (used to amplify epank1 gene fragment of *A. phagocytophilum*).

Primers:

- Forward primer (LA6): 5′-GAG AGA TGC TTA TGG TAA GAC-3′
- Reverse primer (LA1): 5′-CGT TCA GCC ATC ATT GTG AC-3′

The PCR reaction was carried out in a final volume of 25 µl. The amplification was done in the thermocycler My Cycler (BioRad®) using the temperatures previously described, adapted to the MyTaqTM Red Mix (BIOLINE®). Amplicons were visualised by electrophoresis in a 1.5 % agarose gel stained with Midori Green fluorescent colour (Nippon Genetics® Europe). The size of the amplified DNA fragment was 444 bp (4, 35).

RESULTS AND DISCUSSIONS

Only one sample out of 29 was positive for *Anaplasma phagocytophilum* infection (3.44%) (Figs. 1, 2).

The study presents important data about the role of wild boars as possible sources of infection with *A. phagocytophilum* for humans and farm animals.

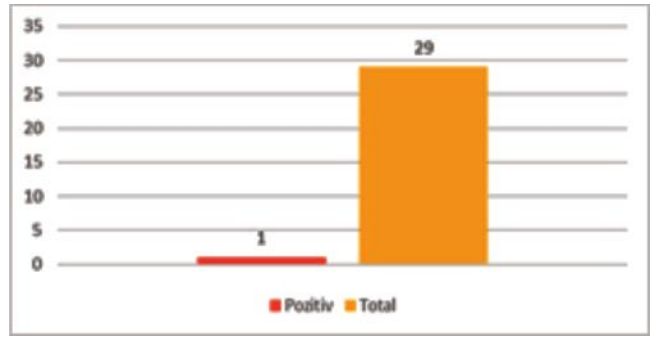

Fig. 1. Graphic representation of the presence of *Anaplasma phagocytophilum* in wild boars from Hunedoara and Timiș counties

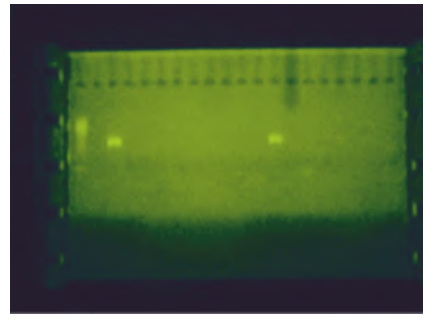

Fig. 2. Agarose (1.5%) gel electrophoresis image of PCR amplicons

Lane [1] - Molecular Weight Markers HyperLadder™ 100 bp DNA ladder (Bioline, United Kingdom); Lane [2] - Negative Control (nuclease-free water); Lane [3] - Positive Control; Lanes – [4-12] - Negative Isolates; Line [13] - Positive Isolate; Lines [14–20] - Negative Isolates (Fig. 2).

The distribution of *A. phagocytophilum* DNA

(3.44%) was low compared to previously reported rates in wild boars from other European countries (Czech Republic, Slovenia, Italy and Japan) with prevalence ranging between 6.2%–14.3% (13, 15, 21, 34). The low infection rate with *A. phagocytophilum* detected in wild boars is in accordance with the previous experimental study of Galindo et al. (2012) which demonstrated the ability of the boar´s innate immune system to control the infection with this pathogen (10).

A low prevalence rate of *A. phagocytophilum* infection has been reported in wild boars (*Sus scrofa*) from The Czech Republic (15) and Slovenia (31). Recently, a prevalence rate of 12% has been detected in wild boars from Poland (22). *A. phagocytophilum* infection rates found in wild boars were identical to those found in humans and *I. ricinus* ticks (31, 22).

In Sicily, evidence suggested that *A. phagocytophilum* infection might occur in domestic pigs as well (34). In south-central Spain, where *I. ricinus* ticks are rare (28), *Anaplasma* spp. has not been reported in wild boars (6, 8, 25), although other tick species feeding on wild boars were found positive for *A. phagocytophilum* DNA (6).

Recently, 16S rDNA genotypes, but not p44/msp2 genotypes, identical to *A. phagocytophilum*, have been found with low prevalence in wild boars in Japan (21), but a study conducted in Mississippi, USA, failed to detect pathogen DNA in boars (3). These results suggested that boars may play a role in the epizootiology of *A. phagocytophilum*, serving as a natural reservoir host only in certain regions.

In addition to the possible role of dogs as potential reservoir hosts, data on the prevalence of *A. phagocytophilum* infection in animals provide important information on the incidence, risk factors, sources of exposure, and real-time risk of exposure for human infection (26). Overall, several studies have documented the utility of using dogs as sentinels for human vector-borne diseases (VBD) (24).

Between 2007 and 2012, Kiss et al. established a prevalence rate of *A. phagocytophilum* infection of 4.48% in 870 blood samples from Transylvania (19).

CONCLUSIONS

The study presents important data on the role of wild boars as possible sources of infection with *A. phagocytophilum* in humans and farm animals.

The distribution rates of *A. phagocytophilum* DNA (3.44%) in our study were low compared to rates previously detected in wild boars from other European countries and indicate the necessity to extend the evaluation of *A. phagocytophilum* occurrence in wild boar populations to establish the role of the reservoir host in disease transmissibility in the studied area.

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