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Short communication

Investigation of the tick-borne pathogens *Rickettsia helvetica* and *Anaplasma phagocytophilum* in the blood of the domestic goat (*Capra hircus*)

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Abstract

The bacterial species *Anaplasma phagocytophilum* and *Rickettsia helvetica* are pathogenic for humans and domestic animals and are transmitted by ticks, e.g., of the *Ixodes* genus. Most of the vertebrate species constituting reservoirs for anaplasmas are known, but the potential reservoirs of rickettsiae are still under discussion. This study presents an analysis of the DNA of tick-borne pathogens isolated from the whole blood of goats grazing on meadows in West Pomerania, Poland. No DNA of *A. phagocytophilum* was found in the blood of the goats, while the DNA of *R. helvetica* was detected in 5.5% of the animals. The potential role of ruminants in the circulation of *R. helvetica* remains unknown.

Key words: tick-borne pathogens, veterinary pathogens, molecular identification

Introduction

Representatives of the order Rickettsiales are intracellular bacteria transmitted to the host mainly through arthropods. For certain genera of bacteria, such as *Rickettsia*, arthropods may not only be vectors, but also reservoirs. In the life cycle of *Anaplasma* sp. and *Rickettsia* sp., a very important role is played by widespread, long-lived arthropods, i.e. ticks, that feed on the blood of vertebrates. The common tick *Ixodes ricinus* is a cosmopolitan vector of *Anaplasma phagocytophilum* and *Rickettsia helvetica* (Raoult and Roux 1997, Petrovec et al. 2003a, Rymaszewska and Grenda 2008, Silaghi et al. 2011, Rymaszewska and Piotrowski

2013). The reservoirs of anaplasmas have been widely studied and wild animals, including the roe deer (*Capreolus capreolus*), the red deer (*Cervus elaphus*) or the wild boar (*Sus scrofa*), have been indicated numerous times as the potential sources of bacteria found in engorged ticks (Petrovec et al. 2003b, Skotarczak et al. 2008). The relationship between *Rickettsia* sp. and their potential vertebrate hosts is not yet known (Raoult and Roux 1997, Inokuma et al. 2008, Jilintai et al. 2008, Hornok et al. 2014).

The aim of this study was to discover whether *Capra hircus* goats in the West Pomerania region are infected with the tick-borne pathogens *Anaplasma phagocytophilum* and *Rickettsia helvetica*. These animals, often in-

fested with ticks, remain healthy and potentially could be reservoirs of these bacteria.

Materials and Methods

The detection of the DNA of tick-borne pathogens was attempted using the blood of 90 individuals of the domestic goat (*C. hircus*), taken in 2004–2009 (all animals in the herd were examined once in a period of 6 years). The animals, aged 3–5 years, originated from a farm located in West Pomerania (Poland) and were farmed in a free-grazing system. The meadows were located in the vicinity of wood complexes, in areas of occurrence of wild ruminants, e.g., *Cervus elaphus* and *Capreolus capreolus*. Both species are considered to be reservoirs for many tick-borne pathogens. This biotope is abundantly inhabited by the common tick *I. ricinus*, a vector of many pathogens.

DNA from blood samples was isolated using a MasterPure™ DNA Purification Kit (Epicentre, Madison, WI, USA). Tick DNA was isolated using the phenol-chloroform method after maceration of individuals. DNA isolates were stored at –70°C until analysis. Bacterial DNA was detected using a fragment of the *msp2* gene of *A. phagocytophilum*, *gltA* and the genes encoding the outer membrane proteins *17-kDa* and *ompB* of *Rickettsia* spp. For PCR, Go Taq®Flexi DNA Polymerase Promega (Madison, WI, USA) was used. The profile of temperature cycles over time was established in accordance with the requirements of the enzyme manufacturer. All positive samples were sequenced by MacroGen Europe (the Netherlands) using the same primer sets, and the results were analysed using Finch TV, BLAST and Mega 6 software.

Results and Discussion

None of the investigated blood samples of goats contained the DNA of *A. phagocytophilum*, while the DNA of *Rickettsia* spp. was detected in the blood of five animals, 5.5% in total. Nucleotide sequence analysis confirmed the presence of *R. helvetica* in the investigated material. The sequences of the *gltA* gene in *R. helvetica* were identical with those previously submitted to GenBank, and the sequences of the *17-kDa* and *ompB* genes were submitted under accession numbers: KT734809, KT734810.

The lack of *A. phagocytophilum* DNA in the blood of goats was not surprising. In a study of ticks *I. ricinus*, which are the main vector of these bacteria, infections with this pathogen were low. In the West Pomeranian region it is an average of 2.65% in the spring and 1.23% in the autumn (Rymaszewska, based on the data of a

three-year monitoring, results unpublished). There are also no reports of livestock infections with *A. phagocytophilum* in the region. Goats from the herds from which blood samples were collected were in good health, thus bacteraemia caused by anaplasmas was not expected. On the other hand, high infection rates of small ruminants were demonstrated by Petrovec et al. (2003a) in a study of animals originating from herds from outdoor pastures (203 individuals; Shkodra, Albania). In goats and sheep, the DNA of *Anaplasma* sp. was reported in 44% and 48% of the animals, respectively, with the animals showing no signs of the disease before slaughter. In contrast, Silaghi et al. (2011) monitored the presence of *A. phagocytophilum* in 6 herds of goats (60 individuals in total; Central Switzerland) over several months (May to October) and revealed the presence of pathogen DNA in half of these herds at the rates of 1.6% to 3.1%. Also in this case, no signs of the disease were observed in the animals, and positive results were obtained only once in each herd in the study period.

The results of this rickettsiae study are very interesting, the first in Poland and one of the few reports worldwide. For many years, it has been known that ticks are important vectors of *Rickettsia* spp. (Raoult and Roux 1997, Rymaszewska and Piotrowski 2013). It was also proposed that they might be reservoirs of these pathogens as well (Raoult and Roux 1997). To date, rickettsiae DNA was sporadically detected in the blood of vertebrates. The first reports of *R. helvetica* found in the blood of ruminants came from Japan in 2008 (Inokuma et al. 2008, Jilintai et al. 2008). Inokuma et al. (2008) examined the blood collected from 102 individuals of the sika deer (*Cervus nippon yeiensis*) and, based on the nucleotide sequences of the *gltA* and *17-kDa* genes, revealed the presence of *R. helvetica* in 7.8% of the animals. Jilintai et al. (2008), using the same genetic markers, detected *R. helvetica* DNA in 63.3% of the tested *C. nippon yeiensis* population, while no rickettsiae were found in calves grazing on a farm in the Hidaka District (Hokkaido, Japan). In Slovakia, Stefanidesova et al. (2008) also detected *R. helvetica* DNA only in one male roe deer (3.3%). In 2014, another article was published, in which the authors demonstrated the presence of *R. helvetica* DNA in the blood of 4.7% of tested birds (Hornok et al. 2014). The material for the tests was collected from several bird species, more than half of which were infested with ticks. However, the genetic material of rickettsiae was detected both in birds infested with ticks and in individuals free of these ectoparasites.

The results of this study, as well as literature data, indicate that rickettsiaemia may occur and remain in vertebrate animals without weakening them or without

provoking pathological signs, and thus such individuals constitute a potential source of bacteria infecting the ticks that infest these animals. Therefore, it is advisable to continue studies to better understand the reservoirs of *Rickettsia* spp.

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