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Molecular evidence for bacterial and protozoan pathogens in hard ticks from Romania

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ABSTRACT

The aim of the present study was to provide a preliminary insight into the diversity of tick-borne pathogens circulating at the domestic host–tick interface in Romania. For this, feeding and questing ticks were analyzed by real-time polymerase chain reaction (PCR) for the presence of *Anaplasma phagocytophilum*, *Anaplasma platys*, *Ehrlichia canis*, *Borrelia burgdorferi* sensu latu, and by PCR and subsequent sequencing for *Rickettsia* spp., *Babesia* spp. and *Theileria* spp. A total of 382 ticks, encompassing 5 species from 4 genera, were collected in April–July 2010 from different areas of Romania; of them, 40 were questing ticks and the remainder was collected from naturally infested cattle, sheep, goats, horses or dogs. Tick species analyzed included *Ixodes ricinus*, *Dermacentor marginatus*, *Hyalomma marginatum*, *Rhipicephalus bursa*, and *Rhipicephalus sanguineus*. Four rickettsiae of the spotted fever group of zoonotic concern were identified for the first time in Romania: *Rickettsia monacensis* and *Rickettsia helvetica* in *I. ricinus*, and *Rickettsia slovaca* and *Rickettsia raoultii* in *D. marginatus*. Other zoonotic pathogens such as *A. phagocytophilum*, *Borrelia afzelii*, and *Babesia microti* were found in *I. ricinus*. Pathogens of veterinary importance were also identified, including *Theileria equi* in *H. marginatum*, *Babesia occultans* in *D. marginatus* and *H. marginatum*, *Theileria orientalis/sergenti/buffeli*-group in *I. ricinus* and in *H. marginatum* and *E. canis* in *R. sanguineus*. These findings show a wide distribution of very diverse bacterial and protozoan pathogens at the domestic host–tick interface in Romania, with the potential of causing both animal and human diseases.

1. Introduction

Ticks (Acari: Ixodidae) are among the most important vectors of diseases in temperate climates (Heyman et al., 2010). In Europe, a wide range of tick-borne pathogens, including viruses, bacteria, and protozoa can cause diseases in both animals and humans. Moreover, some tick-borne pathogens, e.g. several *Rickettsia* spp. or *Ehrlichia* spp, are recognized as important zoonotic pathogens (Walker and Dumler, 1996; Raoult and Roux, 1997). Ticks play also an important role in the maintenance of several of these agents in nature as reservoirs due to the transovarial transmission (Parola and Raoult, 2001).

The first step towards planning tick-borne diseases (TBDs) surveillance should consist in assessing the diversity of pathogens occurring in a given area and their relative epidemiological importance (Capelli et al., 2012). Despite of a great concern on tick-borne diseases worldwide, up to now only a single survey on *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks from Romania has been carried out (Coipan and Vladimirescu, 2011). Besides of

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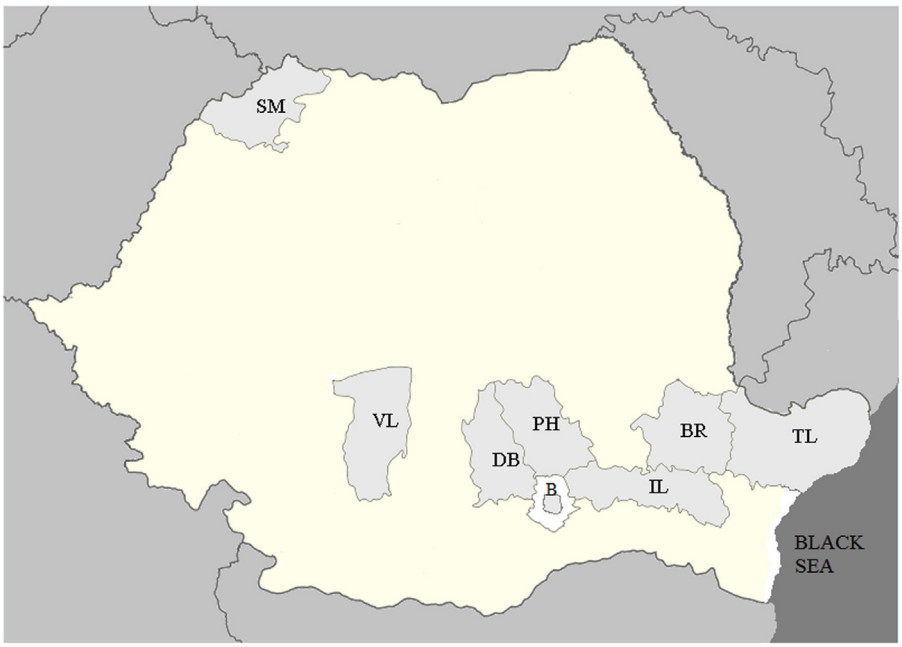

Fig. 1. Map of Romania showing the counties where ticks were collected, SM – Satu Mare; VL – Valcea; DB – Dambovita; PH – Prahova; B – Bucharest; IL – Ialomita; BR – Braila; TL – Tulcea.

Lyme borreliosis, other TBDs, such as ehrlichioses, granulocytic anaplasmosis, spotted fever group rickettsioses, and babesioses likely play an important role in infectious pathology in southeastern Europe (Christova et al., 2003). However, the prevalence and distribution of these pathogens in the diverse tick fauna occurring in Romania has not yet been studied.

Therefore, to provide a preliminary insight into the diversity of tick-borne pathogens circulating at the domestic host–tick interface in Romania, questing and feeding ticks were analyzed by molecular methods to determine the presence of *Anaplasma phagocytophilum*, *Anaplasma platys*, *Ehrlichia canis*, *Rickettsia* spp., *Borrelia* spp., *Babesia* spp., and *Theileria* spp.

2. Materials and methods

2.1. Tick collection

Questing and feeding ticks were collected in April–July 2010 directly from the vegetation and from host species in seven counties of Romania: Satu-Mare (SM), Valcea (VL), Dambovita (DB), Prahova (PH), Ialomita (IL), Braila (BR), Tulcea (TL), and Bucharest area (Fig. 1). Ticks feeding on domestic animals were manually collected from cattle, sheep, goats, horses or dogs. Questing ticks were collected by dragging at a single site in a wooded area in Valcea county (Table 1). For all ticks the species, stage, and sex were determined under a stereomicroscope (Pomerantzev, 1959; Arthur, 1960; Hillyard, 1996).

2.2. DNA extraction, PCR, and DNA sequence analysis

DNA was extracted from each tick individually using the Qiagen DNA MiniKit as described (Schorn et al., 2011a). All ticks were screened for DNA of the *gltA* gene of *Rickettsia* spp. and DNA of the *18S rRNA* gene of *Babesia* spp. as previously described (Schorn et al., 2011b). *I. ricinus* ticks were additionally screened for the *msp2* gene of *A. phagocytophilum* and the *flagellin* gene of *Borrelia* spp. by real-time PCR as described (Schwaiger et al., 2001; Silaghi et al., 2011a). *Rhipicephalus sanguineus* ticks were additionally screened with a real-time PCR for the *16S rRNA* gene of *A. platys* (Teglas et al., 2005) and the *p30* gene of *Ehrlicha canis* For rapid detection of *E. canis* in tick and canine blood samples, a real-time PCR assay had been developed in the diagnostic laboratory of the Institute of Comparative Tropical Medicine and Parasitoloy (Munich, Germany) based on a conserved region of the p30 gene of *E. canis* (Messerer, 2006). On the basis of this gene segment, a new primer pair had been developed: Ecp30-f: 3′-TGGATACTACCATGGCGTTATTGG-5′; Ecp30-r: 3′-GAGGAGCATCATTTAATACTACAGGAGTT-5′ as well as a TaqMan® MGB probe Ecp30-p: FAM-3′-CAGGTATCTTCTCAAATTT-5′-NFQ. For the validation of specificity and sensitivity of the *E. canis* real-time PCR assay, DNA of an unpublished South African cultured *E. canis* strain was kindly provided as positive control by Dr. Erich Zweygarth. Specificity of the assay was tested by testing cross reactions with field samples of *Theileria equi*, *Babesia caballi*, *Babesia divergens*, *Babesia canis canis*, *Babesia canis vogeli*, *Babesia gibsoni*, *Hepatozoon canis*, *Mycoplasma haemominutum*, *Mycoplasma haemofelis*, *Candidatus* M. turicensis, *B. burgdorferi* s. l., *A. phagocytophilum*, *A. platys*, *Rickettsia helvetica*, *Leishmania infantum* and a clone of *Bartonella henselae*. The efficiency of the assay was tested with a standard curve for 5 ten-fold dilution steps of DNA extracted from the *E. canis* cell culture and set up in triplicates. A relative quantification of the dilution series