

V 46 Circulation and genetic variability of *Anaplasma* spp. and other tick-borne intracellular microorganisms in the natural foci of Slovakia

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Anaplasmoses are common tick-borne zoonotic bacterial diseases. The causative agents are intracellular Gram-negative bacteria that belong to the family *Anaplasmataceae*. In moderate climatic regions of Europe, *Anaplasma phagocytophilum* is the most frequently occurring and the most important pathogen of this family. The main aim of this study was to analyze the prevalence and genetic variability of members from the *Anaplasmataceae* family in questing ticks, small domestic ruminants, and wild rodents in Slovakia, to understand the circulation of these tick-borne bacteria in natural foci. Anti-anaplasma antibodies were detected using modified ELISA protocol. In extracted DNA from blood, skin biopsies, and ticks, *A. phagocytophilum* and *A. ovis* were detected using species-specific *msp4* PCR and real-time PCR. Genetic variability within the genus *Anaplasma* was studied using variable region of 16S rRNA followed by SSCP analysis and/or DNA sequencing. 40.4% of tested sheep, goats, and 13% of rodents carried anti-anaplasma IgG antibodies. In south-eastern Slovakia, 66.1% ruminants were infected with *A. ovis* in contrast to one positive animal from northern Slovakia. It was represented by 2 different genotypes. The presence of *A. ovis* in sheep and goat blood represents the most northern occurrence of this pathogen documented in Europe, so far. *A. phagocytophilum* was present in all sheep and goat flocks over Slovakia with an infection prevalence ranging from 1.8% to 20%. 12% of ticks feeding on rodents were infected with members of the *Anaplasmataceae* family representing *Ehrlichia muris* and *Neoehrlichia mikurensis*. Moreover, the 16S rRNA primers detected the presence of *Rickettsia massiliae* and *R. helvetica*. 1.8% of ear biopsies and 0% of the rodent-feeding *I. ricinus* ticks carried *A. phagocytophilum*. In questing ticks, none of the tested ticks carried *A. ovis*. *A. phagocytophilum* was detected in 1.1% and 7.8% of *I. ricinus*. Questing *I. ricinus* were infected also with *N. mikurensis* and *E. muris*. *msp4* gene of *A. phagocytophilum* revealed higher intraspecific diversity than in *A. ovis*. The overall pairwise uncorrected p distances among all obtained sequences varied from 0.25% to 12%. Overall, sequences from questing ticks were more divergent as compared to the sequences obtained from ruminants, however all except one clustered within a clearly supported monophyletic group together with *A. phagocytophilum* strains from ruminants. The circulation of *Anaplasma*, *Ehrlichia*, and *Rickettsia* in the enzootic foci of Slovakia warrants further studies on their possible interaction as well as clarification of the ecological cycles; in means of identifying the tick vector for *A. ovis* and all possible reservoir hosts for tick-transmitted intracellular pathogens. Studies on the involvement of birds in the natural cycling of these agents are currently in progress.

The study was supported by the Vega project nos. 2/0055/11 and 2/0128/09.