LETTER-TO-THE EDITOR

AGENTS OF *EHRLICHIA PHAGOCYTOPHILA* GROUP AND OTHER MICROORGANISMS CO-INFECTING TICKS IN SOUTHWESTERN SLOVAKIA

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A total of 3221 adult ticks of three species, namely *Ixodes ricinus* (2554), *Haemaphysalis concinna* (593), and *Dermacentor reticulatus* (74) were collected by flagging the vegetation in a locality of southwestern Slovakia, Martinský Forest, from February 2000 to August 2001. Altogether 3186 ticks were tested by a hemocyte test (*1*); 51 (1.60%) of them were positive for rickettsiae (*Rickettsia* sp., *Coxiella burnetii*, and other rickettsia-like organisms). *I. ricinus* ticks were more frequently infected (48/2530, 1.90%) than *H. concinna* ticks (3/582, 0.52%). A randomly chosen group of 174 ticks, namely 137 *I. ricinus* (16 positive, 121 negative), 32 *H. concinna* (2 positive, 30 negative), and 5 *D. reticulatus* ticks (all negative) were tested by a polymerase chain reaction (PCR) for *Rickettsia* sp., *C. burnetii, B. burgdorferi sensu lato,* and microorganisms of *E. phagocytophila* group.

Total DNA was prepared from ticks using the DNeasy Tissue Kit (Qiagen, Germany) or the Invisorb Spin Tissue Kit (Invitek, Germany). Less than 1 μg of DNA was used in PCR. Primers RpCS.877p and RpCS.1258n targeting the *gltA* gene of *Rickettsia* sp. (*3*) and primers CBCOS and CBCOE targeting the *comI* gene of *C. burnetii* were used in PCR under conditions described earlier (*4*). The following primer pairs targeting the *ospA* gene of borreliae were used: SL for *B. burgdorferi s. l.*, GI for *B. burgdorferi sensu stricto*, GII for *B. garinii*, and GIII for *B. afzelii* (*5*). The conditions for PCR for borreliae were the same as those for PCR for *C. burnetii*. Primers GER3 and GER4 targeting the 16 S rDNA, specific for *E. phagocytophila*/HGE/*E. equi*, were used in PCR under conditions described earlier (*6*). Final concentration of each primer was 1 μmol/l. Controls for the PCR amplifiability of DNA samples were done using general eukaryotic 28 S rDNA primers (*2*). Composition of the reaction mixture was that recommended by the supplier of PCR components (Finnzymes, Finland). PCR was performed in a thermal cycler Techne (Progene, UK) or PTC-200 (MJ Research, USA). Amplified PCR products were resolved by electrophoresis in 1% agarose gels in TAE buffer. The PCR products amplified with the primers for rickettsiae were digested with restriction endonuclease *Alu*I. The obtained fragments were electrophoresed in 12% polyacrylamide gels in TBE buffer. An 100 bp ladder as DNA size marker and ethidium bromide staining were used in both types of gel electrophoresis.

Rickettsia sp. was found in 13 hemocyte-positive and 5 hemocyte-negative *I. ricinus* ticks. A restriction endonuclease digestion of PCR products indicated that 15 of them belonged to the spotted fever group (SFG); however, their exact identification requires further studies. The species *R. slovaca, R. helvetica, R. sibirica* or some new rickettsiae with the same *Alu*I restriction profile may be supposed

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Abbreviations: PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; SFG = spotted fever group

according to the current knowledge. The remaining three species of rickettsiae were not identified. *C. burnetii* was found in one hemocyte-negative *I. ricinus* and one hemocyte-negative *H. concinna* ticks by PCR and digestion of its products with *Sau*3AI and *Msp*I. *B. afzelii* was detected in four *I. ricinus* ticks and *B. garinii* in one *I. ricinus* tick. Genospecies of *B. burgdorferi* s.l. were not identified in the three *I. ricinus* ticks tested. Spirochetes have been detected and isolated from *I. ricinus* ticks collected in the locality investigated in 1999 (*7*). PCR/RFLP performed in that study revealed *B. afzelii, B. garinii, B. valaisiana* and *B. lusitania*. The prevalence of borreliae of 48% given in that report belongs to the highest ones reported to date for European Lyme borreliosis areas.

Therefore we can assume the presence of *B. valaisiana* and *B. lusitania* in the three ticks, in which the genospecies of *B. burgdorferi* s.l. were not identified. We detected also *E. phagocytophila* group microorganisms in six *I. ricinus* and one *H. concinna* ticks.

In this study we confirmed that Martinský forest, a locality in the south western Slovakia, is a natural focus of the following tick-borne agents: SFG rickettsie, *C. burnetii, B. afzelii, B. garinii* and microorganisms of the *E. phagocytophila* group. A co-infection of SFG rickettsiae and microorganisms of the *E. phagocytophila* group was identified in one *I. ricinus* tick. It should be emphasized that this is the first report on ehrlichiae in ticks in Slovakia.

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