

## LETTER TO THE EDITOR

DETECTION OF SPOTTED FEVER GROUP (SFG) RICKETTSIA  
IN *IXODES RICINUS* TICKS IN AUSTRIA

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Altogether, 1027 adult *Ixodes ricinus* ticks were collected during May – July 1997 from vegetation in Vienna Forest, Vienna, Austria. Forty-nine (4.8%) of them were positive in haemocyte test for rickettsia-like microorganisms. DNA amplification by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis of PCR product were used for closer characterization of rickettsia-like microorganisms obtained from 12 positive ticks.

Two different primer pairs were used for DNA amplification: primers DV1 (5'-GGCTTTATCGGCAGCAAC/TACTGT-3') and DV2 (5'-TCAAACAGGAATTTT/GGAGTAA/GCC-3') for *Coxiella burnetii* detection designed by I. Barák, (Institute of Molecular Biology, Bratislava), and primers Rp CS 877p (5'-GGGGGCCTGCTCACGGCGG-3') and Rp CS 1258n (5'-ATTGCAAAAAGTACAGTGAACA-3') (1) for SFG rickettsia detection and differentiation. The latter primers concern the SFG rickettsia citrate synthase gene.

DNA for amplification was prepared with QiAmp Tissues Kit (Qiagen, Germany) according to the producer's recommendations. The PCR for *C. burnetii* detection was carried out in LKB Gene ATAQ Controller (Pharmacia) using Taq DNA polymerase and chemicals from Advance Biotechnologies (UK) as follows: 30 cycles of denaturation

at 95°C for 20 secs, annealing at 50°C for 30 secs, and extension at 72°C for 2 mins, the last extension prolonged for 10 mins. The protocol for PCR for SFG rickettsia detection was already described (1).

The DNA products were analysed by 1.5% agarose gel electrophoresis using Tris-acetate-EDTA (TAE) buffer, 100 bp ladder as molecular size standard and ethidium bromide for DNA visualization.

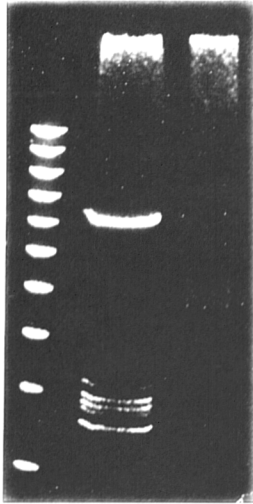
In addition, the PCR products amplified with SFG rickettsia primers was subjected to RFLP analysis using *AluI* restriction endonuclease. The DNA fragments obtained were analysed by 8% polyacrylamide gel electrophoresis.

The results of PCR with *C. burnetii*-specific primers were negative, showing that the investigated rickettsia-like microorganisms were not *C. burnetii* (data not shown).

RFLP analysis of the PCR products obtained with SFG rickettsia-specific primers revealed one positive tick with the restriction pattern different from other patterns typical for SFG rickettsiae. The figure shows RFLP analysis (from the left to the right) of a negative and the positive sample, and the 100 bp ladder. The pattern containing one 600 bp fragment and four fragments of about 230-160 bp was clearly different from those of *R. slovaca* as well as *R. helvetica* found in ixodid ticks (2) collected in Middle and Eastern Europe, and also from those of other SFG rickettsiae described previously (1) or recently in Sweden (3).

Based on these results, we can conclude that we have found a new rickettsia related to SFG rickettsiae in female

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



*I. ricinus* tick and that SFG rickettsiae may comprise various species. Moreover, the RFLP analysis of PCR products derived from the citrate synthase gene can be recommend-

ed for identification, differentiation and classification of this species.

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## References

1. Regnery RL, Spruill CL, Plikaytis BD, *J. Bacteriol.* **173**, 1576–1589, 1991.
2. Beati L, Péter O, Burgdorfer W, Aeschlimann A, Raoult D, *Int. J. Syst. Bacteriol.* **43**, 521–526, 1993.
3. Nilsson K, Jaenson TGT, Uhnöo I, Lindquist O, Pettersson B, Uhlén M, Friman G, Pahlson C, *J. Clin. Microbiol.* **35**, 243–247, 1997.