First Isolation of Rickettsia conorii from Human Blood in Croatia

Sanda Sardelić, Pierre-Edouard Fournier1, Volga Punda Polić, Nikola Bradarić2, Dušanka Grgić2, Ivo Ivić2, Dragan Ledina2, Boris Lukšić2, Ivo Milas2, Didier Raoult1

Department of Clinical Microbiology, Split University Hospital, Split, Croatia; 1WHO Rickettsial Reference Centre, School of Medicine, University of Marseille, Marseille, France; and 2Department of Infectious Diseases, Split University Hospital, Split, Croatia

Aim. To detect and isolate rickettsial strains from blood samples of patients with presumptive diagnosis of Mediterranean spotted fever (MSF) in the coastal region of south Croatia, and to compare the results with routine serology.

Methods. A “suicide” polymerase chain reaction (PCR), and a shell vial culture were done on samples of ethylenediamine tetra-acetic acid (EDTA) and citrate-anticoagulated blood samples. Indirect immunofluorescence was performed on sera collected from 17 patients clinically diagnosed with MSF during summer in three consecutive years, from 1998 to 2000.

Results. The primers used in PCR amplified the expected part of the rickettsia genomic DNA and Rickettsia conorii grew from the shell vial-cultured blood of a single patient. In 13 patients, the diagnosis was confirmed serologically by paired sera, whereas in 4 patients the diagnosis remained presumptive, since no paired sera were available. Analyzing sequences of the ompA and citrate synthase gene, respectively, derived from the shell vial isolate, a 100% similarity with Rickettsia conorii, strain Seven (Malish), was found.

Conclusion. To the best of our knowledge, this is the first isolation of Rickettsia conorii from a human sample in Croatia, and the first proof of a causative agent of MSF in the country. Beside PCR-based methods and isolation, correct diagnosis of MSF could be still routinely reached by serology.

Key words: Boutonneuse fever; polymerase chain reaction; Rickettsia conorii; rickettsia infections; sequence analysis; serology