SUPPLEMENTAL INFORMATION

Materials and methods. Molecular detection of rickettsial DNA. Two hundred microliters of the whole blood samples from each patient were used for DNA extraction using a QIAamp DNA mini kit (Qiagen, Hilden, Germany) in accordance with the instructions of the manufacturer. The samples were first screened for rickettsial DNA using polymerase chain reaction (PCR) primers CS78/CS323 (gltA) or CS-239/ CS-1069 (gltA-1) targeting 410 base pairs (bp) and 830 bp of the rickettsial citrate synthase gene, respectively.¹ Positive samples were then subjected to amplification using primers Rr190.70p/Rr190.602n, targeting a 532-bp fragment of the 190-kDa outer membrane protein gene (ompA)² and primers 120-M59/120-807, targeting a 866-bp fragment of 135-kDa outer membrane protein gene (ompB).³ All PCR assays were performed in a final volume of 20 µL containing 2 µL of DNA template, 1× ExPrime Taq DNA polymerase (GENET BIO, Daejeon, South Korea), and 0.2 µM of each primer, in a Veriti thermal cycler (Applied Biosystems Foster City, CA). DNA extracted from Rickettsia conorii antigen slides (Fuller Laboratories, Fullerton, CA) was used as positive control for the PCR assay. Sterile distilled water was used as the negative control in each PCR reaction. PCR products were purified using a GeneAll Expin[™] Combo GP kit (GeneAll Biotechnology, Seoul, South Korea). The purified DNA was then subjected to sequencing on an ABI PRISM 377 Genetic Analyzer (Applied Biosystems Foster City, CA), using both forward and reverse primers of each PCR assay. The sequences obtained were subjected to Basic Local Alignment Search Tool analysis (http://blast.ncbi.nlm .nih.gov/Blast.cgi) to search for homologous sequences in the GenBank database.⁴

SUPPLEMENTAL REFERENCES

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