8. Chaudhry R, Mahajan RW, Diwan A, Khan S, Singhal R, Chaudel DS, et al. Unusual presentation of enteric fever: three cases of splenic and liver abscesses due to *Salmonella typhi* and *Salmonella paratyphi A*. Trop Gastroenterol. 2003;24:198–9.
9. Tasar M, Ugurel MS, Kocaoglu M, Saglam M, Somuncu I. Computed tomography guided percutaneous drainage of splenic abscesses. Clin Imaging. 2004;28:44–8.

Address for correspondence: Sandro Contini, Department of Surgical Sciences, University of Parma, Via Gramsci 14, 43100 Parma, Italy; fax: 39-0521-940-125; email: sandro.contini@unipr.it

Rickettsioses in South Korea, Materials and Methods

To the Editor: We read with interest the article by Choi et al. (*1*), which describes the molecular detection of *Rickettsia typhi* and 4 spotted fever group rickettsiae by nested polymerase chain reaction (PCR) in the serum of febrile Korean patients. The value of the study, however, is limited by imprecision, inconsistencies, and the impossibility of verifying data. First, neither epidemiologic nor clinical data are provided for studied patients, although these are essential for interpreting PCR results. Second, multiplex nested PCR is hampered by a high risk of contamination (*2*). Alternatively, nested PCR techniques that use a closed assay or single-use primers without positive controls limit such a risk (*3*). In all cases, the use of negative controls is critical (*2*,*3*). In this study, negative controls are neither described in the Materials and Methods section nor shown on the gels. In addition, the authors used as positive controls 4 of the 5 *Rickettsia* species they detected. Therefore, apart from *R. felis*, which was not used as a positive control, positive products may result from cross-contamination. Finally, technically, the data are impossible to reproduce: 1) primer sets WJ77/80 and WJ79/83/78 cited in the legends of Figures 2 and 3 are neither described nor referenced in the text, 2) sequence of the RpCS.877p primer in Table 1 differs from that in the referenced article (*4*), 3) described sequences have not been deposited in GenBank, and 4) all *rompB* primers described in Table 1 exhibit 1–6 nucleotide mismatches with *ompB* sequences of at least 1 of the detected species. Based on these errors, the 7 cases of dual infections with *R. conorii* and *R. typhi*, which have never been reported before, are doubtful, and these data need to be confirmed.

Pierre-Edouard Fournier,*
Jean-Marc Rolain,*
and Didier Raoult*

*Université de la Méditerranée, Marseille, France

References

1. Choi YJ, Jang WJ, Kim JH, Ryu JS, Lee SH, Park KH, et al. Spotted fever group and typhus group rickettsioses in humans, South Korea. Emerg Infect Dis. 2005;11:237–44.
2. Hayden RT. In vitro nucleic acid amplification techniques. In: Persing DH, Tenover FC, Versalovic J, Tang YW, Unger ER, Relman DA, et al., editors. Molecular microbiology. Washington: ASM Press; 2003. p. 43–69.
3. Fournier PE, Raoult D. Suicide PCR on skin biopsy specimens for diagnosis of rickettsioses. J Clin Microbiol. 2004;42:3428–34.
4. Roux V, Rydkina E, Eremeeva M, Raoult D. Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. Int J Syst Bacteriol. 1997;47:252–61.

Address for correspondence: Didier Raoult, CNRS UMR 6020, IFR 48, Faculté de Médecine, Université de la Méditerranée, 27 Blvd Jean Moulin, 13385 Marseille CEDEX 5, France; fax: 33-491-38-77-72; email: didier.raoult@medecine.univ-mrs.fr

Rickettsioses in South Korea, Data Analysis

To the Editor: Choi et al. (*1*) conducted a study on sequence analysis of a partial *rompB* gene amplified from sera of humans who were seropositive for spotted fever group (SFG) and typhus group rickettsioses. They write, "These finding suggested that several kinds of rickettsial diseases, including boutonneuse fever, rickettsialpox, *R. felis* infection, and Japanese spotted fever… are occurring in Korea."

These claims propagate some errors and may lead to an inadequate conclusion. First, *rompB* is conserved in *Rickettsia* spp. and consists of 4,968 bp with respect to the published sequence of the *R. conorii* strain Seven (*2*,*3*). Fournier et al. (*4*) amplified 4,682 bp of *rompB* and showed a high degree of nucleotide sequence similarity (99.2%) between *R. africae* and *R. sibirica*, *R. pakeri*, and *R. slovaca*. Choi et al. amplified ≈420 bp of *rompB* (position 3562–4077) for sequence analysis. This segment is located in a highly conserved region of the gene, which may decrease the ability to differentiate particular species from other SFG rickettsiae. This study cannot prove the existence of specific SFG rickettsioses until the results are confirmed further by, for example, isolating these SFG rickettsiae from humans, animals, or ticks in South Korea. Recently, the authors analyzed nucleotide sequences of 267-bp amplicons of *rompB* (position 4762–4496) obtained from patient sera and found that *R. conorii* could not be differentiated from *R. sibirica* (*5*). This finding also supports our concerns.

Second, although partial *rompB* nucleotide sequence analysis of rickettsiae obtained from 1 patient's serum showed 98.87% similarity with *R. conorii* strain Seven, the finding does not indicate boutonneuse fever is

occurring in South Korea because high similarities (98.6%–99.8%) are found among 4 subspecies of *R. conorii*. Multilocus sequence typing can help differentiate among these subspecies (*6*).

This study provided a model to amplify SFG rickettsial DNA from sera of patients, and it will be helpful in surveillance of these diseases. However, the results should be interpreted more carefully in the context of clinical and epidemiologic data and combined with different gene sequence analyses to obtain a reliable and specific diagnosis.

Jui-Shan Ma*

*Show-Chwan Memorial Hospital, Changhua, Taiwan

References

1. Choi YJ, Jang WJ, Kim JH, Ryu JS, Lee SH, Park KH, et al. Spotted fever group and typhus group rickettsioses in humans, South Korea. Emerg Infect Dis. 2005;11:237–44.
2. Roux V, Raoult D. Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer-membrane protein rOmpB (*ompB*). Int J Syst Evol Microbiol. 2000;50:1449–55.
3. Ogata H, Audic S, Barbe V, Artiguenave F, Fournier PE, Raoult D, et al. Selfish DNA in protein-coding genes of *Rickettsia*. Science. 2000;290:347–50.
4. Fournier PE, Dumler JS, Greub G, Zhnag J, Wu Y, Raoult D. Gene sequence–based criteria for identification of new *Rickettsia* isolates and description of *Rickettsia heilongjiangensis* sp. nov. J Clin Microbiol. 2003;41:5456–65.
5. Choi YJ, Lee SH, Park KH, Koh YS, Lee KH, Baik HS, et al. Evaluation of PCR-based assay for diagnosis of spotted fever group rickettsioses in human serum samples. Clin Diagn Lab Immunol. 2005;12:759–63.
6. Zhu Y, Fournier PE, Eremeeva M, Raoult D. Proposal to create subspecies of *Rickettsia conorii* based on multi-locus sequence typing and an emended description of *Rickettsia conorii*. BMC Microbiol. 2005;5:11.

Address for correspondence: Jui-Shan Ma, Department of Pediatrics, Show-Chwan Memorial Hospital, 542, Section 1, Chung-Shang Rd, Changhua, 500, Taiwan; fax: 886-4-723-6226; email: morrison@show.org.tw

"*Mycobacterium tilburgii*" Infections

To the Editor: Advanced molecular biologic methods have improved the species differentiation and taxonomic classification of microorganisms, including nontuberculous mycobacteria. Identifying and characterizing an increasing number of "new" mycobacterial species of medical importance is now possible. Often, these newly described mycobacteria have been isolated from immunocompromised patients (*1*,*2*). Some of those have been difficult to cultivate, and 16S rRNA gene sequencing or similar methods have become of major importance to allow species identification and clinical diagnosis. Here, we report 2 patients with disease likely caused by a novel mycobacterial species that could not been previously cultivated. Diagnosis relied on molecular identification of acid-fast organisms in tissues. We also briefly review 2 similar cases published previously and note that all 4 known patients were from central Europe.

A 43-year-old woman without evidence of immunodeficiency reported recurrent episodes of dysuria, hematuria, and abdominal discomfort for >1 year. On cystoscopy, a hyperemic bladder with yellow plaques was observed. Biopsy of the plaques showed granulomatous infiltration of histiocytes. No definite diagnosis was made, and symptomatic relief occurred after a trial of empiric antimicrobial drug therapy. When the patient sought treatment again with persistent abdominal discomfort, endoscopy showed a lesion in the stomach that resembled a healed ulcer and numerous elevated yellow plaques throughout the colon and ileum. Microscopically, a granulomatous inflammation with macrophages filled with many acid-fast rods was seen, but mycobacterial growth did not occur in different media or in a guinea pig. Antituberculous treatment was initiated, and the patient slowly improved. A repeat colonoscopy showed fewer and smaller lesions. Efforts to culture the organism from biopsy specimens were again unsuccessful (different solid and liquid media, blood or chocolate agar, guinea pig, Balb/c mice). Sequencing of polymerase chain reaction (PCR) products of the 16S rRNA gene (*3*) from the organism represented a previously unknown mycobacterial species (EMBL DNA database: accession number Z50172). "*Mycobacterium tilburgii*" was proposed as the designation of this species because the novel mycobacterium was identified in the city of Tilburg (*4*). Retrospective analysis of the initial bladder biopsy specimen and of 2 lymph nodes taken during abdominal surgery (which became necessary because of a complicating ileal obstruction) confirmed the presence of "*M. tilburgii*" 16S rRNA gene sequences. All samples yielding PCR fragments hybridized with a "*M. tilburgii*"–specific biotinylated DNA probe.

A 34-year-old AIDS patient sought treatment for involuntary weight loss. Endoscopy showed white superficial embossments of the duodenal mucosa. Biopsy specimens were negative for acid-fast bacteria and mycobacterial growth (again, different solid and liquid media, extended incubation periods). Antiretroviral therapy was begun, but asthenia, persistent fever, diarrhea, vomiting, and cachexia developed. Repeat endoscopy showed yellow, plaquelike lesions in the duodenum (online Appendix Figure; available at http://www.cdc.gov/ncidod/EID/vol12no03/05-1139-G1.htm) and esophagus with periodic acid–Schiff (PAS) and acid-fast intracellular bacteria that were nonculturable. PCR of 16S rRNA gene (*3*) confirmed the presence of mycobacterial DNA; sequencing showed 100% homology to the