View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

ORIGINAL ARTICLE 10.1111/j.1469-0691.2007.01889.x

Detection of new genotypes of *Orientia tsutsugamushi* infecting humans in Thailand

P.-E. Fournier[1], S. Siritantikorn[1,2], J.-M. Rolain[1], Y. Suputtamongkol[2], S. Hoontrakul[3], S. Charoenwat[4], K. Losuwanaluk[5], P. Parola[1] and D. Raoult[1]

[1]Unité des Rickettsies, IFR 48, CNRS UMR6020, Faculté de Médecine, Université de la Méditerranée, Marseille, France, [2]Faculty of Tropical Medicine, Mahidol University, Bangkok, [3]Chumphon Hospital, Chumphon Province, [4]Chaitapoom Hospital, Chaiyapoom Province and [5]Banmai Chaiyapod Hospital, Bureerum Province, Thailand

ABSTRACT

PCR screening of blood specimens taken from 195 patients with serologically confirmed scrub typhus in three Thai provinces detected the 56-kDa protein-encoding gene from *Orientia tsutsugamushi* in ten (5%) patients. Significant genetic diversity was found among the ten amplicons, with nine new genotypes identified that were different from those found previously in Thailand. Phylogenetically, the ten sequences obtained in the present study and sequences from 71 strains characterised previously were distributed into several clusters that included the Karp, Gilliam, Kuroki, Saitama, Kawasaki and Kato clusters. Two of the new genotypes found in the present study clearly belonged to the Karp cluster. However, the other new genotypes formed three different clusters, including one cluster that appeared to be distant from all previously known clusters, and which may therefore be representative of a previously undescribed serotype. Other genotypes formed two other clusters that may also be associated with undescribed serotypes.

Keywords Genotypes, *Orientia tsutsugamushi*, PCR, scrub typhus, serotypes, Thailand

Original Submission: 10 July 2007; **Revised Submission:** 12 August 2007; **Accepted:** 30 August 2007

Clin Microbiol Infect 2008; **14:** 168–173

INTRODUCTION

Scrub typhus, also named tsutsugamushi disease, is caused by infection with *Orientia tsutsugamushi*, which is transmitted by the bite of trombiculid mites. The disease is a public health threat to a population of over a billion humans in a geographical triangle extending from northern Japan and far eastern Russia in the North, to northern Australia in the south, and to Pakistan and Afghanistan in the west [1]. The estimated incidence of the disease in endemic areas is one million cases/year [1]. *O. tsutsugamushi* is an obligate intracellular bacterium that belongs to the family Rickettsiaceae in the order Rickettsiales. This organism has established a specific association with chigger mites, in which it is stably transmitted vertically [2], although horizontal transmission may also occur occasionally [3]. As chigger mites are habitat-specific, it is likely that *O. tsutsugamushi* strains have evolved mostly in separate biotopes, and thus have followed a divergent evolutionary path [4], as indicated by 16S rRNA nucleotide sequence divergence of up to 4.0% among strains [5]. This diversity has resulted in the occurrence of different serotypes, depending on their location [1].

Initially, the great antigenic diversity of *O. tsutsugamushi* was described for the Gilliam, Karp and Kato strains [6]. Later, additional antigenic types were described, with representative strains including the Kawasaki type [7], Kuroki type [8], Shimokoshi type [9] and other types [10]. This antigenic variation has been linked to the sequence diversity of the immunodominant 56-kDa type-specific antigen [11]. This protein is exposed at the surface of the bacterium [7], and has been suspected to play a role in the early

Corresponding author and reprint requests: P. Parola, Unité des Rickettsies, IFR 48, CNRS UMR6020, Faculté de Médecine, Université de la Méditerranée, 27 Blvd Jean Moulin, 13385 Marseille cedex 5, France
E-mail: philippe.parola@medecine.univ-mrs.fr

© 2007 The Authors
Journal Compilation © 2007 European Society of Clinical Microbiology and Infectious Diseases

stages of cell infection [12]. In addition, its importance as a major immune response-eliciting protein has been demonstrated by its ability to prevent infection when used as a vaccine [13]. However, the great antigenic diversity of *O. tsutsugamushi* may limit the sensitivity of serological tests; indeed, it has been demonstrated that the addition of a serotype to the panel of normally tested *O. tsutsugamushi* antigens improves the sensitivity of antibody detection in patients significantly [14]. As a consequence, within a given area, newly detected pathogenic strains should be added to the panel of antigens for serological tests.

Given its antigenic diversity and the apparent geographical specificity of strain distribution, the population structure of *O. tsutsugamushi* remains poorly understood. Serotyping studies were performed as early as the 1940s, using complement fixation, serum neutralisation, vaccination challenge and toxin neutralisation tests [15]. More recently, immunofluorescent testing with strain- or type-specific hyper-immune sera or monoclonal antibodies that recognise the 56-kDa antigen has been preferred over other serotyping methods [1]. However, serotyping is time- and labour-consuming, and is limited by the need to include all prototype strains when testing a new strain. Therefore, molecular typing methods, particularly PCR amplification of the 56-kDa protein gene, followed by restriction fragment length polymorphism analysis and, more recently, sequencing, have been used extensively for investigations of scrub typhus [11,16].

To date, most studies concerning the genetic diversity of *O. tsutsugamushi* have been conducted in Japan and South Korea. In Thailand, scrub typhus has been reported for >50 years [17]. Several mite vectors have been identified in Thailand, including *Leptotrombidium* spp., *Blankaartia* spp. and *Ascoschoengastia* spp. [18]. Serotyping studies have revealed the antigenic diversity of *O. tsutsugamushi* in Thailand, with eight prototype strains being identified, namely Karp, Kato, Gilliam, TA678, TA686, TA716 (Chon), TA763 (Fan) and TH1817 [15]. In addition, a genotyping study has revealed a high degree of heterogeneity among 12 clinical isolates [19]. However, no global study has compared *O. tsutsugamushi* isolates from Thailand with all the isolates for which sequences are available in GenBank. In addition, no genotypic study of this agent in southern Thailand has been performed previously.

In the present study, genotypic variations among *O. tsutsugamushi* strains detected in patients with scrub typhus in the Bureerum and Chaiyapoom provinces in northeastern Thailand and the Chumphon province in southern Thailand were investigated. The 56-kDa protein gene sequences of these isolates were compared with those of all genotypes available in GenBank. The relationships among the strains were then determined by phylogenetic analysis based on sequence homologies.

MATERIALS AND METHODS

Patients and serological tests

Between 1 July 2003 and 31 December 2004, all patients admitted to the Bureerum, Chaiyapoom and Chumphon Hospitals in Thailand with clinically suspected and serologically documented scrub typhus were enrolled in the study. Informed consent was obtained from all patients. The Weil–Felix *Proteus* agglutination assay, using the *Proteus vulgaris* OX-19 and OX-2 strains and the *Proteus mirabilis* OX-K strain (Wellcome Diagnostics, Dartford, UK), was performed on each sample, with a titre ≥80 being considered as positive. Serum specimens were stored at −20°C before testing in Marseille, France, for *O. tsutsugamushi* antibody levels using micro-immunofluorescence with a panel of three *O. tsutsugamushi* antigens (Gilliam, Kato and Kawasaki strains). The micro-immunofluorescence assay was considered positive if antibody titres were >128 for IgG and >64 for IgM, or if seroconversion was demonstrated [20].

PCR and sequencing methods

DNA was extracted from the blood sample (buffy coat layer) using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Two amplification reactions were performed. First, real-time quantitative PCR with a *Taq*Man probe targeting the 47-kDa outer-membrane protein, using previously described primers and probe [21], was used as a screening test in Thailand. The positive results from the first PCR were then confirmed using a standard PCR targeting a 372-bp fragment of the 56-kDa protein gene with forward primer OtsuF (5′-AATTGCTAGTGCAATGTCTG) and reverse primer OtsuR (5′-GGCATTATAGTAGGCTGAG). The resulting gene fragment encompassed the VDI to VDIII hyper-variable fragments [11]. Primers were synthesised by Eurobio (Paris, France). Water was used as a negative control. PCR products were resolved by electrophoresis on agarose 1% w/v gels in 1× Tris-borate-EDTA buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA). The sizes of the PCR amplicons were determined by comparison with a molecular size standard (Boehringer, Manheim, Germany) under UV light after staining with ethidium bromide. PCR products were purified using a QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions. Sequencing reactions were performed using a dRhodamine Terminator Cycle Sequencing Ready Reaction Mix (Applied Biosystems, Foster City, CA, USA). Sequencing

© 2007 The Authors
Journal Compilation © 2007 European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **14**, 168–173

was performed on an ABI Prism 310 DNA Sequencer (Applied Biosystems), and the resulting sequences were compared with those available in GenBank by using BLAST software [22].

Phylogenetic analysis

All available *O. tsutsugamushi* 56-kDa protein-encoding gene sequences were first obtained from GenBank. Only sequences that matched the amplified fragments, and for which the host and geographical origins were described, were retained for analysis. Sequences were aligned using CLUSTALW software [23]. Phylogenetic relationships among the strains detected in the present study, and those for which sequences were available in GenBank, were inferred using MEGA software [24] for the neighbour-joining and maximum-parsimony methods, and PHYLIP software [25] for the maximum-likelihood method. Bootstrap analysis was used to estimate the reliability of nodes.

RESULTS

Serum specimens from 195 enrolled patients were collected during the study period, comprising 72 patients from Bureerum, 47 from Chaiyapoom and 76 from Chumphon. All patients who were initially positive according to the Weil–Felix assay were also positive according to immunofluorescence assay serology. In total, ten specimens were positive according to the real-time quantitative PCR targeting the 47-kDa outer-membrane protein. Similarly, the PCR targeting a fragment of the 56-kDa protein-encoding gene amplified a product of the expected size for the same ten samples, comprising two from Bureerum (2.8%), five from Chaiyapoom (10.6%) and three from Chumphon (3.9%) (Table 1). All negative controls yielded negative PCR results. Sequences were deposited in GenBank under the accession numbers detailed in Table S1 (see Supplementary material).

Table 1. Serological results for ten patients from Thailand who were positive according to PCR for the 47-kDa outer-membrane protein gene and a fragment of the 56-kDa protein-encoding gene of *Orientia tsutsugamushi*

	Serological titres		
Patient origin	Gilliam IgG/IgM	Kato IgG/IgM	Kawazaki IgG/IgM
CHB71[a]	512/256	512/256	512/256
CHB74[a]	512/0	512/0	512/0
CHB75[a]	128/256	512/256	128/256
CIB3[b]	128/256	128/128	128/256
CIB11[b]	64/128	64/128	64/128
CIB33[b]	512/1024	512/1024	512/1024
CPB2[b]	2048/1024	2048/1024	2048/1024
CPB3[b]	2048/1024	512/1024	1024/1024
BB23[c]	512/64	512/64	512/64
BB29[c]	256/256	256/256	256/256

[a]Chumphon province.
[b]Chaiyapoom province.
[c]Bureerum province.

In total, 71 *O. tsutsugamushi* 56-kDa protein-encoding gene sequences were identified in GenBank that could be aligned with the sequences from the present study, and for which the host and geographical origin were described. Another 41 sequences that were too short, were other fragments of the 56-kDa protein-encoding gene, or for which epidemiological data were not available, were not used in the analysis. When comparing the ten amplicons from the Thai patients in the present study to each other and to the 71 GenBank sequences, the percentages of nucleotide sequence similarity ranged from 73.0% between amplicon CPB3 and strain Shimokoshi [11] to 100% between amplicon BB29 and strains TW201 and Twyu81 [26].

The phylogenetic organisation was similar regardless of the analysis method used (Fig. S1, see Supplementary material). Two of the new genotypes found in the present study (CIB3 and CIB11) clustered together with strains related to the Karp type, organised into the JP1 and JP2 subgroups according to previous studies of Tamura *et al*. [27]. Four more of the new genotypes (CPB3, CHB74, CPB2 and BB29) clustered together in a group containing genotypes identified previously in Taiwan. Two new genotypes (BB23 and CHB71) clustered together with a strain from Taiwan; this cluster branched from the cluster formed by Gilliam strains. The remaining two new genotypes (CHB75 2 and CIB33) clustered together with strains from Taiwan, and this group branched from a cluster formed by Kato strains.

DISCUSSION

The present study detected *O. tsutsugamushi* in the blood of ten patients from three provinces in Thailand, and performed a comprehensive phylogenetic analysis of these strains by comparison with a large collection of sequences contained in GenBank. The public health importance of *O. tsutsugamushi*, with geographical differences in pathogenicity [1] and the emergence of antibiotic-resistant strains [28], together with its significant antigenic and genotypic diversity, have stimulated numerous studies of this organism. However, the antigenic diversity of *O. tsutsugamushi* is problematical for serological investi-

© 2007 The Authors
Journal Compilation © 2007 European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **14**, 168–173

gations, as cross-reactions may not occur among all serotypes, and thus infection may not be diagnosed when the causative strain is not included in the panel of tested antigens.

The antigenic diversity of this organism depends on variations in the 56-kDa outer-membrane protein [29], also named Sta56 (56-kDa scrub typhus antigen) [30] and Tsa (tsutsugamushi-specific antigen), and therefore depends on variations in the nucleotide sequence of the 56-kDa protein-encoding gene. This gene has been used in recent years for genotyping *O. tsutsugamushi* isolates [11,16,26], and 112 sequences of the 56-kDa protein-encoding gene are currently available in GenBank, many of which have not appeared in the scientific literature, and for which few or no data are available. Using sequences from 71 strains, in addition to the ten sequences from the present study, several phylogenetic clusters were identified, including the six clusters described previously [27]. Two of the ten genotypes from the present study clearly belonged to the Karp cluster. The other genotypes were grouped into three different clusters, including one that appeared to be distant from all other clusters that include type strains, and which may therefore be representative of a previously undescribed serotype. It is difficult to decide whether the other two clusters belonged to the previously described Gilliam and Kato clusters, respectively, or whether they also constituted clusters that were associated with undescribed serotypes.

In Thailand, scrub typhus accounts for *c.* 20% of all cases of febrile illness, and ranks second among identified infectious diseases after leptospirosis [14]. Few genotyping studies of *O. tsutsugamushi* strains in Thailand have been published previously [18,19,31], but it has been demonstrated that Thai strains can be classified within the Karp and Kato types, with Karp-type strains being predominant. The present study revealed a great genetic diversity among the ten amplicons, with nine new genotypes being identified. All genotypes identified in the present study differed from those found previously in Thailand [19,31,32], with only two being related to the Karp type. The other eight genotypes were classified within other clusters, which represent putative new serotypes. The association of Thai and Taiwanese strains within new clusters is as yet unexplained, as *O. tsutsugamushi* genotypes were considered previously to be restricted to a specific geographical area and to a specific mite vector [1]. The identification of nine new genotypes in Thailand supports this assumption, but the mite vectors that transmitted scrub typhus to the patients were not investigated. It has been demonstrated previously that the antigenic diversity of *O. tsutsugamushi* reduced the sensitivity of serological tests, although this was greatly enhanced by the diversification of antigens tested [14]. The identification of new genotypes, especially those classified within new phylogenetic clusters, suggests that the genotypic diversity of *O. tsutsugamushi* strains in Thailand should warrant the addition of representatives of the new clusters to the panel of antigens used to test patients suspected of having scrub typhus or who have unexplained fever.

In conclusion, this study identified nine new genotypes among *O. tsutsugamushi* strains in Thailand, thereby indicating the possible existence of previously undescribed serotypes. These results not only demonstrate a greater diversity of strains in Thailand than was suspected previously, but also highlight the necessity to test more strains as antigens in serological tests. Additional human, rodent and mite specimens will help in the cultivation of these strains and in obtaining a more precise understanding of the distribution of *O. tsutsugamushi* genotypes in Thailand.

ACKNOWLEDGEMENTS

The authors of this manuscript declare that they have no conflicts of interest in relation to this research, and disclose no commercial relationships.

SUPPLEMENTARY MATERIAL

The following Supplementary material is available for this article online at http://www.blackwell-synergy.com:

Table S1. *Orientia tsutsugamushi* strains used in this study, together with their geographical origin

Fig. S1. Phylogenetic tree constructed using the neighbour-joining method and MEGA software.

REFERENCES

1. Kawamura A, Tanaka H, Tamura A, eds. *Tsutsugamushi disease*. Tokyo: University of Tokyo Press, 1995.
2. Urakami H, Takahashi M, Hori E, Tamura A. An ultrastructural study of vertical transmission of *Rickettsia tsutsugamushi* during oogenesis and spermatogenesis in

© 2007 The Authors
Journal Compilation © 2007 European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **14**, 168–173

Leptotrombidium pallidium. *Am J Trop Med Hyg* 1994; **50**: 219–228.
3. Frances SP, Watcharapichat P, Phulsuksombati D, Tanskul P. Transmission of *Orientia tsutsugamushi*, the aetiological agent for scrub typhus, to co-feeding mites. *Parasitology* 2000; **120**: 601–607.
4. Saxena VK. Chigger mite infestation of small mammals in a feral biotope of a public park area of south Delhi. *J Commun Dis* 1989; **21**: 360–364.
5. Roux V, Raoult D. Phylogenetic analysis of the genus *Rickettsia* by 16S rDNA sequencing. *Res Microbiol* 1995; **146**: 385–396.
6. Shishido A. Identification and serological classification of the causative agent of scrub typhus in Japan. *Japn J Sci Biol* 1962; **15**: 308–321.
7. Yamamoto S, Kawabata N, Tamura A *et al*. Immunological properties of *Rickettsia tsutsugamushi*, Kawasaki strain, isolated from a patient in Kyushu. *Microbiol Immunol* 1986; **30**: 611–620.
8. Ohashi N, Tamura A, Sakurai H, Yamamoto S. Characterization of a new antigenic type, kuroki, of *Rickettsia tsutsugamushi* isolated from a patient in Japan. *J Clin Microbiol* 1990; **28**: 2111–2113.
9. Tamura A, Takahashi K, Tsuruhara T *et al*. Isolation of *Rickettsia tsutsugamushi* antigenically different from Kato, Karp, and Gilliam strains from patients. *Microbiol Immunol* 1984; **28**: 873–882.
10. Ohashi N, Koyama Y, Urakami H *et al*. Demonstration of antigenic and genotypic variation in *Orienta tsutsugamushi* which were isolated in Japan, and their classification into type and subtype. *Microbiol Immunol* 1996; **40**: 627–638.
11. Ohashi N, Nashimoto H, Ikeda H, Tamura A. Diversity of immunodominant 56-kDa type specific antigen (TSA) of *Rickettsia-Tsutsugamushi*. Sequence and comparative analyses of the genes encoding TSA homologues from 4 antigenic variants. *J Biol Chem* 1992; **267**: 12728–12735.
12. Tamura A. Invasion and intracellular growth of *Rickettsia tsutsugamushi*. *Microbiol Sci* 1988; **5**: 228–232.
13. Ni YS, Chan TC, Chao CC, Richards AL, Dasch GA, Ching WM. Protection against scrub typhus by a plasmid vaccine encoding the 56-KD outer membrane protein antigen gene. *Am J Trop Med Hyg* 2005; **73**: 936–941.
14. Suttinont C, Losuwanaluk K, Niwatayakul K *et al*. Causes of acute, undifferentiated, febrile illness in rural Thailand: results of a prospective observational study. *Ann Trop Med Parasitol* 2006; **100**: 363–370.
15. Elisberg BL, Campbell JM, Bozeman FM. Antigenic diversity of *Rickettsia tsutsugamushi*: epidemiologic and ecologic significance. *J Hyg Epidemiol Microbiol Immunol* 1968; **12**: 18–25.
16. Enatsu T, Urakami HL, Tamura A. Phylogenetic analysis of *Orientia tsutsugamushi* strains based on the sequence homologies of 56-kDa type-specific antigen genes. *FEMS Microbiol Lett* 1999; **180**: 163–169.
17. Amano K, Tamura A, Ohashi N, Urakami H, Kaya S, Fukushi K. Deficiency of peptidoglycan and lipopolysaccharide components in *Rickettsia tsutsugamushi*. *Infect Immun* 1987; **55**: 2290–2292.
18. Tanskul P, Strickman D, Eamsila C, Kelly DJ. *Rickettsia tsutsugamushi* in chiggers (Acari: Trombiculidae) associated with rodents in central Thailand. *J Med Entomol* 1994; **31**: 225–230.
19. Kollars TM, Bodhidatta D, Phulsuksombati D, Tippayachai B, Coleman RE. Short report: variation in the 56-kD type-specific antigen gene of *Orientia tsutsugamushi* isolated from patients in Thailand. *Am J Trop Med Hyg* 2003; **68**: 299–300.
20. La Scola B, Raoult D. Laboratory diagnosis of rickettsioses: current approaches to the diagnosis of old and new rickettsial diseases. *J Clin Microbiol* 1997; **35**: 2715–2727.
21. Jiang J, Chan TC, Temenak JJ, Dasch GA, Ching WM, Richards AL. Development of a quantitative real-time polymerase chain reaction assay specific for *Orientia tsutsugamushi*. *Am J Trop Med Hyg* 2004; **70**: 351–356.
22. Altschul SF, Madden TL, Schaffer AA *et al*. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997; **25**: 3389–3402.
23. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994; **22**: 4673–4680.
24. Kumar S, Tamura K, Nei M. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* 2004; **5**: 150–163.
25. Felsenstein J. PHYLIP—Phylogeny Inference Package (version 3.2). *Cladistics* 1989; **5**: 164–166.
26. Qiang Y, Tamura A, Urakami H *et al*. Phylogenetic characterization of *Orientia tsutsugamushi* isolated in Taiwan according to the sequence homologies of 56-kDa type-specific antigen genes. *Microbiol Immunol* 2003; **47**: 577–583.
27. Tamura A, Yamamoto N, Koyama S *et al*. Epidemiological survey of *Orientia tsutsugamushi* distribution in field rodents in Saitama Prefecture, Japan, and discovery of a new type. *Microbiol Immunol* 2001; **45**: 439–446.
28. Watt G, Chouriyagune C, Ruangweerayud R *et al*. Scrub typhus infections poorly responsive to antibiotics in northern Thailand. *Lancet* 1996; **348**: 86–89.
29. Tamura A, Ohashi N, Urakami H, Takahashi K, Oyanagi M. Analysis of polypeptide composition and antigenic components of *Rickettsia tsutsugamushi* by polyacrylamide gel electrophoresis and immunoblotting. *Infect Immun* 1985; **48**: 671–675.
30. Stover CK, Marana DP, Carter JM, Roe BA, Mardis E. The 56-kilodalton major protein antigen of *Rickettsia tsutsugamushi*—molecular cloning and sequence analysis of the Sta56 gene and precise identification of a strain-specific epitope. *Infect Immun* 1990; **58**: 2076–2084.
31. Manosroi J, Chutipongvivate S, Auwanit W, Manosroi A. Determination and geographic distribution of *Orientia tsutsugamushi* serotypes in Thailand by nested polymerase chain reaction. *Diagn Microbiol Infect Dis* 2006; **55**: 185–190.
32. Tamura A. Genetic diversity of *Orientia tsutsugamushi*. In: Raoult D, Brouqui P, eds, *Rickettsiae and rickettsial diseases at the turn of the third millenium*. Paris: Elsevier, 1999; 67–73.
33. Majahan SK, Rolain JM, Kashyap K *et al*. Scrub typhus in Himalayas. *Emerg Infect Dis* 2006; **12**: 1590–1592.
34. Tamura A, Makisaka Y, Kadosaka T *et al*. Isolation of *Orientia tsutsugamushi* from *Leptotrombidium fuji* and its characterization. *Microbiol Immunol* 2000; **44**: 201–204.
35. Shim SK, Shin YK, Choi EN *et al*. Analysis of cellular fatty acids in *Orientia tsutsugamushi* as taxonomic markers. *Microbiol Immunol* 2005; **49**: 343–347.

© 2007 The Authors
Journal Compilation © 2007 European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **14**, 168–173

36. Seong SY, Park SG, Huh MS *et al*. T-track PCR fingerprinting for the rapid detection of genetic polymorphism. *FEMS Microbiol Lett* 1997; **152**: 37–44.
37. Chen X, Niu H, Zhang Y, Zhang X, Yu Q. Typing of *R. tsutsugamushi* isolated in Shanxi province and determination of nucleotide sequence encoding 56-kDa protein gene. *Chin J Zoonoses* 1998; **14**: 21–23.
38. Kim IS, Seong SY, Woo SG, Choi MS, Chang WH. High-level expression of a 56-kilodalton protein gene (*bor56*) of *Rickettsia tsutsugamushi* boryong and its application to enzyme-linked immunosorbent assays. *J Clin Microbiol* 1993; **31**: 598–605.
39. Derrick EH, Brown HE. Isolation of the Karp strain of *Rickettsia tsutsugamushi*. *Lancet* 1949; **ii**: 150–151.

© 2007 The Authors
Journal Compilation © 2007 European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **14**, 168–173