RESEARCH ARTICLE

Genetic typing of the 56-kDa type-specific antigen gene of contemporary *Orientia tsutsugamushi* isolates causing human scrub typhus at two sites in north-eastern and western Thailand

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Abstract

Orientia tsutsugamushi is the causative agent of scrub typhus, a major cause of febrile illness in the rural areas of Southeast Asia. Twenty-three strains of *O. tsutsugamushi* were isolated from patients with scrub typhus in north-east (Udorn Thani province) and western Thailand (Tak province) between 2003 and 2005. The isolates were characterized by sequencing the entire ORF of the 56-kDa-type-specific antigen gene, followed by phylogenetic analysis. The majority (15/23) of isolates clustered with the Karp-type strain, six with a Gilliam-type strain and one each with the TA716- and TA763-type strains. Overall, there was considerable diversity in sequence, comparable to that seen in strains from across the rest of the scrub typhus-endemic world. There was no significant difference in the distributions of strains between the two provinces ($P = 0.08$, Fisher's exact) nor a temporal change in distribution with year of isolation ($P = 0.80$, Fisher's exact). Within this diversity there were also examples of isolates with identical 56-kDa genotypes that were cultured from patients from the same geographical areas.

Introduction

Orientia tsutsugamushi, formerly known as *Rickettsia tsutsugamushi*, is the cause of scrub typhus, a major cause of febrile illness in rural Southeast Asia (Leelarasamee *et al.*, 2004; Phongmany *et al.*, 2006; Suttinont *et al.*, 2006). Infection is largely confined to the Asia-Pacific region, where the pathogen is vertically maintained in the *Leptotrombidium* spp. mite population and transmitted to humans by the bite of the larval stage (Watt & Parola, 2003). No vaccine is currently available, but antibiotic therapy with either doxycycline or azithromycin achieves an effective cure.

The 56-kDa type-specific antigen (TSA) of *O. tsutsugamushi* is located on the outer membrane surface, is the primary immunogen, and is responsible for eliciting neutralizing antibodies (Hanson, 1985; Tamura *et al.*, 1985; Ohashi *et al.*, 1989; Stover *et al.*, 1990; Seong *et al.*, 1997, 2000). The gene encoding the 56-kDa TSA has an ORF of *c.* 1600 bp (Ohashi *et al.*, 1989, 1992; Stover *et al.*, 1990). *Orientia tsutsugamushi* isolates are conventionally classified on the basis of reactivity with hyperimmune serum raised against prototype strains (e.g. Karp, Kato, and Gilliam), and the four hypervariable regions within the 56-kDa TSA are considered to play a significant role in type strain assignment.

Genetic and antigenic characterization of contemporary *O. tsutsugamushi* isolates causing human disease in scrub typhus-endemic regions is limited. A major contributing factor is that isolation involves growth in tissue culture lines within a BSL3 facility. However, variability of the 56-kDa TSA gene and its product within the population of natural isolates could have a major bearing on both the accuracy of diagnostic tests and vaccine development. Previous studies have noted a dominance of Karp-like strains in human and ecological studies in Thailand (Elisberg *et al.*, 1968; Shirai *et al.*, 1981; Kollars *et al.*, 2003; Manosroi *et al.*, 2006). The

aim of this study was to determine the dominant contemporary strains and variability of the 56-kDa gene from *O. tsutsugamushi* isolated from scrub typhus patients at two sites in north-east and western Thailand to determine (1) distribution of strains compared with previous studies, (2) genetic relatedness of strains and (3) comparison with type strains from other countries.

Materials and methods

Patient specimens and isolates

Whole blood samples were collected from scrub typhus patients in Udon Thani (535 km north-east of Bangkok) and Tak (512 km north-west of Bangkok) provinces between September 2003 and August 2005. Samples were collected as part of studies investigating the causes of fever at these sites. Ethical clearance was obtained from the Faculty of Tropical Medicine, Mahidol University (Tak), the Thai Ministry of Public Health (Udon Thani) and the Oxford Tropical Research Ethics Committee (both studies). Patients provided informed written consent before sample collection. Twenty-three *O. tsutsugamushi* isolates were grown *in vitro* (Table 1) (Luksameetanasan *et al.*, 2007). Nineteen isolates (19/23; 83%) were from Udon Thani patients and four (4/23; 17%) isolates were from Tak patients.

Genotyping of the 56-kDa TSA gene

Orientia tsutsugamushi-infected VERO cells were harvested at the peak of infection as determined by the indirect immunofluorescence assay (IFA) (Luksameetanasan *et al.*, 2007). Infected cells were scraped into the tissue culture supernatant (TCSN) and centrifuged to pellet the cells. The TCSN was removed by decanting and the cell pellet was washed twice with PBS. Genomic DNA was extracted from the cells using the Wizard SV Genomic DNA purification system (Promega Co.) according to the manufacturer's instructions. DNA was eluted in double-distilled, deionized H_2O to a final volume of 200 μL and stored at $-20\,^{\circ}C$ until use.

The complete ORF of 56-kDa TSA gene was amplified by PCR using previously described primers – Q1, Q2 and Q4 (Enatsu *et al.*, 1999; Qiang *et al.*, 2003) – and primers designed specifically for this study – R3 (5′-GCCTAATAGTGCATCTGTCG-3′), R4 (5′-GCCTATAAGTATAGCTGATCG)-3′, R5 (5′-GCTGCTGTGCTTGCTGCG-3′) and R6 (5′-GGCCAAGTTAAACTCTATGC-3′). The three primer sets gave sufficient overlap to enable determination of a contiguous ORF following nucleotide sequencing; Q1–Q2 (position 266–856), R4–R5 (position 454–1056) and R3–Q4 (position 909–1575). R6 was used as a sequencing primer at position 1318 for R3–Q4 amplicon products.

The PCR reaction mixture contained 1 μL extracted DNA, 2.5 μL 10 × PCR buffer (Promega Co.), 0.5 μL deoxynucleotide phosphate (dNTP) (10 mM each), 1.75 μL of each primer (5 μM), 0.25 μL (2.5 U) of *Taq* polymerase (Promega Co.), and distilled deionized water in a final volume of 25 μL. The cycling parameters used were denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 60 s for 30 cycles, with a final extension of 72 °C for 5 min. The PCR product (3 μL) was analyzed by 0.8–1.5% agarose gel electrophoresis in TBE buffer at 100 V. After completion the gel was stained with

Table 1. Description of *Orientia tsutsugamushi* isolates and reference strains examined in this study

Isolate	Month/ year	Source	District	Province/ prefecture	Country	Bases	Complete or partial gene sequence	GenBank accession number	Strain	Isolation/56-kDa gene references
UT76	09/2003	Human	Muang	Udorn Thani	Thailand	1611	Complete	EF213078	Karp	Luksameetanasan *et al.* (2007). This publication
UT125	10/2003	Human	Muang	Udorn Thani	Thailand	1596	Complete	EF213096	Gilliam	Luksameetanasan *et al.* (2007). This publication
UT144	06/2004	Human	Muang	Udorn Thani	Thailand	1596	Complete	EF213091	Gilliam	Luksameetanasan *et al.* (2007). This publication
UT150	06/2004	Human	Muang	Udorn Thani	Thailand	1611	Complete	EF213086	Karp	Luksameetanasan *et al.* (2007). This publication
UT167	06/2004	Human	Phen	Udorn Thani	Thailand	1611	Complete	EF213080	Karp	Luksameetanasan *et al.* (2007). This publication
UT169	06/2004	Human	Muang	Udorn Thani	Thailand	1608	Complete	EF213092	Karp	Luksameetanasan *et al.* (2007). This publication
UT176	07/2004	Human	Ban Phu	Udorn Thani	Thailand	1602	Complete	EF213081	Karp	Luksameetanasan *et al.* (2007). This publication
UT177	07/2004	Human	Muang	Udorn Thani	Thailand	1605	Complete	EF213084	Karp	Luksameetanasan *et al.* (2007). This publication
UT196	07/2004	Human	Muang	Udorn Thani	Thailand	1596	Complete	EF213079	Gilliam	Luksameetanasan *et al.* (2007). This publication

Table 1. Continued.

Isolate	Month/ year	Source	District	Province/ prefecture	Country	Bases	Complete or partial gene sequence	GenBank accession number	Strain	Isolation/56-kDa gene references
UT213	07/2004	Human	Sang Khom	Udorn Thani	Thailand	1611	Complete	EF213088	Karp	Luksameetanasan *et al*. (2007). This publication
UT219	07/2004	Human	Muang	Udorn Thani	Thailand	1611	Complete	EF213100	Karp	Luksameetanasan *et al*. (2007). This publication
UT221	08/2004	Human	Muang	Udorn Thani	Thailand	1614	Complete	EF213097	Karp	Luksameetanasan *et al*. (2007). This publication
UT302	08/2004	Human	Muang	Udorn Thani	Thailand	1587	Complete	EF213095	TA763	Luksameetanasan *et al*. (2007). This publication
UT316	10/2004	Human	Muang	Udorn Thani	Thailand	1611	Complete	EF213082	Karp	Luksameetanasan *et al*. (2007). This publication
FPW2016	05/2004	Human	Pho Pra	Tak	Thailand	1608	Complete	EF213085	Gilliam	Luksameetanasan *et al*. (2007). This publication
FPW1038	10/2004	Human	Mae Ramat	Tak	Thailand	1593	Complete	EF213087	TA716	Luksameetanasan *et al*. (2007). This publication
FPW2031	12/2004	Human	Pho Pra	Tak	Thailand	1614	Complete	EF213098	Karp	Luksameetanasan *et al*. (2007). This publication
UT329	07/2005	Human	Na Yang	Udorn Thani	Thailand	1596	Complete	EF213099	Gilliam	Luksameetanasan *et al*. (2007). This publication
UT332	07/2005	Human	Muang	Udorn Thani	Thailand	1611	Complete	EF213083	Karp	Luksameetanasan *et al*. (2007). This publication
UT336	07/2005	Human	Wang Sam	Udorn Thani	Thailand	1599	Complete	EF213089	Karp	Luksameetanasan *et al*. (2007). This publication
UT395	07/2005	Human	Muang	Udorn Thani	Thailand	1611	Complete	EF213094	Karp	Luksameetanasan *et al*. (2007). This publication
FPW2049	07/2005	Human	Pho Pra	Tak	Thailand	1596	Complete	EF213093	Gilliam	Luksameetanasan *et al*. (2007). This publication
UT418	08/2005	Human	Muang	Udorn Thani	Thailand	1605	Complete	EF213090	Karp	Luksameetanasan *et al*. (2007). This publication
Gilliam	1943	Human		–	Burma	1575	Complete		Gilliam	Ohashi *et al*. (1992)
Kawasaki	1981	Human	–	Miyazaki	Japan	1569	Complete	M63383	Kawasaki	Ohashi *et al*. (1992)
Kato	1955	Human	–	Niigata	Japan	1590	Complete	M63382	Kato	Ohashi *et al*. (1992)
Karp	1943	Human	–	–	New Guinea	1599	Complete	M33004	Karp	Stover *et al*. (1990)
Boryong	1998	Human	–	–	South Korea	1602	Complete	L04956	Kuroki	Kim *et al*. (1993)
Shimogoshi	–	–	–	–	Japan	1566	Complete	M63381	Shimogoshi	Ohashi *et al*. (1992)
TA686	1963	Tupaia glis	–	–	Thailand	1599	Complete	U80635	TA686	Genbank submission
TA763	1963	*Rattus rajah*	–	–	Thailand	1581	Complete	U80636	TA763	Genbank submission
TA678	1963	*Rattus rattus*	–	–	Thailand	1548	Complete	U19904	TA678	Genbank submission
TA716	1963	*Menetes berdmorei*	–	–	Thailand	1575	Complete	U19905	TA716	Genbank submission
Matsuzawa		–	–	–	Japan	1458	Partial	AF173043	JP1	Enatsu *et al*. (1999)
402I		–	–	–	Japan	1468	Partial	AF173047	JP2	Enatsu *et al*. (1999)
Hirahata		*Leptotrombidium pallidum*	–	Aichi	Japan	1474	Partial	AF201835	JP2	Tamura *et al*. (2001)
HSB1	1996–97	Rodent	N/A	Saitama	Japan	1454	Partial	AF302983	Saitama	Tamura *et al*. (2001)
Kuroki		–	–	–	Japan	1599	Complete	M63380	Kuroki	Ohashi *et al*. (1992)
Ikeda		Humn	–	Niigata	Japan	1424	Partial	AF173033	JG	Tamura *et al*. (2001)
TW26-1	1990	*Rattus norvegicus*		Lan-Yu Island	Taiwan	1448	Partial	AY222636	Karp-like	Qiang *et al*. (2003)
TW73R	1999	*Rattus* spp.	–	Kinmen Island	Taiwan	1478	Partial	AY222628	Karp-like	Qiang *et al*. (2003)
TWYu8-1	1990	*Leptotrombidium pallidum*	–	Lan-Yu Island	Taiwan	1454	Partial	AY222640	Karp-like	Qiang *et al*. (2003)
TW12-1	1990	*Rattus norvegicus*	–	Lan-Yu Island	Taiwan	1517	Partial	AY222639	Karp-like	Qiang *et al*. (2003)
TW38-1	1990	*Rattus norvegicus*	–	Lan-Yu Island	Taiwan	1412	Partial	AY222635	TA763	Qiang *et al*. (2003)
TWYu1-1	1990	*Leptotrombidium pallidum*	–	Lan-Yu Island	Taiwan	1421	Partial	AY222641	TA716	Qiang *et al*. (2003)
TW46-1	1986	*Rattus rattus*	–	Chengkung	Taiwan	1415	Partial	AY222631	JG	Qiang *et al*. (2003)

2 μg mL^{-1} ethidium bromide for 5 min and destained in distilled water for 10 min. The DNA band was visualized and photographed under UV light and products were compared to the positive control sample and a known molecular size marker (EZ-load, Biorad Co., UK). Nucleotide sequencing was performed using the MegaBACE Model 1000 automated sequencer (Amersham Bioscience, UK).

Phylogenetic analysis

Multiple sequence alignment and analysis to determine genetic relationships of isolates and reference type strains was performed using BIONUMERICS version 4.6 (Applied Maths, Belgium) and the CLUSTAL X program (Thompson *et al.*, 1997). Complete and partial 56-kDa TSA ORF nucleotide sequences for reference and prototype strains were obtained from GenBank (Table 1). The exception was the Gilliam (M33267) 56-kDa gene nucleotide sequence, kindly provided by Dr Hiroshi Urakami, which had been referenced by a previous study (Enatsu *et al.*, 1999). A percentage nucleotide identity matrix was constructed by the PAM250 method using the DNASTAR MEGALIGN 6.1 program. Phylogenetic analysis was performed and the resulting dendrogram was constructed by the MEGA 3.1 program (Kumar *et al.*, 2004) using UPGMA and neighbor joining algorithms bootstrapped for 1000 replications. Fisher's exact test was performed to determine the significance of the relationship ($P < 0.05$) between the strains of *O. tsutsugamushi* isolates and their geographical distribution (i.e. Tak and Udon Thani provinces). Fisher's exact test was also performed to determine whether there was significant association ($P < 0.05$) between the strains of *O. tsutsugamushi* isolates and the annual distribution (i.e. 2003, 2004 and 2005).

Results

Characteristics of contemporary strains of *O. tsutsugamushi* causing human disease in Thailand

Genetic analysis of the 56-kDa TSA ORF demonstrated that the 23 Thai *O. tsutsugamushi* isolates obtained during this study were related to either the Karp- or Gilliam-type strains, or to the historical Thai-type strains TA716 and TA763 (Fig. 1 and Table 2). The majority (65%; 15/23) of contemporary Thai *O. tsutsugamushi* isolates were related to the Karp-type strain [percentage nucleotide identity range (PNIR) with prototype Karp: 93.1–96.1% (Fig. 1 and Table 2)]. Six (26%; 6/23) isolates demonstrated highest similarity to the Gilliam strain cluster (Gilliam PNIR 88.9–91.8%). Isolate UT302 was most closely related to the TA763-type strain (TA763 PNIR: 90.1%) and FPW1038 grouped with type strain TA716 (95.9% identity). Isolates UT144, UT196 and UT125 (all clustering with the Gilliam strain) demonstrated 100% identity, as did UT150, UT167, UT316 and UT332 (in the Karp strain cluster).

Geographical and temporal relationships between the contemporary Thai *O. tsutsugamushi* strains

The majority of *O. tsutsugamushi* isolates were from Udon Thani province (83%; 19/23). The majority of Udon Thani province isolates were related to the Karp-type strain (74%; 14/19) with a lower proportion of Gilliam-type strain-related isolates (21%; 4/19) and a single isolate that was related to type strain TA763 (5%; 1/19). The Tak province isolates were composed of two Gilliam-like strains (50%), a Karp-like strain (25%) and an isolate clustering with type strain TA716 (25%). There was no significant difference in the distributions of strains (i.e. Karp, Gilliam, TA716 and TA763) between the two provinces ($P = 0.08$, Fisher's exact) nor a temporal change in distribution with year of isolation [2003 (Karp 1, Gilliam 1), 2004 (Karp 10, Gilliam 3) and 2005 (Karp 4, Gilliam 2) ($P = 0.80$, Fisher's exact)].

Relationship between contemporary Thai *O. tsutsugamushi* isolates and strains in other countries

Of the Karp-related strains, the 56-kDa TSA sequence of Thai isolates was most similar in terms of Taiwanese strains from 1990 (TW26-1, 97.6% identity with UT336; TWYu8-1, 98.7% identity with UT76, 98.8% identity with UT150, UT167, UT316 and UT332) and 1999 (TW73R, 95.8% identity with UT176, 95.5% identity with UT177) and were distinct from the JP1, JP2 and Saitama Japanese Karp strains (Fig. 1). Similarly, within the Gilliam-related strains, the Thai isolates grouped with a 1986 Taiwan strain (TW46-1, 98.8% identity with UT125, UT144 and UT196) and were distinct from the Japanese JG Ikeda strain.

Discussion

This study demonstrates the diverse nature of contemporary isolates of *O. tsutsugamushi* causing human disease at locations in north-eastern and western Thailand. Phylogenetic analysis clearly differentiated the isolates into Karp, Gilliam, TA763 and TA716 type strain-associated clusters based on conventional antigenic/genotypic classifications (Enatsu *et al.*, 1999; Qiang *et al.*, 2003; Tay *et al.*, 2005).

This is the first study to genetically characterize *O. tsutsugamushi* isolates from a consecutive series of patients with scrub typhus infections from a defined geographical area. This is also the first study to genetically characterize the entire *O. tsutsugamushi* 56-kDa TSA gene ORF from Thai patients, which necessitated the design of new amplification and sequencing primers to amplify all Thai type strains that

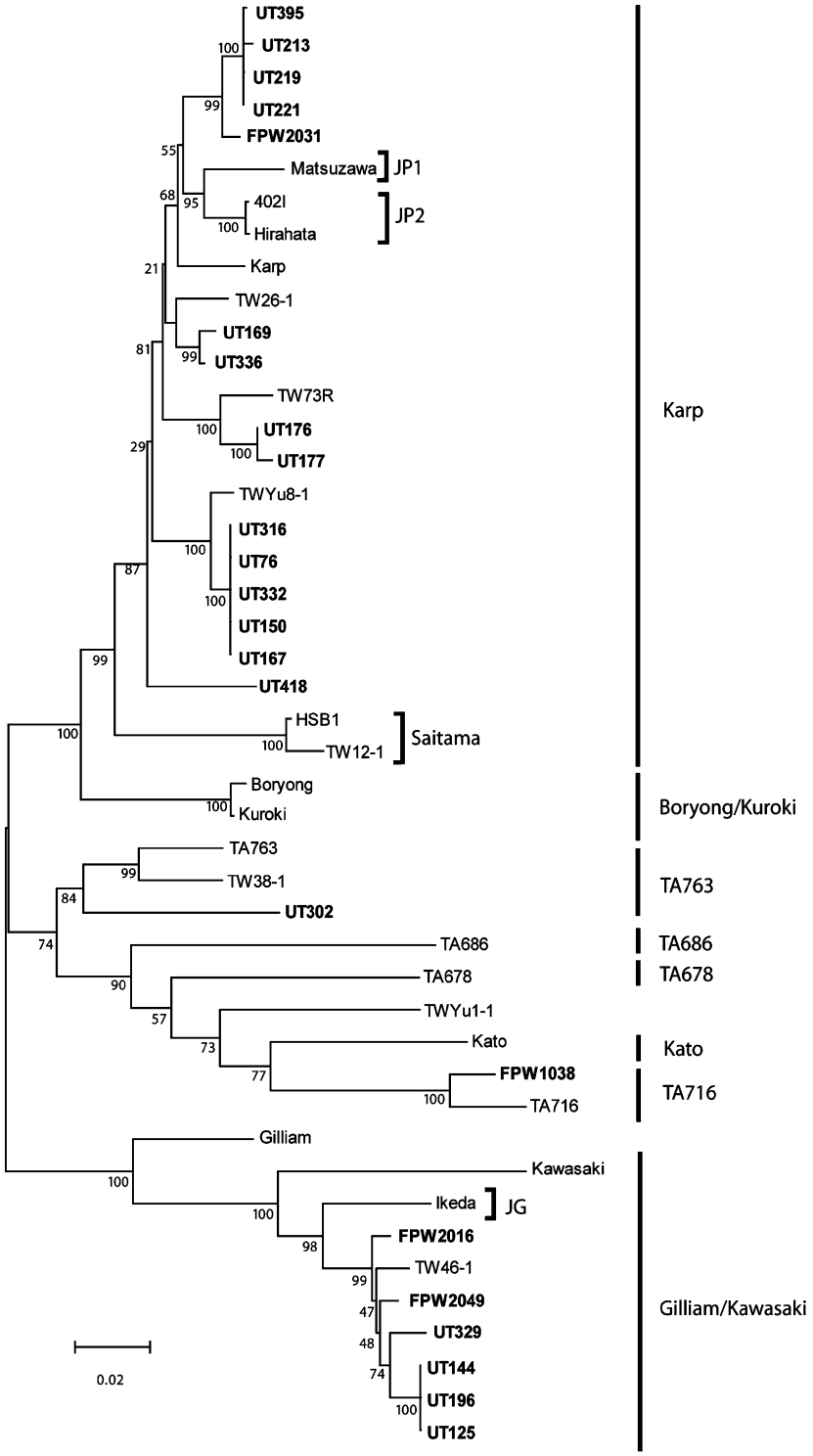

Fig. 1. Dendrogram representing the genetic relationships of Thai (represented in boldface type) and reference *Orientia tsutsugamushi* type strains based on the nucleotide sequence of the partial ORF (1412 bp) of the 56-kDa TSA gene demonstrating the relationships between Thai and non-Thai strains. The designations JP1, JP2, JG and Saitama are from the study by Tamura *et al*. (2001).

Table 2. Percentage nucleotide identity of the entire 56-kDa TSA gene ORF of contemporary Thai *Orientia tsutsugamushi* isolates compared with reference type strains

	Reference *O. tsutsugamushi* type strains							
Isolate	Karp	Gilliam	Kato	TA763	TA716	TA686	Kawasaki	Boryong
UT76	93.1	87.4	82.5	88.4	80.8	80.9	80.7	88.9
UT150	93.2	87.7	82.6	88.7	80.9	81.2	81.0	89.1
UT167	93.2	87.7	82.6	88.7	80.9	81.2	81.0	89.1
UT169	95.7	87.4	82.3	88.9	81.1	82.1	80.4	91.0
UT176	94.6	88.4	82	88.5	81.8	81.4	80.4	90.8
UT177	94.4	88.5	81.8	88.0	81.8	81.3	79.9	90.3
UT213	95.3	88.3	81.4	87.9	81.1	81.5	80.1	92.2
UT219	95.5	88.5	81.6	88.1	81.3	81.7	80.2	92.2
UT221	95.4	88.4	81.5	88.0	81.2	81.6	80.1	92.1
UT316	93.2	87.7	82.6	88.7	80.9	81.2	81.0	89.1
FPW2031	94.7	86.1	81.0	86.6	79.2	81.4	77.9	91.8
UT332	93.2	87.7	82.6	88.7	80.9	81.2	81.0	89.1
UT336	96.1	87.9	81.6	89.4	80.9	81.1	80.3	91.1
UT395	95.4	88.4	81.6	88	81.3	81.6	80.2	92.1
UT418	93.6	87.1	80.5	89.6	81.5	81.1	80.0	90.9
UT125	82.3	91.4	82.3	83.1	80.6	80.6	88.5	82.0
UT144	82.3	91.4	82.5	83.1	80.6	80.6	88.5	82.0
UT196	82.3	91.4	82.5	83.1	80.6	80.6	88.5	82.0
UT329	82.5	91.8	82.3	83	80.7	80.7	88.5	81.9
FPW2049	83.3	89.8	82.5	83.5	80.1	81.1	87.7	81.8
FPW2016	80.8	88.9	80.8	81.3	78.4	79.9	86.1	81.2
UT302	85.9	85.5	81.0	90.3	82.9	83.1	79.1	84.6
FPW1038	80.6	79.8	88.0	83.3	95.9	84.9	77.8	79.5

Highest identity with reference strains is highlighted.

were isolated *in vitro*. The 56-kDa TSA gene ORF sequencing results (~1600 bp) presented here provide an improved level of phylogenetic detail than previous genetic studies that only examined selected hypervariable regions of the same gene (300 bp) (Kollars *et al*., 2003; Manosroi *et al*., 2006) and serologically based characterization methods, which can be difficult to standardize and require reference serum and antigens. The majority of *O. tsutsugamushi* isolates examined in this study belonged to Karp- and Gilliam-type strains. This study also describes the first reported cases of human disease caused by strains that are related to TA716- and TA763-type strains, which were originally isolated from small mammals in Thailand in 1963 (Elisberg *et al*., 1968). Karp-like strains were the dominant group of contemporary Thai isolates in this study, which concurs with a recent study of Thai *O. tsutsugamushi* samples (Manosroi *et al*., 2006). Previous antigenic studies of *O. tsutsugamushi* isolated from trombiculid mites in north-eastern Thailand demonstrated the dominance of Karp-like strains and, to a lesser extent, TA716- and TA763-like strains (Shirai *et al*., 1981). Interestingly, there were a higher proportion of Gilliam-like strains from Tak province in western Thailand, although this observation should be treated with caution because of the low number of samples analyzed. A previous study from northern Thailand that examined 12 patient isolates suggested no relationship of any members to the Karp strain group, although this was based on a 300-bp sequence of only one of four hypervariable regions of the 56-kDa TSA gene (Kollars *et al*., 2003). Nucleotide sequencing of three Karp (UT150/167/316) and three Gilliam (UT144/125/196) strain isolates from Udon Thani demonstrated 100% identity and raised the possibility of a common source of infection given the relatively close temporal and geographical relatedness of the patients. Japanese isolates (Mori, Kamimoto and Okazaki) from human scrub typhus cases in Tokushima in 1998 also demonstrated identical nucleotide sequences for 1455 bp (the extent of the sequence submitted to Genbank) of the 56-kDa gene, although this observation was not discussed in the original study (Enatsu *et al*., 1999). The geographic clustering of isolates and limited genetic variation may be attributable to the vertical transovarial transmission of the *O. tsutsugamushi* bacterium maintained within the mite vector and the existence of 'mite islands' (Audy & Harrison, 1951). The possibility of cross-contamination between cultures in this study was unlikely as the samples were not processed at the same time and stringent biocontainment measures were in place during *in vitro* propagation. Further evidence that cross-contamination is not the cause of the apparent clonality of the isolates is provided by significant (Coleman *et al*., 2002) high

admission immunofluorescence antibody titers (i.e. $\geq 1:400$ IgM) against *O. tsutsugamushi* in every patient suggesting true scrub typhus infection, and by the high homologous binding titers of patient sera when tested by indirect immunofluorescence (results not presented). The 56-kDa TSA sequences of contemporary Thai *O. tsutsugamushi* isolates were more similar to those of Taiwanese strains than Japanese strains. However, only a small number of entire 56-kDa TSA nucleotide sequences are currently deposited on genetic databases and further characterization of previously collected and future *O. tsutsugamushi* isolates from other scrub typhus-endemic countries is required for a complete understanding of the geographical diversity of this important immunogen.

Results from this study provide opportunities to improve serological and molecular diagnosis of scrub typhus infections as well as the raw materials for future studies. Serodiagnosis for scrub typhus infections is performed by indirect IFA (gold standard), immunoperoxidase (IIP) assay, commercial enzyme-linked immunosorbent assays or rapid immunochromatographic assays that incorporate a mixture of antigen strain types. Strain results presented here demonstrate that antigen pools should contain at least Karp, Gilliam, TA716 and TA763 strain antigens, as well as the Kato strain, which has been previously recognized in Thailand (Shirai *et al*., 1981; Khuntirat *et al*., 2003; Manosroi *et al*., 2006). The nucleotide sequencing results from the entire 56-kDa TSA gene ORF of the studied isolates have been deposited on genetic databases and can be used for the design of improved diagnostic PCR primers. However, further genetic studies of the 56-kDa TSA and other diagnostically important genes are required in Thailand and other scrub typhus-endemic locations to make the information deposited on the databases more representative.

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