Chapter 16
Scrub Typhus and Its Causative Agent, *Orientia tsutsugamushi*

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1 Background

During World War II, Allied and Japanese Forces serving in the Asia-Pacific region were afflicted by outbreaks of a serious, acute, febrile disease. More than 16,000 cases were observed among Allied troops and 20,000 cases were recorded in members of the Japanese forces (Bavaro et al. 2005; Philip 1948). Mortality rates were variable, ranging from only 0.6 % in a large outbreak in the Schouten Islands to 35 % in a smaller outbreak in Papua New Guinea (Philip 1948). In some areas, these disease outbreaks were as disabling to a regiment as the combat itself and placed a huge burden on medical facilities (Philip 1948). For example, following a 4-day jungle exercise in Sri Lanka, 756 British soldiers were hospitalized with fever on their return to India (Philip 1948). The disease became known as scrub typhus by the Allied troops, after recognition that infection seemed to be associated with exposure to the secondary vegetation termed "scrub" (Traub and Wisseman 1974).

The disease experienced by the armed forces had been recognized as early as the third century A.D. in China (Fan et al. 1987), but it was first documented in the medical literature as tsutsugamushi disease in 1879 (Nagayo et al. 1917). The name was derived from the Japanese "tsutsuga" meaning illness and "mushi" meaning insect, in reference to the source of infection, the trombiculid mite. The tsutsugamushi disease of Japan and scrub typhus were confirmed to be same disease through studies in laboratory animals (Philip 1948). It has been known by many other descriptive and colloquial names, such as tropical typhus, mite bite fever, Japanese river fever, and Kedani fever (Hayashi 1920; Corbett 1943), but scrub typhus is the name now in common use.

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World War II accelerated research into scrub typhus, necessitated by the need to understand and prevent the disease, which was feared by the troops (Philip 1948). This research dwindled after the war, following the discovery that chloramphenicol was an effective treatment (Smadel et al. 1948). However, scrub typhus remains a serious health threat in the Asia-Pacific region. Based on its geographic distribution, an estimated one billion people are at risk of contracting the disease and more than one million cases occur each year (Watt and Parola 2003). Over 10 years ago the World Health Organization identified scrub typhus as "probably one of the most under-diagnosed and under-reported febrile illnesses requiring hospitalization in the region," and this statement is still valid today (World Health Organization Department of Communicable Disease Surveillance and Response 1999; Paris et al. 2013). The discovery of antibiotic resistant strains, the reemergence of the disease in areas where it had been absent for years and its emergence in new areas, both in the endemic region and beyond, demonstrates that research into scrub typhus is still important. This review summarizes current knowledge.

2   The Causative Agent of Scrub Typhus

2.1   *Classification*

The causative agent of scrub typhus is *Orientia tsutsugamushi*, an alpha proteobacterium belonging to the order *Rickettsiales* and the family *Rickettsiaceae*. When it was first isolated in 1930, it was placed in the genus *Rickettsia* and named *Rickettsia orientalis* (Nagayo et al. 1930), with a name change to *Rickettsia tsutsugamushi* shortly afterward (Ogata 1931). However, it was recognized early on that scrub typhus differed from other rickettsial diseases, in terms of the arthropod vector responsible for infection and clinical presentation (Ashburn and Craig 1908). Later research demonstrated several phenotypic differences between the scrub typhus agent and other species of *Rickettsia* (Table 16.1).

Sequencing of the 16S rRNA gene showed that while strains of *R. tsutsugamushi* shared more than 98.5 % sequence similarity, the level of similarity with other rickettsial species was only 90.2–90.6 % (Ohashi et al. 1995). This genotypic difference, along with the phenotypic differences, provided sufficient evidence for the scrub typhus agent to be reclassified in 1995 into its own genus, *Orientia* (Tamura et al. 1995). A second member of the *Orientia* genus has been proposed, although it is not yet officially recognized. The organism was isolated from an Australian patient who became infected while in Dubai in the United Arab Emirates. Sequencing of the 16S rRNA, 56-kDa and 47-kDa genes demonstrated significant genetic diversity (1.5 %, 17.7 %, and 46.9 %, respectively) from strains of *O. tsutsugamushi*. Thus, a new species name, *O. chuto* was proposed, with "chuto" derived from the Japanese for "Middle East" (Izzard et al. 2010).

Table 16.1 Phenotypic differences between the scrub typhus agent, *Orientia (Rickettsia) tsutsugamushi* and *Rickettsia* species

Feature		*Orientia (Rickettsia) tsutsugamushi* Scrub typhus agent	*Rickettsia* species	Reference
Size	Width	0.5–0.8 μm	0.2–0.6 μm	Tamura et al. (1991)
	Length	1.2–3.0 μm	0.5–2.5 μm	
Cell wall structure		Thin inner leaflet Thick outer leaflet	Thick inner leaflet Thin outer leaflet	Silverman and Wisseman (1978)
Peptidoglycan and lipopolysaccharide		Absent	Present	Amano et al. (1987)
Capsule layer		Absent	Present	Tamura et al. (1991)
Protein composition (Major protein sizes in kDa)		110, 80, 70, 60, 54–56, 46–47, 42, 35, 28 and 25	155, 120, 49, 32, 27.5, 17.5 and 16.5	Tamura et al. (1991)
In vitro susceptibility to antibiotics	Tetracyclines	Sensitive	Sensitive	Miyamura et al. (1989)
	Benzylpenicillin (1 mg/mL)	Resistant	Sensitive	
	Ofloxacin (quinolone; 1 μg/mL)	Resistant	Sensitive	

2.2   Genomics

The first full *O. tsutsugamushi* genome to be sequenced was the Boryong strain, isolated from a Korean scrub typhus patient (Cho et al. 2007). Shortly after, the genome of the Ikeda strain from a Japanese patient was fully determined (Nakayama et al. 2008) and data from a further eight Whole Genome Shotgun (WGS) projects are now available (Benson et al. 2009). The median genome size of the sequenced genomes is 2.00334 Mb as a single circular chromosome, making the *O. tsutsuga-mushi* genome the largest of any member of the order *Rickettsiales*, with the largest number of protein coding genes (Cho et al. 2007; Nakayama et al. 2008). Although its genome is larger than those of *Rickettsia* spp.*, O. tsutsugamushi* has undergone a greater degree of reductive evolution associated with adaptation to an obligate intra-cellular lifestyle (Andersson and Kurland 1998). It lacks some genes involved in nucleotide metabolism, DNA recombination, DNA repair, and fatty acid biosynthe-sis, making *O. tsutsugamushi* more dependent on the functions of its host cell than *Rickettsia* species in which these genes are conserved (Nakayama et al. 2008).

A unique feature of the *O. tsutsugamushi* genome is the high proportion of repet-itive sequences present. Repeat sequences (>200 bp) make up around 40 % of the genome, which is 200-fold greater than the density of repeats observed in the *R. prowazekii* genome and around tenfold greater than in the genomes of most other members of the order *Rickettsiales*. In fact, the repeat sequence density in the *O. tsutsugamushi* genome is more than double that of most other bacterial genomes (Cho et al. 2007; Darby et al. 2007; Nakayama et al. 2008, 2010).

The high repeat density is due to extensive amplification of mobile genetic ele-ments that can be classified into three main types: (1) integrative and conjugative elements termed *O. tsutsugamushi* amplified genetic elements (OtAGEs), that spread by conjugative transfer between cells and integrate into the genome; (2) transposable elements (TEs); and (3) short repetitive elements (Nakayama et al. 2008). The OtAGEs make up almost 30 % of the genome (Nakayama et al. 2008, 2010) and comprise a cluster of *tra* genes for conjugative Type IV secretion systems (T4SSs), flanked on one side by an integrase gene and on the other side by putative effector genes with possible roles in signaling and interaction with the host cell (Cho et al. 2007; Nakayama et al. 2008). Conjugation systems, which facilitate the transfer of DNA between cells, are rare in intracellular bacteria. For example, the *R. bellii* genome contains a single *tra* gene operon, similar in sequence and gene orga-nization to the OtAGEs (Ogata et al. 2006) and the plasmid of *R. felis* contains four *tra* gene fragments (Ogata et al. 2005). In contrast, the genome of the Boryong strain of *O. tsutsugamushi* has 359 *tra* genes and the Ikeda strain contains 185 rem-nants of OtAGEs (Cho et al. 2007; Nakayama et al. 2008). The mechanism for the amplification of the OtAGEs is not known but it has been hypothesized that the process may have been important for the adaptive evolution of *O. tsutsugamushi,* by selecting for components involved in the modulation of host cell functions (Cho et al. 2007; Nakayama et al. 2008). Since many of the genes in the OtAGEs have been pseudogenized by insertions or deletions, the genetic elements are no longer

transmissible and their current function is unclear (Cho et al. 2007). The TEs comprise five families of insertion sequence elements, four types of miniature inverted-repeat transposable elements, and a Group II intron, none of which has been identified in the genomes of *Rickettsia* spp. (Nakayama et al. 2008, 2010). They contribute 13–14 % of the genome sequence, although the amount of each type of TE varies between strains, suggesting significant decay and expansion (Nakayama et al. 2010). The huge amplification of repetitive elements has induced extensive shuffling within the *O. tsutsugamushi* genome and as such, little colinearity is observed between strains or with other rickettsial genomes (Nakayama et al. 2008, 2010). However, a set of around 500 genes is conserved between *O. tsutsugamushi* strains and five species of *Rickettsia* and thus may represent the core genes of the *Rickettsiaceae* family (Nakayama et al. 2010). In addition to intragenomic shuffling, a high rate of homologous recombination has been demonstrated to occur among *O. tsutsugamushi* strains, which also contributes to the diversification of the population (Sonthayanon et al. 2010). There is also evidence of horizontal gene transfer, through which *O. tsutsugamushi* has acquired viral and protist genes encoding ankyrin-repeat proteins and a gene from *Cyanobacteria* encoding a transposase (Georgiades et al. 2011). Overall, the genomic evolution of *O. tsutsugamushi* is unique and may be driven by the population bottlenecks created by its unique life cycle (Nakayama et al. 2008).

2.3   Strain Variation

From the early days of research into scrub typhus it was recognized that there were considerable differences in virulence between strains of *O. tsutsugamushi,* in both humans and laboratory animals (Bengston 1945). Studies using the complement fixation test (Bengston 1945; Shishido 1964), cross-neutralization (Bell et al. 1946; Bennett et al. 1947), and cross-vaccination (Rights and Smadel 1948) demonstrated serological heterogeneity between strains and three serotypes of Karp, Kato, and Gilliam were defined (Shishido 1964). Following the development of further immunological tools such as immunofluorescence assays (Bozeman and Elisberg 1967; Shirai et al. 1979) and monoclonal antibodies (Eisemann and Osterman 1985; Chang et al. 1990), and more diverse geographical studies (Elisberg et al. 1968), additional variants were identified that differed from the three original serotypes. At least nine major antigenic types are now recognized (Fig. 16.1 and Table 16.2), some of which contain subtypes (Ohashi et al. 1996).

   The antigenic diversity of *O. tsutsugamushi* is due to variation of the 56-kDa outer membrane protein (Eisemann and Osterman 1985; Stover et al. 1990; Tamura et al. 1985), which is considered to be species specific (Kelly et al. 2009). Characterization of the gene encoding the 56-kDa antigen from different strains demonstrated variation in its length, resulting in the protein size ranging from 516 to 541 amino acids (Ohashi et al. 1992; Kelly et al. 2009). Comparison of different strains showed that throughout the protein sequence there are insertions, deletions,
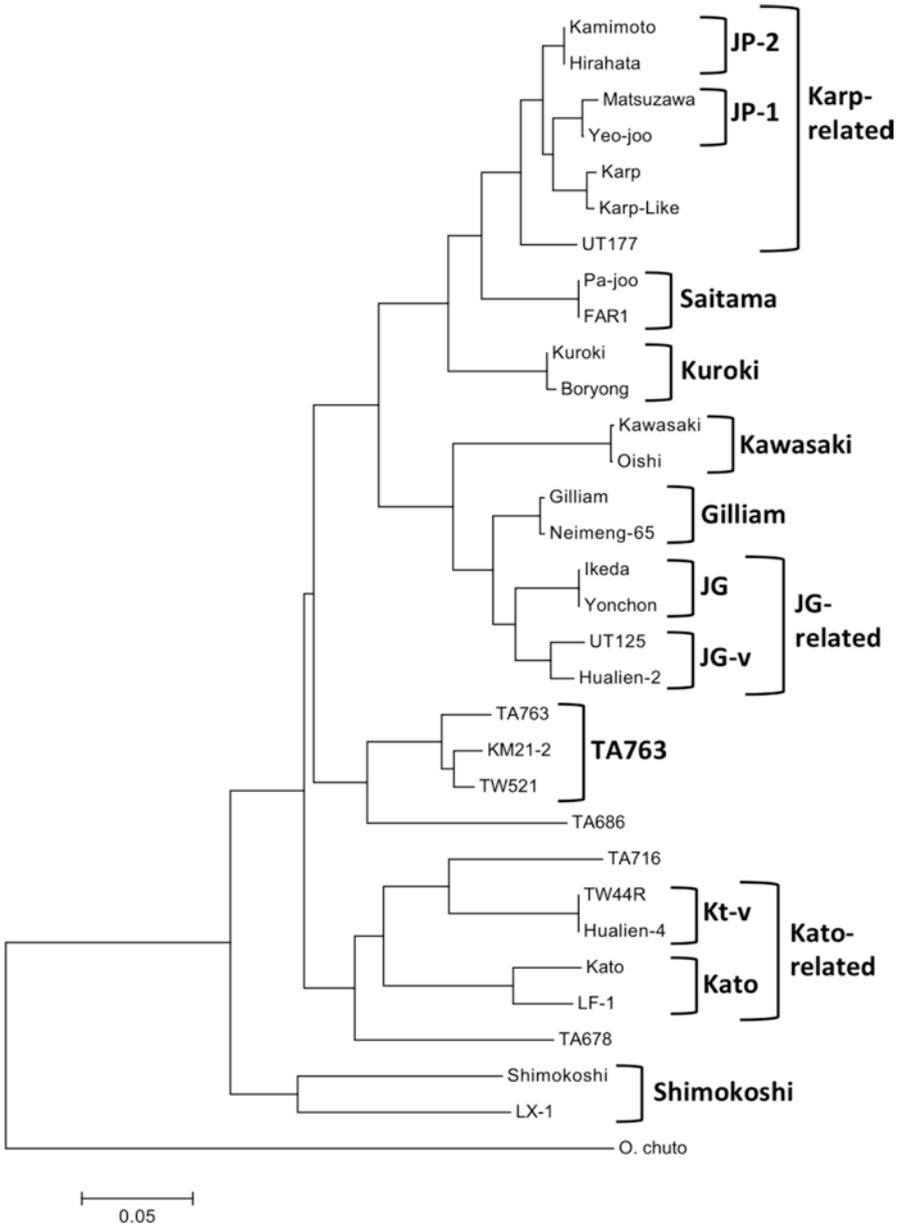
Fig. 16.1 Major antigenic types of *O. tsutsugamushi*. Phylogenetic tree of representative strains of *O. tsutsugamushi* antigenic types based on the nucleotide sequences of the 56-kDa antigen gene. The tree was constructed from a multiple sequence alignment using the neighbor-joining method, using the algorithm implemented by MEGA6 (Tamura et al. 2013). Clusters relating to the major antigenic types are indicated in *bold type*. Scale represents base substitutions per site

Table 16.2 Prevalence of major antigenic types of *O. tsutsugamushi*

Serotype		Prevalence	Comment
Karp		39.5%	Found throughout the endemic area.
JG-related	JG-v JG	10.3% 8.1%	Referred to as "Gilliam-type in Japan" based on serological reactivity, although sequences are quite divergent from Gilliam. JG-v is a closely related variant.
TA763		9.6%	First identified in Thailand but now found in China, Taiwan, India, Australia, and southeast Asia. May not occur in Japan and Korea.
Kato	Kato	6.3%	Found throughout the endemic region.
	Kt-v	5.2%	
Saitama		5.5%	Similar to Karp-related strains. Found mostly in Japan but also identified in Korea and China.
Kuroki		4.1%	Includes the fully sequenced Boryong strain.
Kawasaki		4.1%	Found in Japan and China.
Gilliam		4.1%	Prototype strain isolated in Myanmar but subsequently identified in isolates from India and China.
Shimokoshi		1.1%	Highly divergent based on 56-kDa antigen gene sequence analysis.

Prevalence among collection of 271 strains for which the complete or almost complete sequence of the 56-kDa antigen gene was available (Kelly et al. 2009)

or substitutions of one or more amino acids. However, there were four particular regions in which higher rates of change were observed (variable domains (VD) I–IV) (Fig. 16.2), with protein sequence similarity between strains ranging from more than 75% to less than 50% (Ohashi et al. 1992). The diversity of the VDs cannot be attributed to point mutations alone and it is likely that recombination has occurred in these regions. This is supported by the fact that some strains seem to be chimeric. For example, in the Kuroki strain, VD I is similar to the Gilliam strain, VD II is Kuroki specific, VD III is similar to the Karp strain, and VD IV is similar to both Gilliam and Karp (Ohashi et al. 1992).

Since gene sequencing has become readily available, determination of the 56-kDa antigen gene sequence has become the major tool for the differentiation and classification of *O. tsutsugamushi* strains (Enatsu et al. 1999; Kelly et al. 2009). However, phylogenies based on these sequences are not accurate and different trees are obtained depending on which of the VD sequences is used (Nakayama et al. 2010). More recently, multilocus sequence typing (MLST) schemes based on seven (Sonthayanon et al. 2010; Wongprompitak et al. 2015) or 11 (Nakayama et al. 2010) housekeeping genes have been used for differentiation. A low level of congruence was observed between the MLST and 56-kDa antigen gene sequencing data (Nakayama et al. 2010; Sonthayanon et al. 2010; Wongprompitak et al. 2015), with MLST providing significantly better discrimination between isolates. For example, among a collection of 22 isolates, 15 sequence types were determined using MLST compared with only three antigenic types determined using the single locus method

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Kato        M K K I M L I A S A M S A L S L P F S A S A I E L G D E G G L E C G P Y A K V G V V G G M I T G V E  50
Karp        . . . . . . . . . . . . . . . . . . . . . . . . . . E . - . . . . . . . . . . . . . . . . . . . . .
Gilliam     . . . . . . . . . . . . . . . . . . . . . . . . E . . . . . . . . . . G . . I . . . . . . . . . . A .
Kawasaki    . . . . . . . . . . . . . . . . . . . . . . . . . . . . V . . . . . . I . . . . . . . . V . . . . .
Kuroki      . . . . . . . . . . . . . . . . . . . . . . . . . . E . V . . . . . . . . . . . . . . . . . . . A .
Shimokoshi  . R . . . . . . . . . . . . . . . . . . . . N . . . F D E N - S . . . Q . . . . . . I . . . V L S . . .

Kato        S T R L D P A D A G G K K Q L P L T T S M P F G G T L A A G M T I A P G F R A E L G V M Y L A N V K  100
Karp        . A . . . . . . . E . . . H . S . . N G L . . . . . . . . . . . . . . . I . . . . . T . I T
Gilliam     . . . S T . S E . . H . S . . G L . . . . . . . . . . . . . . . . . . . . . . . . . R . I S
Kawasaki    . A . . . . . . V D C . . H . S . . M L . . . . . . . . . . . . . . . . . . . . . R . I N
Kuroki      . . . S T . S E . . H . S . . G L . . . . . . . . . . . . . . . . . . . . . . R . I S
Shimokoshi  . A . . . . . S E . . . H . . . I K G . . . . V . . . . . . . . . T . . V . . I S A . . M . . .

                                      Variable domain I
Kato        A E V E S G - - K T G S D - - - - A D I R S G A D S P M P Q R Y K L T P P Q P T I M P I S I A D R D  150
Karp        . Q . . E . K V . A D . V G E T K . S V G . K . A . I R K . F . . . . . . . . . . . . . . . V . . .
Gilliam     . . . . V . K G . V D . K G E I K . . S G G . T . T . I R K . F . . . . . . . . . . . . . . . . . .
Kawasaki    . . . . L . E G . . . G - - - A . N A A I D T G A . I R K . F . . . . . . . . . . . . . . . . . .
Kuroki      . . . . V . K G E V D . K G E I K . . S G G . T . A . I R K P F . . . . . . . . . . M . . . . . .
Shimokoshi  . . . . L . K M G S D A N T G T T . . A S A - - - V I R K H K . . . . . . . . . N . . . . . . . .

                                    Variable domain II
Kato        L G V D I P N V P Q G G A N H L G D N L G A N D I R R A D D R I T W L K N Y A G V D Y M V P D P N N  200
Karp        F . I . . . Q T S A A S T S R - - S . R L . . E Q . . A A . . A . . . . . C . . I . . R . K N . . D
Gilliam     V . . . T D - - I L A Q . A A G - - Q P R L T V E Q . . A . . A . . . . . . . . . . . . . . . . Q .
Kawasaki    . . . . T D - - I L A Q . A V G - - Q Q Q L T V E Q . E . . A . . . . . . . . I . . . . . S Q .
Kuroki      F . I . . . I . A Q . Q A A - - Q P P L . . Q K . . A A . . A . . . . . C . . I . . . K . . . .
Shimokoshi  I A . . . . . . - - - - A . G Q G - - - - - - V D V . . A A . . A . . . . . . I . . Y . . . S . .

                                      Variable domain III
Kato        P - Q A R I V N P V L L N I P Q G P P N A N P R Q A M Q P - - - - - C S I L N H D H W R H L V V G I  250
Karp        . N G P M V I . . I . . . . . . . N . . . P V G N P P Q R A N P P A G F A . H . . E Q . . . . . . L
Gilliam     . - N . . V I . . . . . . . . T . . . . V Q . . P R Q - - - - - N L D . . D . G Q . . . . . . V
Kawasaki    . - N . . V . . . . . . . . . T . A . . V . . P R Q - - - - - N L N . . D . . Q . . Y . . . V
Kuroki      . . - G H M M . . . . . . . . . N . . . P V G Q P P Q R A N Q P A N F A . H . . E Q . . . S . . . L
Shimokoshi  . - . G . V . . . . . . . . . . . N . . . P A G G G G R A A P - - A A F D . . D . A Q . . D V . . . .

Kato        T A M S N A N K P S V S P I K V L S E K I V Q I Y R D V K P F A R V A G I E V P S D P L P N S A S V  300
Karp        A . L . . . . . . . A . . V . . . D . . T . . . S . I . H L . D I . . . D . . D T S . . . . . .
Gilliam     . . L . H . . . . . T . V . . . D . . T K . . S . I . . D I . . . D . . D T G . . . . . . . . .
Kawasaki    . . L . . . . . . . S V . . . D . . T . . . S . I R Q . . K I . N . . . G A . . . . . . . . . .
Kuroki      A . L . . . . . . . A . . V . . . D . . I . . . S . I . . . D I . . . N . . D T G . . . . . . I
Shimokoshi  . . L . . . . N . . A V . . . D . . S . . A . I . . . N . . S V Q I S E T . . . D . . . . . . . .

Kato        E Q I Q N K M Q E L N D I L D E I R D S F D G C I G G N A F A N Q I Q L N F R I - P Q A Q Q Q G Q G  350
Karp        . . . . . . . . . . . L . E . L . E . . . . Y L . . . . . . . . . . . V M P Q . . . . . - G Q
Gilliam     . . . . S . . . . . V E D L . . . . . . Y M - . . . . . . . . . . . V M P Q . . . . . Q G Q
Kawasaki    . . . . T . . . . . V . E . L . E . . . . Y L A . . . . . . . . . Q . - Q . . . . . . - - Q
Kuroki      . . . . S . I . . G . T . E . L . . . . Y . N - . . . V . . . H . . . V M P . . . . . . Q G Q
Shimokoshi  D . . . . . . K V . E D V . E . . . F . L . . . . Q P V R . . . Q . P Q V V . G . . . - - -

Kato        Q Q Q Q Q A Q A T A Q E A A A A A A V R V L N N - - N D Q I I K L Y K D L V K L K R H A G I K K A M  400
Karp        G . . . . . . . . . . . . . V . . . . . L . . G - - . . . . A Q . . . . . . . . Q . . . . . . .
Gilliam     G . . . . . . . . . . . . . V . . . . . L . . G - - . . . . A Q . . . . . . . . Q . . . . V . . .
Kawasaki    . . . . . . G . V . . . . . . . . . A . . G - - . E . . Q . . . . . . . . Q . . . . . . R . . .
Kuroki      G . . . . . . . . . . . . . V . . . . . L . . G - - S . . . A Q . . . . . . . . Q . . . . . R . . .
Shimokoshi  G . . P . . A . . . . . . . . . . . . I . A . . D G E . N G . . Q . . . . . Y . . Q . N V A L . . S .

                                      Variable domain IV
Kato        E E L A A Q - - - D G G C N G G G D N K K K R G A S E D S D A G G A S K G G K G K E T K E T E F D L  450
Karp        . K . . . . Q E E . A K N Q . E . . C . Q Q Q . T . - - - - - - K . . K . . D . - - . A . . . .
Gilliam     . K . . . . Q E E . A K N Q C E . . C . Q Q Q . . . . . - . . . . K . . E . . . . - . - . . . .
Kawasaki    . K . . . . . E E G . D Q S Q V S C N D . . Q Q A V A . . K . . - S . . E . . N . . - - . V . L . .
Kuroki      . K . . . . Q E E . A K N Q . K . . C . Q Q Q . . . . . . . . K . . E . . V . - - - . . I . . . .
Shimokoshi  K Q . G D E L G V . Q . Q E . . C S K D . . Q S D T T A E E - - - S K . E . . K G - . . . I . . . .

Kato        S M I V G Q V K L Y A D L F T T E S F S I Y A G L G A G L A Y T S G K I D G V D I K A N T G M V A S  500
Karp        . . . . . . . . . . . . . V M I . . . V . . . . . V . . . . . . . . . . N K . . . G H . . . . .
Gilliam     . . . . . . . . . . . . . . . . . . . . . V . . . . . H . Y . . . D K . . G H . . . .
Kawasaki    . . . . . . . A . . . . . . V V A . . . . . . . I . G . V . R . Y . D . . K S V . - H I . V . .
Kuroki      . . . V . . . . . . . . . . V . . . . . G . V . . . . . Y . . . . N K . V . G Y . . . .
Shimokoshi  H . A . . . . . . . . . . . I D . . V . . I . . . . . . H . . . . . K . . H . . . . G .

Kato        G A L G V A I N A A E G V Y V D I E G S Y M H S F S K I E E K Y S I N P L M A S L G V R Y N F  547
Karp        . . . . . . . . . . . . . . . . . . . . . . . . Y . . . . . . . . . . . . . . V S . . . . .
Gilliam     . . . . . . . . . . . . . . . . . . . . . L . . . . . . . . . . . . . . . . . . . . . . . .
Kawasaki    . V . . . . . . . V . D . C . . . D . G . . . . . . . . . . . . D . . . V . A F I . N A . . . .
Kuroki      . . . . . . . . . . . . . . . . . . . . . . V . D . . Q V . A F I . . A C . . . . .
Shimokoshi  L . . . . . . A . V . D . . . M . V D A G . L Y . . . . . . . . . . Q M . A F V . . A . I . . . .
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Fig. 16.2 Alignment of 56-kDa antigen sequences. Protein sequences were aligned using the ClustalW algorithm in MEGA version 6 (Tamura et al. 2013). *Colored shading* indicates the variable domains I–IV

(Sonthayanon et al. 2010). In contrast to the strongly clustered trees created from 56-kDa antigen gene sequences (Fig. 16.1), phylogenies based on MLST data have poor bootstrap support and the limited clustering observed does not correlate with the 56-kDa gene clusters (Wongprompitak et al. 2015). This limited correlation of clusters was also observed when comparing phylogenetic trees created from sequences of the *groES* and *groEL* genes with 56-kDa antigen gene phylogenies (Arai et al. 2013). While the 56-kDa antigen gene sequence may be appropriate for investigation of local outbreaks, it is less useful for the phylogenetic analysis of *O. tsutsugamushi*, since this antigen gene is under selection pressure from host immune systems (Sonthayanon et al. 2010).

Regardless of which typing method is used, there is little evidence to support the grouping of a specific antigenic or sequence type to a particular geographical location (Enatsu et al. 1999; Kelly et al. 2009; Wongprompitak et al. 2015). However, in some areas, one prevalent antigenic type is observed, which may be linked to the prevalence of a particular mite vector species (Ogawa and Ono 2008). Of the main serotypes, Karp is the most common, accounting for approximately 40 % of all isolates (Kelly et al. 2009). Many of these isolates are described as "Karp related" as they have slight differences in the 56-kDa gene sequence compared to the prototype Karp strain, but the same reading frame is maintained (Kelly et al. 2009). The prevalence of the other serotypes ranges between 1.1 and 10.3 % (Table 16.2). While some cross-reactivity is observed between antibodies raised against one serotype with antigens from another, it is important for diagnostic serological methods to incorporate a mixture of antigens to ensure cases due to an antigenically variant strain of *O. tsutsugamushi* are not missed.

2.4   *Biology of* O. tsutsugamushi

The typical morphological form of an *O. tsutsugamushi* cell is a short rod or coccobacillus, with a length of 1.2–3.0 μm (Tamura et al. 1991) (Fig. 16.3). It is Gram negative (Tamura et al. 1995), although the Giemsa or Gimenez stain is more useful for observing the organism grown in tissues (Gimenez 1964). Propagation of *O. tsutsugamushi* must be performed in a biosafety level 3 (BSL3) laboratory (U.S. Department of Health and Human Services 2009) and as it is an obligate intracellular bacterium, its growth in vitro requires a host cell. Successful growth has been achieved in the yolk sac membrane of embryonated chicken eggs (Clancy and Cox 1946) and a variety of cell lines including mouse lymphoblasts (Bozeman et al. 1956), mouse fibroblasts (Urakami et al. 1982), and human endothelial cells (Kim et al. 1999). However, intracellular growth studies of *O. tsutsugamushi* have been hampered by its slow replication and comparatively low yields in vitro. Studies have shown the bacterial load can be improved by the addition of up to 20 % fetal bovine serum and 0.2–0.8 μg/mL daunorubicin (an inhibitor of host cell replication) to the culture medium, although the cell doubling time is still slow, at around 9 h (Giengkam et al. 2015; Hanson 1987). While cell-free media have been developed
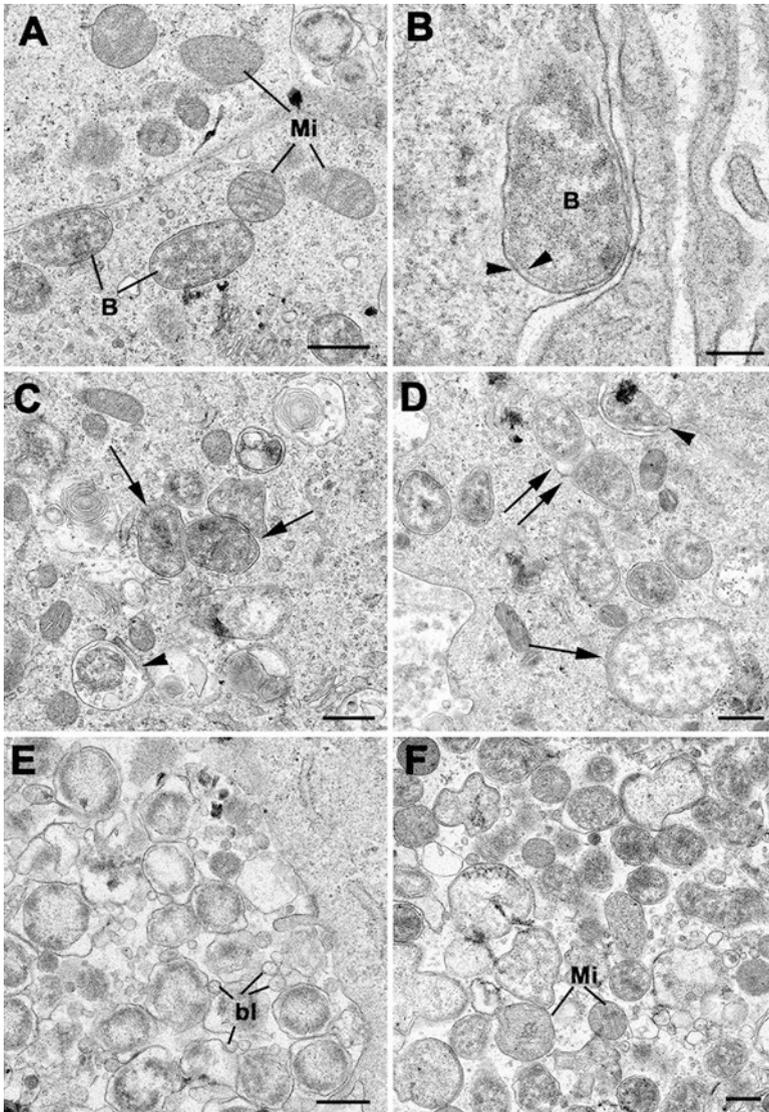
Fig. 16.3 *Orientia tsutsugamushi* morphology after infection of Vero cells (green monkey kidney epithelial cells, *O. tsutsugamushi* Thai strain UT76). (Images courtesy of Daniel Paris, Mahidol Oxford Tropical Medicine Research Unit, Mahidol University, Thailand and Centre for Tropical Medicine and Global Health, University of Oxford, UK). (**a**) Intact cytosolic *O. tsutsugamushi* (**B**), in close vicinity to mitochondria (Mi), both with and without cristae. Bar length corresponds to 500 nm. (**b**) Submembrane location of a single *O. tsutsugamushi*; the membrane protrusion is suggestive of budding in progress. Distinguishing feature of *O. tsutsugamushi*—double membrane with double leaflets (*arrowheads*). Bar length corresponds to 200 nm. (**c**) Intact *O. tsutsugamushi* (*arrows*) and degenerating *O. tsutsugamushi* in vacuoles (*arrowhead*). Bar length corresponds to 500 nm. (**d**) Vero cell with intact and free cytosolic *O. tsutsugamushi*. In the lower right is a large (approximately 2 mm in diameter) *O. tsutsugamushi* featuring a double-membrane and diffuse

for other species that were previously considered to be strictly intracellular, such as *Tropheryma whipplei* and *Coxiella burnetii*, axenic culture of *O. tsutsugamushi* has not yet been possible (Singh et al. 2013).

The in vivo target cells for human infection by *O. tsutsugamushi* are initially dendritic cells, monocytes, and macrophages in the skin at the site of the mite bite (Paris et al. 2012). The infection becomes disseminated, initially by migration of monocytes and dendritic cells to regional lymph nodes and via the lymphatic system (Paris et al. 2012), and subsequently via the blood to other organs, where endothelial cells and macrophages are the main bacterial targets (Moron et al. 2001; Keller et al. 2014).

Adherence and uptake of the bacteria by nonphagocytic host cells occurs through the process of endocytosis, which exploits host cell signaling pathways, leading to rearrangement of the host actin cytoskeleton. Several interactions between *O. tsutsugamushi* ligands and host receptor molecules have been implicated in the adhesion of bacteria to the host. The 56-kDa antigen of *O. tsutsugamushi* has been shown to bind to syndecan-4, a cell surface heparan sulfate proteoglycan (Kim et al. 2004a), and fibronectin (Cho et al. 2010; Lee et al. 2008). Inhibition of these interactions results in reduced internalization of bacteria by the host (Kim et al. 2004a; Cho et al. 2010; Lee et al. 2008). The bacterial autotransporter protein, ScaC, also binds to fibronectin, although this interaction seems only to enhance bacterial adherence, as it does not result in bacterial invasion (Ha et al. 2011). The formation of the 56-kDa antigen–fibronectin complex leads to engagement of integrin α5β1 molecules at the host cell surface and the subsequent activation of focal adhesion kinase (FAK) and Src tyrosine kinase through tyrosine phosphorylation (Cho et al. 2010). As a result, focal adhesions are formed and focal adhesion signaling adaptor proteins, such as talin and paxillin, are recruited to the site of attachment (Cho et al. 2010). In vitro observations by confocal microscopy have shown that within 10 min of infection, *O. tsutsugamushi* organisms are surrounded by membrane protrusions from the host cell surface. These are caused by rearrangements of the actin cytoskeleton, induced by the small GTPase, RhoA, which is activated following focal adhesion (Cho et al. 2010).

In addition to activation of the integrin-mediated signaling pathway, *O. tsutsugamushi* causes a transient activation of the signaling enzyme PLC-γ, which leads to the mobilization of calcium ions (Ca^{2+}) from intracellular calcium stores in the host. As a result, the host cytoplasmic calcium concentration is increased. It is cur-

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Fig. 16.3 (continued) bacterial cytoplasm (*arrow*). A single *O. tsutsugamushi* is in the process of budding and potentially invading the adjacent cell (*arrowhead*). Another *O. tsutsugamushi* is in the process of mitosis and binary fission (*double arrow*). Bar length corresponds to 500 nm. (**e**) Multiple *O. tsutsugamushi* within a phagosome. There is abundant production of blebs (bl), protrusions and cleaving of the outer membrane of *O. tsutsugamushi*. Bar length corresponds to 500 nm. (**f**) *O. tsutsugamushi* can appear in various forms and sizes, with condensed or grainy and loose cytoplasm. Note the close vicinity with mitochondria (Mi). Bar length corresponds to 500 nm

rently unclear what role this plays in *O. tsutsugamushi* infection, but in vitro*,* inhibition of PLC-γ reduces bacterial invasion by around 80 %. It is thought that activation of the Ca^{2+} signaling pathway may occur prior to or in parallel with integrin-mediated signaling and may contribute to the rearrangement of host actin (Ko et al. 2011).

O. tsutsugamushi cells that induce host cytoskeleton rearrangement are internalized by clathrin-dependent endocytosis, similar to several other intracellular pathogens such as *Chlamydia* spp. and *Neisseria gonorrhoeae* (Chu et al. 2006). Once internalized, bacteria are contained within clathrin-coated vesicles, which move through the endocytic pathway as evidenced by colocalization of *O. tsutsugamushi* with the early endosome marker EEA1 and late endosome and lysosome marker LAMP2 (Chu et al. 2006). This colocalization is only observed early in the infection process. By 2 h postinfection, the bacteria have escaped from the endocytic compartment and are free in the cytoplasm of the cell (Chu et al. 2006). This process is pH dependent, as demonstrated by studies in which *O. tsutsugamushi* failed to traffic to late endosomes if the normal acidification of the endosome was prevented (Chu et al. 2006). The mechanism by which the bacteria promote their release from the intracellular compartment is not yet elucidated but it may involve a hemolysin or phospholipase D (Chu et al. 2006; Ge and Rikihisa 2011). The endocytic pathway is not involved during *O. tsutsugamushi* infection of professional phagocytes such as macrophages (Chu et al. 2006). Instead, the bacteria become internalized within a phagosome, from which some may escape into the host cytoplasm (Rikihisa and Ito 1979).

O. tsutsugamushi free within the cytoplasm move from the periphery of the cell to the microtubule organizing center in the region around the nucleus, using the microtubule network. Bacterial movement is mediated by the motor protein dynein. This is in contrast to other rickettsiae, which move within the cytoplasm using the host cell actin (Kim et al. 2001). Although intranuclear replication of *O. tsutsugamushi* has been observed (Urakami et al. 1982; Pongponratn et al. 1998), replication occurs more typically in the perinuclear region of the cytoplasm by binary fission (Urakami et al. 1984) (Fig. 16.3d). As the host cell becomes more heavily infected with *O. tsutsugamushi,* micro-colonies are observed, which may be biofilm-like structures, aggregated by matrices of the bacterial polysaccharide antigen, NT19 (Lee et al. 2009). In vitro, by 72 h postinfection, *O. tsutsugamushi* cells are observed throughout the host cell cytoplasm, including at the cell periphery. The bacteria push on the host cell membrane from the inside (Fig. 16.3b, d) and as the infection proceeds, numerous bud-like projections containing bacteria are observed on the host cell surface (Urakami et al. 1984). The bacteria are released from the host cell, either in host membrane coated buds (Ewing et al. 1978) or as free bacteria, following lysis of the host (Rikihisa and Ito 1979). Both forms have been shown to be capable of infecting other new cells, although one form may be more infective than the other for some host cell types. For example, in a study performed in mice, mesothelial cells of the peritoneal cavity were able to phagocytose the host membrane coated vesicles containing *O. tsutsugamushi* but not uncoated bacteria (Ewing et al. 1978). A similar budding process has been observed in mites infected

with *O. tsutsugamushi*, where bacteria are released from the cells of the mite salivary glands, when triggered by mite feeding (Kadosaka and Kimura 2003).

The interaction of *O. tsutsugamushi* with its host is a complex process. A study of human monocytes showed that the expression of more than 4500 genes was altered in response to infection by *O. tsutsugamushi.* The most up-regulated genes (>20 % enrichment) were those involved in the immune response and more than 15 % of these genes were cytokines or chemokines (Tantibhedhyangkul et al. 2011). While a normal host response elicits the production of cytokines, if this production is excessive it can cause a severe systemic disorder in the host (Ge and Rikihisa 2011). The hyperproduction of cytokines and chemokines has been observed in a scrub typhus susceptible mouse model (Yun et al. 2005) and in scrub typhus patients, and is likely linked to some of the systemic symptoms observed (Kramme et al. 2009; Chung et al. 2008). Infection with *O. tsutsugamushi* can result in the subversion of functions within the host cell, which promotes bacterial survival. For example, the process of autophagy plays an important role in both the innate and adaptive immune response against several intracellular pathogens (Choi et al. 2013). Although *O. tsutsugamushi* induces autophagy, it actively escapes the autophagosomes, therefore its growth is unaffected by the process (Choi et al. 2013; Ko et al. 2013). Whole genome sequencing of *O. tsutsugamushi* revealed a large number of genes encoding proteins with eukaryotic ankyrin-repeat (Ank) domains (Cho et al. 2007; Nakayama et al. 2008). These proteins have been found in other intracellular bacteria such as *Coxiella burnetii* and *Anaplasma phagocytophilum*, in which they have been implicated in the regulation of host cell processes. Recent studies have shown that the *O. tsutsugamushi* Ank proteins are substrates for the Type I secretion system (Beyer et al. 2015; Min et al. 2014; Viebrock et al. 2014). Several of these proteins traffic to the endoplasmic reticulum of the host cell, where they may be involved in modulation of the host response to infection (Viebrock et al. 2014) and one (Ank9) interacts with the host cell polyubiquitination machinery, although the host cell protein targets are yet to be determined (Beyer et al. 2015). *O. tsutsugamushi* may also affect the metabolism of its host. For example, in L929 cells, infection with *O. tsutsugamushi* resulted in altered lipid metabolism, with the accumulation of organelles capable of storing triglycerides, known as lipid droplets (Ogawa et al. 2014). The role of this alteration has not yet been determined, but lipid droplets are essential for the growth of *Chlamydia trachomatis* (Cocchiaro et al. 2008), and may also be involved in the growth or survival of *O. tsutsugamushi* (Ogawa et al. 2014).

3   Disease Transmission

Like other rickettsial diseases, scrub typhus is arthropod borne. Its vector is the larval stage of more than 50 species of trombiculid mites belonging to several genera, but species of the genus *Leptotrombidium* are considered to be the primary cause of disease transmission in most countries (Lee et al. 2011). These larvae (Fig. 16.4) are commonly referred to as chiggers.

Fig. 16.4 Larval (chigger) stage of the trombiculid mite. Micrograph of an unfed larva of the species *Eutrombicula hirsti*. Although not a vector of scrub typhus, this larva is typical of the trombiculid mites. (Image courtesy of Stephen Frances, Vector Surveillance and Control, Australian Army Malaria Institute)

Fig. 16.5 Life cycle of trombiculid mites. The life cycle comprises four main stages (egg, larva, nymph, and adult) with three intermediate, quiescent stages. Humans are an incidental host of the larvae, which more commonly feed on mammals or small birds (Santibáñez et al. 2015)
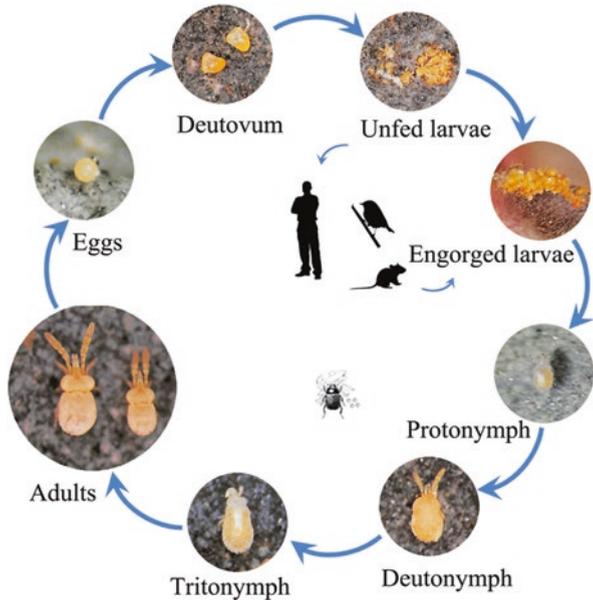
The life cycle of trombiculid mites encompasses four main stages: egg, larva, nymph, and adult (Fig. 16.5). Females lay their eggs loosely in soil over a period of 6–12 weeks, at a rate of one to five eggs per day. After a break of a similar time, a second phase of oviposition takes place. During this whole 3–5 month period, a single female will lay around 400 eggs. The egg stage lasts only 5–7 days, then the shell ruptures, exposing the deutovum (an immature, quiescent form), which develops into the larva within another week. The six-legged larvae that emerge are barely visibly to the naked eye. Within 2 days of emergence, they are ready to feed, with most species attaching to any mammal or bird with which they can make contact.

The larvae feed on serum exudate and cellular debris rather than blood, taking 2–12 days to become fully engorged. After detaching from their host, they enter a second quiescent, pupa-like phase (protonymph), and develop into the eight-legged nymph within 7–10 days. The nymphs do not require a host, but instead feed on the eggs of other arthropods and quiescent or deceased soft-bodied insects. Within about 2 weeks, the nymphs enter another quiescent phase (tritonymph) and emerge as adult mites around 2 weeks later. Male adults deposit spermatophores, which are collected by the adult females, and oviposition begins within a fortnight. The life cycle of most *Leptotrombidium* mites typically takes 2–3 months, but it may be as long as 8 months in some species. Adult mites live for around 4–6 months, although some have been observed to survive for more than 14 months in the laboratory (Neal and Barnett 1961; Traub and Wisseman 1974; Walker et al. 1975).

Since only the larvae bite humans, for the mite to act as a vector for scrub typhus it is essential that the causative bacteria are transmitted trans-stadially to the subsequent nymph and adult stages, then transovarially from the female to the eggs (Burgdorfer and Varma 1967). This results in a new population of infected larvae capable of transmitting disease. The transovarial transmission of *O. tsutsugamushi* has been demonstrated and studied in many species of trombiculid mites (Rapmund et al. 1969, 1972; Traub and Wisseman 1974; Roberts and Robinson 1977; Frances et al. 2001; Phasomkusolsil et al. 2009; Shin et al. 2014). This process is very efficient, with transovarial transmission rates (percentage of infected females that transmit the bacteria to their progeny) often as high as 100 %. Filial infection rates (percentage of infected larvae derived from a single infected female) are more variable and seem to vary between parents (Rapmund et al. 1969; Urakami et al. 1994a). In laboratory reared mites, three main patterns of filial infection rates have been observed: (1) all the progeny are infected, (2) all except one or two individuals are infected, and (3) the offspring are mostly uninfected (Rapmund et al. 1969). In some laboratory-reared colonies, filial infection became less efficient over time. Commonly, 100 % of the first generation of larvae from *O. tsutsugamushi* infected females was also infected, whereas in later generations, the overall filial infection rate dropped to less than 65 % (Urakami et al. 1994a; Frances et al. 2001; Phasomkusolsil et al. 2009).

In infected mites, *O. tsutsugamushi* is distributed throughout the body and found in most tissues, although not all studies have demonstrated its presence in the muscles (Urakami et al. 1994a, b; Wright et al. 1984). Particularly high concentrations of the organism are found in the salivary glands of the larvae and in the salivary glands, excretory bladder, epidermal layer, digestive tissues, and reproductive organs of adults (Wright et al. 1984; Urakami et al. 1994a). Although the bacteria have been observed in the testes of infected adult males, they are not transmitted to the spermatophores (Takahashi et al. 1988; Urakami et al. 1994b), thus only infected females are capable of passing the infection to their offspring. In some species, infection with *O. tsutsugamushi* has been shown to affect the development of infected mites. Infected larvae of *L. chiangraiensis* and *L. imphalum* were shown to feed and detach from their host quicker than larvae that were not infected. This may be advantageous as the period where the mites are vulnerable to antimite behavior

by the host is reduced (Phasomkusolsil et al. 2012). Egg production in several species of mites is higher when the adult females are infected (Roberts et al. 1977; Frances et al. 2001), again conferring an advantage to the mite, by increasing the number of progeny. However, in other species, a reduction in both egg production and hatching is observed in infected mites (Roberts et al. 1977; Phasomkusolsil et al. 2012; Shin et al. 2014), which would be expected to decrease their survival. In some species of mite studied, infection with *O. tsutsugamushi* alters the sex ratio of the offspring from around 2:1 in favor of females, to exclusively females (Roberts et al. 1977; Shin et al. 2014). This phenomenon has not been observed in uninfected mites.

The primary hosts of the trombiculid mite larvae are wild rodents. Many studies have demonstrated the presence of *O. tsutsugamushi* in these animals (Jackson et al. 1957; Walker et al. 1973; Glazebrook et al. 1978; Ishikura et al. 1985; Lerdthusnee et al. 2008; Cosson et al. 2015) and the larval mites successfully transmit the bacterium to their rodent hosts (Frances et al. 2001; Lerdthusnee et al. 2002). Although the larval acquisition of *O. tsutsugamushi* from an infected animal and subsequent trans-stadial transmission have been demonstrated, the infection is very rarely passed on to the mites' offspring (Traub et al. 1975; Walker et al. 1975; Takahashi et al. 1994). It is thought that the bacteria may not penetrate the walls of the gut, thus never reaching the correct part of the mite's body to be transmitted by the transovarial route (Walker et al. 1975; Traub et al. 1975). The reservoir of a disease must be capable of maintaining "a regular or permanent source of infection in nature" (Traub et al. 1975). Since uninfected mites acquiring *O. tsutsugamushi* from their hosts are not able to transmit the bacteria to another host, wild rodents are not a reservoir of scrub typhus. Instead, trombiculid mites are considered to be both the disease reservoir and vector. However, rodents are important in the ecology of scrub typhus and it is likely that infected rodents provide the explanation for the occurrence of multiple strains of *O. tsutsugamushi* within individual larvae (Shirai et al. 1982; Frances et al. 2000).

4 Epidemiology

Traditionally, the worldwide distribution of scrub typhus has been defined by the 'tsutsugamushi triangle.' This describes the region in which the disease is endemic and encompasses an area of approximately 13 million square kilometers, with the northern most point in Korea and the far east of Russia, reaching to tropical northern Australia in the south and Afghanistan in the west (Paris et al. 2013) (Fig. 16.6). However, in recent years, this concept has been challenged by cases of the disease outside of the endemic region. The aforementioned case of scrub typhus caused by the proposed new species *O. chuto* was acquired in Dubai in the United Arab Emirates, around 500 km west of the previously recognized area (Izzard et al. 2010) and in 2006, a patient bitten by a leech on a Chilean island developed a scrub typhus-like illness, with an *Orientia*-like agent confirmed by DNA
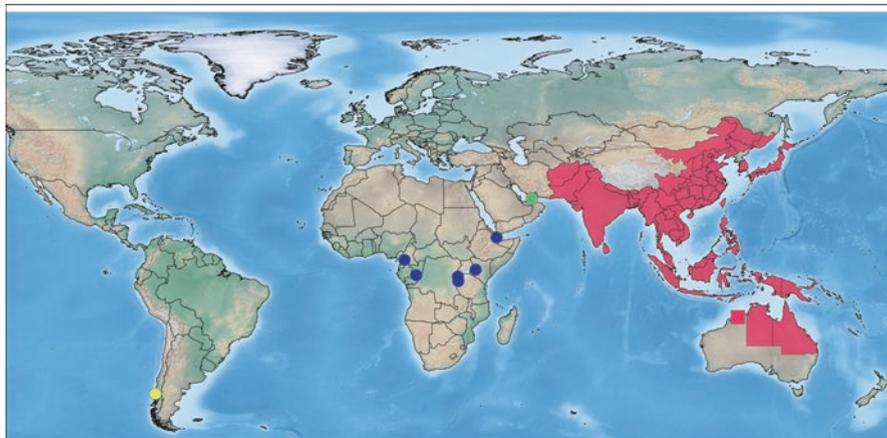
Fig. 16.6 Worldwide distribution of scrub typhus. Map indicates the regions in which scrub typhus is known to be endemic (*red*) and the locations of scrub typhus cases caused by *O. chuto* (*green dot*) and an Orientia-like species (*yellow dot*). Possible locations of scrub typhus infection in Africa are also indicated (*blue dots*)

sequencing (Balcells et al. 2011). If the leech is shown to be an alternative vector for scrub typhus, the distribution of the disease may be increased. A further 3 confirmed cases of scrub typhus have more recently been reported from the same island, in patients that had never travelled outside of Chile, therefore the infection may now be endemic in this region (Weitzel et al. 2016). There have also been reports of scrub typhus cases and seroprevalence studies in which infection with *O. tsutsugamushi* was believed to have occurred in Africa (Osuga et al. 1991; Ghorbani et al. 1997; Thiga et al. 2015; Luce-Fedrow et al. 2015). It is important for clinicians in nonendemic countries to consider the disease in travelers with compatible symptoms returning from the endemic region, and in light of these recent cases to be aware that scrub typhus may also be acquired outside of the 'tsutsugamushi triangle.'

Humans become infected with *O. tsutsugamushi* via the bite of an infected chigger, when they encroach on the habitats in which the mites are found. Therefore, the epidemiology of scrub typhus is closely linked to the distribution and behavior of the trombiculid mite species that are vectors of the disease. Unsurprisingly, positive correlations have been demonstrated between the number of human scrub typhus cases in an area with both the coincidence of mite vector species (Ishikura et al. 1985; Roh et al. 2014) and the prevalence of *O. tsutsugamushi* infection in wild rodents (Ishikura et al. 1985; Lin et al. 2014). The description of the disease as scrub typhus is a misnomer as trombiculid mites are not restricted to the scrub vegetation of subtropical regions but are found in a diverse range of climates and habitats. These include subarctic regions, semidesert (Traub and Wisseman 1968), woody vegetation (Traub and Wisseman 1974), deep jungle (Cadigan et al. 1972), and rice paddies (Tanskul et al. 1998). Some of the scrub typhus vector mite species have a

very restricted distribution. For example, *L. arenicola* is only found in Malaysia (Upham et al. 1971). Other species, such as *L. deliense*, appear to be more adaptable and their distribution is more widespread (Traub and Wisseman 1974). Within the areas in which the mites are found, their distribution can be highly focal. Localized units of mites are termed "mite islands" and they range in size from a few centimeters to more than a meter (Traub and Wisseman 1968). They can be explained by the observation that the larvae are rarely observed individually, but occur mostly as clusters, which wait on stems or leaves to attach to a host (Gentry et al. 1963).

The seasonal occurrence of scrub typhus varies with the climate in different countries. For example, on the Korean peninsula, the peak incidence is during October and November (Noh et al. 2013), whereas in southern China, the number of cases peaks first in the summer months of June and July, then second in September and October (Wei et al. 2014a). In contrast, in some areas of Taiwan, no seasonal variation in the number of cases is observed (Tsai and Yeh 2013). Early observations of *L. deliense* and *L. akamushi* in Malaysia showed that the larvae in their natural habitats were sensitive to changes in temperature and humidity (Gentry et al. 1963), thus seasonal variation in disease occurrence is linked to fluctuations in temperature and rainfall. Many studies have described increased numbers of chiggers during periods of increased rainfall (Traub and Wisseman 1974), therefore scrub typhus outbreaks are often associated with monsoon and rainy seasons (Gurung et al. 2013) or periods of heavy rain (Faa et al. 2003). In temperate regions, scrub typhus cases are correlated more with temperature variations than with rainfall (Van Peenen et al. 1976; Kuo et al. 2011). There is evidence that climate change may be affecting the temporal occurrence and number of scrub typhus cases in some areas. For example, in Pingtan Island in eastern China, the known vector of scrub typhus is *L. deliense*, which previously appeared in late May, resulting in cases of scrub typhus in the summer. Since 2000, several cases of the disease have been reported in the spring, with mites observed as early as March. This could be linked to a rise in the average March temperatures since 1997 (Cao et al. 2006). Increases in temperature, sunshine, and rainfall were also linked to an increase in scrub typhus incidence in some areas of northern and southern China (Li et al. 2014; Yang et al. 2014).

Traditionally, scrub typhus is considered to be a disease associated with agricultural activities in rural areas (Kuo et al. 2011). Seroprevalence and outbreak studies have identified various occupational and behavioral risk factors for exposure to *O. tsutsugamushi*. These include being a farmer (particularly on dry, cultivated land), working in vegetable fields, bundling waste straw, living at the edge of a village, sitting on grass while taking breaks, and having close contact with rats (Mathai et al. 2003; Kweon et al. 2009a; Vallée et al. 2010; Kuo et al. 2011; Lyu et al. 2013; Wei et al. 2014b; Hu et al. 2015). A higher prevalence of antibodies against *O. tsutsugamushi* has been observed in older people (>50 or 60 years) in several studies (Bang et al. 2008; Brown et al. 1978a; Kuo et al. 2011; Vallée et al. 2010; Zheng et al. 2015), probably reflecting increased opportunities for exposure over the course of their lifetime. Many studies have demonstrated an increased risk of exposure for females compared to males (Bang et al. 2008; Kweon et al. 2009b; Kuo et al. 2011; Noh et al. 2013; Zheng et al. 2015), which may be linked to differences in the work-

ing behaviors of men and women in some areas. In South Korea, for example, men tend to work in the rice fields, where they use tools in a standing position. Women are more likely to be employed in dry fields, where they work with bare hands, typically in a squatting position in which they are more likely to come into contact with infected mites (Kweon et al. 2009b). However, in some areas such as Japan, there is no significant difference in exposure risk between the two sexes and equal numbers of scrub typhus cases are seen in males and females. This is believed to reflect cultural differences between different countries in terms of work and clothes (Bang et al. 2008). Military personnel deployed in endemic areas also have a high risk of contracting scrub typhus, due to their exposure to vegetation harboring infected mites. Subsequent to the aforementioned cases observed during World War II, outbreaks occurred during the Vietnam conflict and numerous cases have been recorded during training exercises and humanitarian operations (Corwin et al. 1999; Bavaro et al. 2005; Likeman 2006).

There is evidence to suggest that the epidemiology of scrub typhus is changing. Certainly, the disease is becoming urbanized and can no longer be considered a problem limited to rural areas. In recent years, numerous cases and outbreaks have been recognized in urban patients (Strickman et al. 1994; Wei et al. 2014a, b; Sethi et al. 2014; Park et al. 2015). One such outbreak, associated with a city park in China resulted in four deaths (Wei et al. 2014b). The urbanization of scrub typhus can be attributed to three main factors. First, the incursion of expanding human populations into agricultural or previously uninhabited areas has resulted in deforestation and the clearing of land, creating more suitable habitats for the vector mite species (Strickman et al. 1994; Vallée et al. 2010). Second, infected chiggers have been found in central urban locations, possibly due to their spread from surrounding areas (Park et al. 2015). Third, changes in the behavior of urban residents have made their contact with trombiculid mites more likely. For example, the working week in Korea was reduced to 5 days in 2004, allowing urban workers more time for recreational activities such as golf and climbing, or for agricultural activities such as harvesting chestnuts (Kweon et al. 2009b). In rural areas, changes in land use may affect scrub typhus epidemiology. For example, following Taiwan's admission to the World Trade Organization, its rice market was exposed to foreign competition, leading to the abandonment of many rice paddies and reduced plowing in agricultural regions. This resulted in overgrowth of secondary vegetation and an increase in rodents and chiggers in these areas, increasing the risk of scrub typhus transmission (Kuo et al. 2012).

The incidence of scrub typhus is increasing in many countries. In part this may be due to improvements in diagnostic testing and better awareness. However, it is clear that the disease has reemerged in areas where cases had not occurred for many years (Lewis et al. 2003; Khan et al. 2012; Sethi et al. 2014) and that it is newly emerging in regions where it had not been recognized previously (Zhang et al. 2010; Hu et al. 2015). For example, in Japan where much of the early scrub typhus research was carried out, the disease was apparently absent for a period of around 10 years prior to its reemergence in 1976, with a subsequent increase in case numbers and expansion of endemic areas (Ishikura et al. 1985). Particularly rapid increases in

disease incidence have been seen in some countries in recent years (Kweon et al. 2009b; Li et al. 2013; Zhang et al. 2013). In South Korea there were almost four times more cases in 2013 compared with 2001 (Lee et al. 2015a) and in China, 12.8 times more cases were reported in 2014 compared with 2006 (Wu et al. 2016). It is important to understand the changing epidemiology of scrub typhus to inform public health policies in both traditional and newly recognized endemic areas.

5 Scrub Typhus, the Disease

5.1 Clinical Manifestations

Scrub typhus presents as a mild disease in some patients, whereas others suffer from a more severe illness, which may even result in death. The bite of the chigger responsible for the transmission of *O. tsutsugamushi* to humans is usually painless and goes unnoticed by the patient (Watt and Parola 2003). Person-to-person transmission of scrub typhus is rare but a few instances have been recorded. Routes of transmission include needle-stick injuries via the placenta from mother to baby and stem cell transfusion (Wang et al. 1992; Kang et al. 2010).

In the days following infection, a small, (2–3 mm), reddish lesion may develop at the bite site and swelling may occur in the adjacent lymph nodes (Hayashi 1920). However, normally the first sign of illness is the sudden onset of a fever accompanied by nonspecific symptoms such as chills, headache, coughing, myalgia, nausea, diarrhea, and vomiting (Corbett 1943; Lee et al. 2013). The incubation period of scrub typhus is typically 7–10 days but can vary between 6 and 21 days.

In some patients, the small lesion at the bite site becomes larger, undergoing necrosis at the center and developing a blackened crust. This larger lesion is known as an eschar and it resembles a cigarette burn (Watt and Parola 2003) (Fig. 16.7).

Eschars are usually found on parts of the body that tend to be warm and damp, such as the groin and axilla and often where pressure from clothing occurs, such as the waistband (Irons and Armstrong 1947). Differences in the distribution of eschars on males and females have been observed. In a study of 162 patients with eschars, the primary area for eschar development in males was within 30 cm below the umbilicus, whereas in females, the lesions were most prevalent on the front chest, above the umbilicus (Kim et al. 2007a). Another study demonstrated that preferential eschar development sites varied in different geographic areas (Zhang et al. 2012). Multiple eschars on a single scrub typhus patient are rare but have been documented (Kim et al. 2007a; Kaushik et al. 2014). Eschars are not present in all cases of scrub typhus and incidence rates are extremely variable. A low prevalence of 1–4 % has been recorded in several case series from India (Mathai et al. 2003; Sharma et al. 2005; Sethi et al. 2014), although another Indian study observed eschars in 45 % of the patients (Chrispal et al. 2010). In cohorts from other Asian countries, eschars have been present in a higher proportion (>60 %) of the patients (Sirisanthana et al. 2003; Kim et al. 2010; Hu et al. 2015). In addition to the eschar, some scrub typhus

Fig. 16.7 Examples of
eschars on scrub typhus
patients. Images courtesy
of Munegowda Koralur;
Kasturba Medical College,
Manipal University, India

patients develop a maculopapular rash on the trunk, which appears 5–8 days after
the onset of fever. The rash may extend to the arms and legs later in the infection
(Irons and Armstrong 1947; Jeong et al. 2007). Other signs of the disease include
generalized lymphadenopathy, acute hearing loss, conjunctival congestion, hepato-
megaly, and splenomegaly (Noad and Haymaker 1953; Premaratna et al. 2006;
Chrispal et al. 2010; Zhang et al. 2012).

   In some patients, the clinical course of scrub typhus is more severe and a range
of serious complications has been reported involving organs of the pulmonary, car-
diac, abdominopelvic, and central nervous systems. These include interstitial pneu-
monia (Choi et al. 2000), acute respiratory distress syndrome (ARDS) (Park et al.
2000; Wang et al. 2007), myocarditis (Levine 1946), acute cholecystitis (Lee et al.
2015b), renal failure (Yen et al. 2003; Kim et al. 2010), acute kidney injury (Attur
et al. 2013), gastrointestinal bleeding (Irons and Armstrong 1947), meningitis or
meningoencephalitis (Kim et al. 2013), severe disseminated intravascular coagula-
tion (Ono et al. 2012), and septic shock (Sethi et al. 2014). The diversity of these
clinical manifestations is explained by the basic pathology of *O. tsutsugamushi*

infection. The bacteria cause a focal or disseminated vasculitis due to the destruction of the endothelial cells (Choi et al. 2000; Chrispal et al. 2010). In cases of severe scrub typhus, it is not uncommon for multiple organs to be affected. For example, in a cohort of 116 patients with severe scrub typhus, 85 % experienced a dysfunction of three or more organ systems (Griffith et al. 2014). Risk factors that correlate with the development of severe disease include older age (>60 years), the absence of an eschar, low serum albumin (≤3.0 g/dL), elevated leukocytes (>10,000 μL), the presence of interstitial pneumonia, and elevated plasma inflammatory markers such as YKL-40 (Song et al. 2004; Chrispal et al. 2010; Kim et al. 2010; Otterdal et al. 2014). There is also evidence of a relationship between bacterial load and severity of the disease. A study of 155 Thai scrub typhus patients demonstrated a positive correlation between the concentration of *O. tsutsugamushi* in the blood taken on admission to hospital and the duration of illness, presence of an eschar, and hepatic enzyme levels. The bacterial load in patients that died was significantly higher than in those that recovered from their illness (Sonthayanon et al. 2009). A large systematic review of more than 19,000 untreated scrub typhus patients in 89 case series showed that overall, median mortality from the disease was only 6 %, although a wide range from 0 to 70 % was observed (Taylor et al. 2015). Variability may be due to numerous factors including the infecting strain, geographic area, and host factors (Taylor et al. 2015). Mortality rates in patients with severe complications are often higher. For example, studies of patients with ARDS have demonstrated mortality rates between 22 and 61 % (Wang et al. 2007; Chrispal et al. 2010; Griffith et al. 2014). Other predictors of mortality include the duration of fever, severity of illness on presentation to hospital, elevated serum creatinine (indicative of renal failure), and shock (Varghese et al. 2006; Chrispal et al. 2010; Griffith et al. 2014).

Coinfections of *O. tsutsugamushi* with several other pathogens have been reported. Diseases shown to occur concurrently with scrub typhus include pneumonia caused by *Mycoplasma pneumoniae* (Lee et al. 2015c), murine typhus (Phommasone et al. 2013), Q fever (Lai et al. 2009), leptospirosis (Watt et al. 2003a; Chen et al. 2007; Sonthayanon et al. 2013), chicken pox (Chandramohan et al. 2015), malaria (Mahajan et al. 2014), and dengue (Kumar et al. 2014). Several of the coinfecting pathogens are susceptible to the antimicrobials used to treat scrub typhus. However, a coinfection should be suspected in scrub typhus patients if defervescence does not occur within 48–72 h of appropriate antimicrobial treatment and combination therapy may be required (Wei et al. 2012). Unusually, one *O. tsutsugamushi* coinfection may be advantageous to the patient. In Thailand, researchers observed that HIV-1 patients with scrub typhus experienced a reduction in viral load, and serum from an HIV-1 negative scrub typhus patient had a potent suppressive effect on the virus in vitro (Watt et al. 2000a). Another study was not able to replicate these results in vitro and showed that instead, HIV-1 replication was induced by *O. tsutsugamushi* (Moriuchi et al. 2003). Further work by the Thai researchers demonstrated that antibodies produced in acute scrub typhus suppressed CXCR4-HIV-1 viruses but not those utilizing the CCR5 coreceptor. However, the suppressive effect on the CXCR4-utilizing viruses was potent and long lasting

(Watt et al. 2013). This was the first description of antibodies to one organism having a toxic effect on another infectious agent.

5.2 Diagnosis

The clinical presentation of scrub typhus is notoriously nonspecific. Since the primary manifestation is an acute, undifferentiated fever, when a patient first presents to a healthcare facility there is little to distinguish scrub typhus from other diseases such as typhoid, leptospirosis, and dengue, which are usually endemic in the same areas (Suttinont et al. 2006; Watt et al. 2003b). A recent Korean study developed a prediction rule for identifying suspected cases of scrub typhus in patients with acute undifferentiated fever. The rule comprised five predictors of disease that were derived from a multiple regression model, with each assigned a points value. Predictors were age ≥65 years old (two points), recent history of fieldwork or outdoor activity (one point), onset during the known outbreak period (September to December in Korea; two points), myalgia (one point), and presence of an eschar (two points). Using a cutoff value of four or more points, the sensitivity and specificity of the prediction rule for diagnosis of scrub typhus were 92.7 % and 90.9 %, respectively. While application of this prediction rule does not replace confirmatory testing, it can be used at the time of admission, allowing for the prompt initiation of treatment. However, it should not be used in more complicated cases of scrub typhus with clinical manifestations such as pneumonia or meningitis, and its use outside of Korea has not yet been validated (Jung et al. 2015).

The laboratory and radiographical findings in scrub typhus patients (Table 16.3 and Fig. 16.8) do not provide a definitive diagnosis, although in combination with other symptoms and signs they may lead to a strong suspicion of the disease. For example, in a cohort of Indian patients, a combination of elevated transaminases, thrombocytopenia, and leukocytosis had the best predictive values for diagnosing patients with acute undifferentiated fever (Varghese et al. 2006).

Diagnostic tests for scrub typhus are mostly based on serological methodologies.

The oldest test, the Weil–Felix OXK, is based on the cross-reaction of the OXK antigen from *Proteus mirabilis* with scrub typhus IgM antibodies, resulting in agglutination of the serum (Amano et al. 1992). It has long been recognized that its sensitivity and specificity are poor, particularly in the early stages of *O. tsutsugamushi* infection (Brown et al. 1983; Isaac et al. 2004). For example, in a comparison between the Weil–Felix OXK and indirect immunoperoxidase (IIP) tests, only 10 % of serum samples positive by IIP gave a positive reaction in the Weil–Felix test within the first 9 days of illness (Amano et al. 1992). However, the Weil–Felix test is very cheap and easy to perform, therefore despite its severe limitations, it is still used in some resource-poor regions in which other serological tests are not available. It can be a useful tool in these settings, provided clinicians are aware of its pitfalls and results are interpreted in the correct clinical context (Mahajan et al. 2006).

Table 16.3 Summary of laboratory and radiographical findings in scrub typhus patients

Investigation	Finding	Reported prevalence	Reference
White blood cell count	Leukocytosis Leukocytes >10,000/μL	9–54 %	Ogawa et al. (2002); Mathai et al. (2003); Song et al. (2004); Chrispal et al. (2010); Hu et al. (2015)
Platelet count	Thrombocytopenia Platelets <10,000/μL	21–44 %	Tsay and Chang (1998); Mathai et al. (2003); Song et al. (2004); Chrispal et al. (2010); Griffith et al. (2014); Hu et al. (2015)
	Severe thrombocytopenia Platelets < 50,000/μL	25–47 %	
Serum albumin	Hypoalbuminemia <3.0 g/dL	16–69 %	Song et al. (2004); Kim et al. (2010)
Serum myoglobin	Elevated	35 %	Choi et al. (2000)
Renal function	Elevated serum creatinine > 1.6 mg/dL or > 120 μmol/L	15–37 %	Mathai et al. (2003); Song et al. (2004)
	Elevated blood urea nitrogen > 20 mg/dL	49 %	Jung et al. (2015)
Hepatic function	Elevated transaminases Aspartate transaminase > 60 IU/L Alanine transaminase > 60 IU/L	75–95 %	Chrispal et al. (2010), Song et al. (2004), Ogawa et al. (2002), Mathai et al. (2003), Choi et al. (2000), Zhang et al. (2012), Tsay and Chang (1998)
	Elevated C-reactive protein > 10 mg/dL	25–96 %	Ogawa et al. (2002); Kim et al. (2010); Zhang et al. (2012); Hu et al. (2015)
	Elevated lactate dehydrogenase	92 %	Ogawa et al. (2002); Kim et al. (2010)
	Elevated bilirubin > 1 mg/dL or > 25 μmol/L	18–29 %	Ogawa et al. (2002); Mathai et al. (2003); Kim et al. (2010); Jung et al. (2015)
Partial oxygen pressure of arterial blood (PaO₂)	Hypoxia PaO₂ < 60 mmHg	24–34 %	Song et al. (2004); Chrispal et al. (2010)
Blood pressure	Hypotension Arterial systolic pressure < 90 mmHg	17 %	Song et al. (2004)
Chest x-ray	Normal	50 %	Choi et al. (2000); Mathai et al. (2003); Song et al. (2004); Jeong et al. (2007)
	Pulmonary abnormalities	37–78 %	
	Pleural effusion	42–55 %	
	Hilar enlargement	14–45 %	
	Cardiomegaly	15–38 %	
	Interstitial pneumonia	51 %	Song et al. (2004)

Fig. 16.8 Chest X-ray of a scrub typhus patient. Radiograph shows a diffuse interstitial pneumonia. The patient developed ARDS but responded well to treatment with doxycycline. Image courtesy of Munegowda Koralur
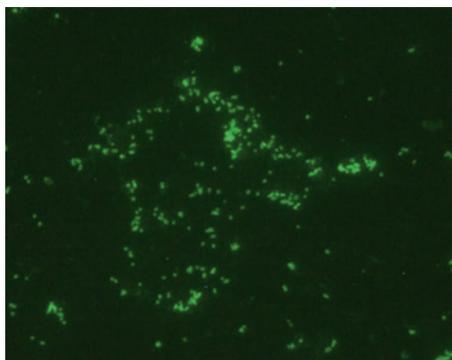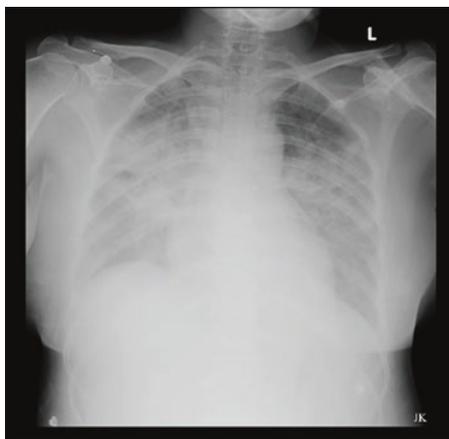
Fig. 16.9 Indirect immunofluorescence assay for scrub typhus diagnosis. Image shows a positive result caused by the binding of antibodies to *O. tsutsugamushi* in the patient serum to antigen from the organism immobilized on the slide. A secondary anti-human antibody labeled with fluorescein isothiocyanate (FITC) enables detection of this reaction by fluorescent microscopy

The currently recognized gold standard test is the indirect immunofluorescence assay (IFA) (Blacksell et al. 2007), in which antibodies to *O. tsutsugamushi* present in the serum of the patient are bound to antigen on a slide, then detected using a fluorescently labeled anti-human antibody (Fig. 16.9).

The most commonly used antigens are a mixture of the Karp, Kato, and Gilliam serotypes (Blacksell et al. 2007), although in some areas, local serotypes are also included (Blacksell et al. 2007; Koh et al. 2010). A positive result is conventionally defined by an IgM titer of ≥1:400 on a single sample taken on admission, or by a fourfold (or greater) rise in titer between paired acute and convalescent samples to ≥1:200 (Brown et al. 1983). However, these conventional cutoff titers may lead to false-positive results, particularly where testing is performed on the admission sam-

ple alone (Blacksell et al. 2007; Lim et al. 2015a). A more recent study proposed revised cutoffs of ≥1:3200 for a single sample, or at least a fourfold rise to ≥1:3200 in the convalescent sample of a pair. These newly proposed optimal cutoffs resulted in an assay sensitivity and specificity of 81 % and 100 %, respectively (Lim et al. 2015a). It is recommended that if IFA is to be used for scrub typhus diagnosis in an endemic country, a local cutoff should be determined based on the scrub typhus seroprevalence rate in the healthy population. This better enables acute infection to be distinguished from previous exposure, which is especially important if testing is performed on only the acute sample (Blacksell et al. 2007). Besides difficulties with determining the appropriate cutoff, the IFA has additional limitations. Since a microscopist reading the slide determines the end-point titer, the test is inherently subjective. Inter- and intraoperator variability has been demonstrated and microscopists must undergo several months of training or be supervised by a more experienced staff member before their results can be considered reliable (Phetsouvanh et al. 2013). The main drawback of the IFA, however, is the relatively high cost and its requirement for a fluorescent microscope. This restricts its use in many of the resource-limited areas in which scrub typhus occurs (Koh et al. 2010). An alternative to the IFA is the IIP, in which the secondary antibody is labeled with peroxidase instead of a fluorochrome (Yamamoto and Minamishima 1982; Kelly et al. 1988). This eliminates the need for a fluorescent microscope, although the method suffers from the same limitations as IFA in terms of cutoff determination.

A scrub typhus IgM ELISA was first developed in 1979, when it was shown to have a similar sensitivity and specificity to the IFA (Dasch et al. 1979). An assay utilizing the *O. tsutsugamushi*-specific recombinant 56-kDa antigen is now available as a commercial kit and more recent studies have demonstrated a similar performance, with sensitivities in the range of 85–93 % and specificities between 94 and 97.5 % (Coleman et al. 2002; Prakash et al. 2006; Koraluru et al. 2015). Although the ELISA does not require specific training or equipment, the cost of the kit may still be too high for some laboratories and its availability is limited in some scrub typhus endemic countries (Isaac et al. 2004).

Numerous assays for the molecular detection of *O. tsutsugamushi* by PCR have been published and used in clinical settings. The most common targets of these assays are the genes encoding the 56-kDa antigen (Furuya et al. 1993; Horinouchi et al. 1996) and 47-kDa surface antigen (Jiang et al. 2004; Singhsilarak et al. 2005), plus the 16S rRNA (Sonthayanon et al. 2006; Kim et al. 2006a, 2016) and *groEL* genes (Park et al. 2005; Paris et al. 2009). PCR is most useful for diagnosis in the early stage of scrub typhus, before antibodies are detectable by serological methods. During this stage of the disease, PCR has been demonstrated to be more sensitive than IFA. In a Korean study, a positive result was obtained by nested PCR on the buffy coat of 19 out of 22 scrub typhus patients that were all negative by IFA on admission (Kim et al. 2006a) and similar results were obtained in a Thai study (Manosroi et al. 2003). The sensitivity of PCR in these two small studies of 21 and 118 patients was reportedly high (82.2 and 90.5 %) (Manosroi et al. 2003; Kim et al. 2006a). However, in larger studies of more than 180 patients, the diagnostic sensi-

tivities of PCRs targeting the 16S rRNA gene, 56-kDa antigen gene, and 47-kDa antigen gene were only 44.8%, 29%, and 28.6%, respectively (Sonthayanon et al. 2006; Watthanaworawit et al. 2013). Conflicting results have also been obtained from studies in which various PCR assays were compared. One demonstrated a real-time PCR targeting the 47-kDa antigen gene to be more sensitive than nested and conventional assays with the same target (Kim et al. 2011a). However, a more recent study showed that a conventional assay targeting the 16S rRNA gene was the most sensitive compared with conventional PCRs targeting different genes, nested and real-time assays (Kim et al. 2016). PCR sensitivity is likely influenced by several factors. Due to the sequence variability between different *O. tsutsugamushi* serotypes, particularly within the 56-kDa antigen gene, primers may not anneal as efficiently to some strains and may not even detect new serotypes. Detection may be more successful if PCR is performed on DNA extracted from the buffy coat of the blood rather than the whole EDTA blood sample, as the bacterial load has been shown to be approximately tenfold higher in the cellular fraction (Paris et al. 2008). Antibiotics have been shown to reduce the sensitivity of PCR detection of *O. tsutsugamushi* in the blood to 10% by the fourth day after initiation of treatment (Kim and Byun 2008). Therefore, the assay should ideally be performed prior to antibiotic administration or at least only within 3 days after treatment initiation. Alternatively, PCR can be performed on eschars (Lee et al. 2006; Kim et al. 2006b). Detection of bacterial DNA from an eschar is less affected by antibiotic treatment (Kim et al. 2006b), but this sample is not available from all scrub typhus patients. Similarly to the IFA, the major disadvantage of PCR as a diagnostic method is its requirement for expensive, specialized equipment and staff training, which is not available in many scrub typhus endemic areas.

Besides serological and molecular diagnostic techniques, lesser used methods include immunohistochemical staining of the eschar (Kim et al. 2008) and isolation of *O. tsutsugamushi*, either in cell culture (Luksameetanasan et al. 2007) or by inoculation into a mouse (Casleton et al. 1998). Since the latter must be performed in a BSL-3 laboratory, it is not attempted routinely. Growth of the organism is often slow; therefore, results are of little use to the patient and treating clinician. However, it is important to obtain isolates of the bacteria to identify new serotypes and to allow further studies to be performed.

The common theme in scrub typhus diagnostics is the lack of resources in many areas in which the disease occurs. For this reason, there has been a focus in recent years on developing rapid, point-of-care tests that could provide an early, accurate diagnosis in these resource-limited settings. An alternative method to traditional PCR for the molecular detection of *O. tsutsugamushi* is the loop-mediated isothermal PCR assay (LAMP) (Paris et al. 2008, 2011). This method utilizes six primers and can amplify from a few copies to 10^9 copies in less than an hour. Amplification takes place at a single temperature (65 °C) and a positive result is determined by naked eye inspection of the reaction tube to visualize turbidity or a pellet (Notomi et al. 2000), therefore the only equipment required is a water bath or heat block. In an acute disease setting, LAMP was shown to have a diagnostic sensitivity of 53% and specificity of 94% (Paris et al. 2011).

Several immunochromatographic tests (ICTs) have been developed to provide point-of-care serological testing for scrub typhus, and some of these are now commercially available. In recent studies, while the specificity of these tests ranged between 68 and 95 %, sensitivity was lower at only 23–68 % (Blacksell et al. 2010a, b, 2012; Watthanaworawit et al. 2015). Diagnostic sensitivity was improved by 10–20 % if an ICT was combined with LAMP (Paris et al. 2011; Blacksell et al. 2012), but further research is required before a point-of-care scrub typhus test can be widely implemented. Most recently, publications have described a dot-ELISA (Rodkvamtook et al. 2015) and another ICT (Kingston et al. 2015), both based on recombinant 56-kDa antigens. Previously, a panel of scrub typhus infection criteria (STIC) was proposed for confirmation of positive cases, which could then be used in the evaluation of new diagnostic methods (Paris et al. 2011). A sample had to satisfy one of the four criteria, which were as follows: (1) positive cell culture isolation of *O. tsutsugamushi*; (2) an admission IgM titer $\geq$ 1:12,800; (3) a fourfold rising IgM titer in paired serum samples; and (4) a positive result in at least two out of three PCR assays. Using STIC as a reference standard assumes that it is 100 % specific and sensitive, when in fact this may not be the case. A recent reanalysis of data from 161 patients using Bayesian latent class models gave different results for the sensitivities and specificities of various diagnostic tests compared to when STIC was used as the reference standard (Lim et al. 2015b). It is now recommended that any new tests for scrub typhus diagnosis be evaluated against a carefully selected set of existing tests using appropriate statistical models (Lim et al. 2015b).

5.3   Treatment and Control

Scrub typhus is readily curable using timely, appropriate antibiotics. Chloramphenicol was the first antibiotic shown to be effective at treating the disease (Smadel et al. 1949), but it was subsequently shown that fever and other symptoms were eliminated more rapidly using tetracycline (Sheehy et al. 1973). The mainstay of scrub typhus treatment is now doxycycline. Defervescence in the patient occurs so promptly (24–36 h) after initial administration of the drug that in some areas with limited access to diagnostic testing, this response to treatment is considered diagnostic for scrub typhus (Watt et al. 1996). The reduction in fever correlates with a reduction in production of inflammatory cytokines such as TNF-α, IL-1β, and IL-6, which are up-regulated in scrub typhus patients (Chung et al. 2008). While short treatment regimens of a single dose (Brown et al. 1978b) or 3 days of doxycycline have been demonstrated to be effective (Song et al. 1995), caution should be exhibited if this treatment is initiated within the first 5 days of disease onset. Since the antibiotics shown to be effective against *O. tsutsugamushi* are bacteriostatic rather than bactericidal, they slow the bacterial growth while the patient mounts an immune response to the infection. Thus, there have been cases of patients relapsing when treatment was terminated too early, before the development of antibodies (Smadel et al. 1950; Olson et al. 1981; Im et al. 2014). Doxycycline failure was also observed in a patient who was administered antacids for the gastrointestinal symptoms of

scrub typhus in addition to the antibiotic. The patient developed scrub typhus meningoencephalitis during the course of the treatment, believed to have been caused by the antacids reducing the doxycycline concentration in the serum to sub-therapeutic levels (Kim et al. 2011b). Delayed fever resolution in some patients treated with doxycycline in northern Thailand led to the discovery of *O. tsutsuga-mushi* strains that were resistant to both doxycycline and chloramphenicol (Watt et al. 1996). The driver for this resistance is unclear, although it was postulated that antibiotics may have reached the chigger reservoir via rats feeding on animal food (Rosenberg 1997).

Alternative antibiotics for the treatment of scrub typhus include rifampin (Im et al. 2014; Watt et al. 2000b) and the macrolides azithromycin, telithromycin (Kim et al. 2007b), and roxithromycin (Lee et al. 2003). Rifampin was shown to reduce fever more rapidly than doxycycline (Watt et al. 2000b), although its efficacy in cases of severe scrub typhus has not been evaluated. Azithromycin has been shown to be as effective as doxycycline for treatment of both mild to moderate and severe scrub typhus (Kim et al. 2004b; Jang et al. 2014). Its use is recommended for the treatment of scrub typhus in pregnant women and children under 8 years old, as doxycycline use in these groups is contraindicated due to association of the drug with fetal risk and permanent discoloration of the teeth (Kim et al. 2006c; Cross et al. 2015). However, evidence for these risks was based on tetracycline rather than the newer, semisynthetic doxycycline and there are calls for the contraindication of doxycycline to be reevaluated (Cross et al. 2015). Conflicting reports are available regarding the efficacy of the fluoroquinolones against *O. tsutsugamushi*. One study showed that levofloxacin was an effective treatment for scrub typhus, although patients took longer to become afebrile compared to those administered tetracycline antibiotics (Tsai et al. 2010). Other studies showed that *O. tsutsugamushi* strains were resistant to ciprofloxacin and ofloxacin, and demonstrated the presence of a mutation (Ser83Leu) associated with resistance in the quinolone resistance-determining region of the *gyrA* gene (Tantibhedhyangkul et al. 2010; Jang et al. 2013). Therefore, the use of fluoroquinolones is not advocated.

In the case of coinfections with *O. tsutsugamushi* and another pathogen, combination therapy may be required for the effective resolution of both diseases. For example, the recommended treatment for a patient with concurrent scrub typhus and leptospirosis is ceftriaxone and doxycycline (Lee et al. 2014). In cases of severe scrub typhus pneumonia and ARDS, empirical treatment with a beta-lactam antibiotic with doxycycline or a macrolide is sometimes used (Lee et al. 2014). This practice may need to be revised in light of the finding that the efficacies of doxycycline, azithromycin, and rifampin against *O. tsutsugamushi* are lessened in the presence of the beta-lactam cefotaxime (Lee et al. 2014). In contrast, the antimalarial agent chloroquine has been shown to increase the antimicrobial effect of doxycycline against *O. tsutsugamushi* in vitro (Son and Chung 2014).

Although most patients make a full recovery from scrub typhus, there is evidence that *O. tsutsugamushi* is not completely cleared and may persist in the body. In an early study, the organism was isolated from an asymptomatic patient who had confirmed scrub typhus infection more than 1 year prior (Smadel et al. 1952). More recently, *O. tsutsugamushi* was isolated from six recovered patients, up to 18

months after disease onset (Chung et al. 2012). The significance of persistent infection is not known but it is possible that recrudescence may occur after primary infection (Im et al. 2014).

Despite extensive research, there is currently no vaccine available to prevent scrub typhus. Chemoprophylaxis of a 200 mg weekly dose of doxycycline has been demonstrated to be effective at preventing the disease and is recommended for people entering a scrub typhus endemic region where they are likely to have a high risk of exposure to mites (Olson et al. 1980; Twartz et al. 1982). Generally however, control of scrub typhus relies on measures to reduce the risk of chigger bites. Toward the end of World War II, military personnel in scrub typhus endemic areas were encouraged to treat their uniforms with the mosquito repellent dimethylphthalate, which was also effective against chiggers. The Australian Army introduced dibutylphthalate to its soldiers as "antimite fluid," which was more resistant to washing (Philip 1948; Frances 2011), and a 90 % reduction in scrub typhus cases was achieved through its use (Mcculloch 1946). It is now recommended that military uniforms be treated with permethrin (Frances et al. 1992; Frances 2011) or dibutylphthalate (Hengbin et al. 2006) to reduce chigger (and other arthropod) attachment.

Local health authorities in endemic areas have implemented education programs and proposed recommendations to civilian populations in order to reduce cases of scrub typhus. Preventative measures include wearing protective clothing such as arm warmers or gumboots during work activities, washing after work, changing into a different set of clothes to sleep, and using insect repellent (Sharma et al. 2009; Kim et al. 2012). The commonly used insect repellent, DEET (*N-N*-diethyl-*m*-toluamide), is effective against chiggers (Buescher et al. 1984) and recent studies have shown some essential oils from plant species, particularly *Syzygium aromaticum* (clove) and *Melaleuca alternifolia* (tea tree) have a chigger repellent effect. These may be cheaper and less harmful than synthetic chemicals (Eamsobhana et al. 2009; Rodkvamtook et al. 2012). A scrub typhus prevention program implemented in several public health centers in Korea was attributed to a decline in the reported cases of disease. A cost–benefit analysis estimated it saved $6.66 million each year by reducing medical costs and productivity losses (Kim et al. 2012), therefore similar programs may be beneficial in other endemic areas. In addition to personal protective measures, steps can be taken to control the environment and rodent populations, making conditions less favorable for mites. These include the trapping or poisoning of rodents, regular cleaning of buildings inside and out, management of human and food waste, and maintenance of the natural habitat such as preventing weed growth around buildings (Hengbin et al. 2006).

6   Conclusions

Scrub typhus caused by *O. tsutsugamushi* remains a serious and potentially life-threatening disease. Despite years of research, there are still many unresolved issues relating to disease ecology, epidemiology, pathogenesis, diagnosis, and immunity

(Paris et al. 2013). Work continues to address these problems and increase our understanding of the disease and its causative agent.

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