Orientia tsutsugamushi, the agent of scrub typhus

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Abstract

Orientia tsutsugamushi is a mite-borne bacterium belonging to the family Rickettsiaceae and is responsible for the disease scrub typhus in humans. It is an obligate intracellular parasite of trombiculid mites, in which natural transmission is maintained from the female to its eggs (transovarial transmission) and from the eggs to adults (transstadial transmission). With a genome of only 2.0–2.7 Mb, it has the most repeated DNA sequences among bacteria. It is transmitted by mite larvae (chiggers) from rodents, the natural hosts of mites, to humans through accidental bites. Naosuke Hayashi first described it in 1920, giving it the name Theileria tsutsugamushi, but it was renamed to Orientia tsutsugamushi in 1995, owing to its unique properties. Unlike other Gram-negative bacteria, its cell wall lacks lipophosphoglycan and peptidoglycan. It instead has a unique 56-kDa type-specific antigen (TSA56), which gives rise to many strains (sub-types) of the bacterium such as Karp, Gilliam, Kato, Shimokoshi, Kuroki, and Kawasaki. It is most closely related to Candidatus Orientia chuto, a species described in 2010. Primarily indicated by undifferentiated febrile illnesses, the infection can be complicated and often fatal. Diagnosis is difficult and requires laborious detection methods such as the Weil–Felix test, rapid immunochromatographic test, immunofluorescence assays, ELISA, or PCR. Eschar, if present on the skin, is a good diagnostic indicator. One million infections are estimated to occur annually in the endemic region called the Tsutsugamushi Triangle, which covers the Russian Far East in the north, Japan in the east, northern Australia in the south, and Afghanistan in the west. However, infections have also spread to Africa, Europe and South America. Antibiotics such as azithromycin and doxycycline are the main prescription drugs. There is no vaccine for the infection.

Biology

O. tsutsugamushi is a Gram-negative bacterium and is a permanent (obligate) parasite in mites. Within a single host cell, O. tsutsugamushi rapidly divides into many individuals as shown in Figure 1. A unicellular organism, it is oval shaped and measures 0.5 to 0.8 µm wide and 1.2 to 3.0 µm long. Due to similarity, it was previously classified in the genus Rickettsia among other bacteria, but later assigned a separate genus, Orientia, which it shares (as of 2010) only with Candidatus Orientia chuto. It is broader but shorter than other rickettsial bacteria, which are rod shaped and on average measure 0.25 to 0.3 µm wide and 0.8 to 1 µm long. During reproduction, it divides (by binary fission) into two daughter cells by the process of budding. While undergoing budding, it accumulates on the host cell surface, unlike other bacteria. One complete budding cycle takes 9 to 18 hours.

The structure of O. tsutsugamushi (revealed by transmission electron microscopy) is shown in Figure 2. The bacterium is enclosed by a cell wall on the outside and cell membrane on the inside. The cell covering takes up

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stains such as Giemsa and Gimenez stains. Although its cell wall has a classic bacterial double layer, its outer leaflet is much thicker than the inner one, which is just the opposite in *Rickettsia* species. A capsule layer that forms a spherical halo in other bacteria is missing. The cell wall is less rigid due to the absence of peptidoglycan, which is otherwise characteristic of the rigid cell walls of other bacteria. Classic bacterial lipopolysaccharides such as muramic acid, glucosamine, hydroxy fatty acids, heptose, and 2-keto-3-deoxyoctonic acid are also absent in the cell wall. Due to the absence of peptidoglycan, the bacterium is naturally resistant to all β-lactam antibiotics (such as penicillin), to which *Rickettsia* species are normally sensitive to. Its genome totally lacks the genes for lipopolysaccharide synthesis, but does contain some for those of peptidoglycan. Important genes essential for peptidoglycan synthesis such as *alr*, *dapF* and *PBP1* are missing: *alr* encodes an enzyme L-alanine racemase, which converts L-alanine to D-alanine in the first step of peptidoglycan synthesis pathway; *dapF* encodes diaminopimelate epimerase, which convert LL-2,6-diaminoheptanediol (LL-DAP) to meso-diaminoheptanediol (meso-DAP); and *PBP1* encodes penicillin-binding protein-1 (PBP1), which converts periplasmic lipid II to peptidoglycan. Thus, the bacterium cannot synthesise a typical peptidoglycan cell wall, and instead makes a peptidoglycan-like structure on its surface. The cell membrane is also chemically different in its protein composition, and this difference gives rise to strain variations within the species itself. The cytoplasm is clear and shows distinct DNA and ribosomes.

The bacterium is highly virulent, such that its isolation and cell culture are done only in a laboratory facility with biosafety level 3. Unlike other bacteria which can easily grow on different culture media, rickettsiales can be cultured only in living cells. *O. tsutsugamushi* specifically can be grown only in the yolk sacs of developing chicken embryos and in cultured cell lines such as HeLa, BHK, Vero, and L929. In contrast to *Rickettsia* species which reside in the nucleus of the host cell, *O. tsutsugamushi* mostly grows within the cytoplasm of the host cell. Genetically, it differs from other *Rickettsia* by only 9%. Even though adaptation to obligate intracellular parasitism among bacteria generally results in a reduced genome, it has a genome size of about 2.0–2.7 Mb depending on the strains (Figure 3), which is comparatively larger than those of other rickettsiales – two times larger than that of *Rickettsia prowazekii*, the most well-known member. The entire genome is distributed in a single circular chromosome. Whole genome sequences are available only for Ikeda and Boryong strains, both from the Republic of Korea. The genome of the Ikeda strain is 2,008,987 base pairs (bp) long, and contains 1,967 protein-coding genes. The Boryong strain is larger with 2,127,051 bp and 2,179 protein-coding genes.

Genome comparison shows only 657 core genes among the different strains. With about 42–47% of repetitive sequences, *O. tsutsugamushi* has the most highly repeated bacterial genome sequenced as of 2013. The repeated DNA sequence includes short repetitive sequences, transposable elements (including insertion sequence elements, miniature inverted-repeat transposable elements, a group II intron), and a greatly amplified integrative and conjugative element (ICE) called the

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*Figure 2 | O. tsutsugamushi* in human (U937) cells. A: Bacteria (B) among mitochondria (Mi). B: Bacteria (B) with associated microparticles (arrowhead). C: Magnification showing a microparticle blebbing on the bacterial surface (arrowhead) and a detached microparticle (mp). D: Details of two microparticles. *Paris et al., 2012 CC-BY 3.0*
Rickettsial amplified genetic element (RAGE). RAGE is also found in other rickettsial bacteria. In *O. tsutsugamushi*, however, RAGE contains a number of genes including *tra* genes typical of type IV secretion systems and gene for *ankyrin repeat*–containing protein. Ankyrin repeat–containing proteins are secreted through a type I secretion system into the host cell. The precise role of type IV secretion system in *O. tsutsugamushi* is not known. It may be involved in horizontal gene transfer between the different strains.

**Life cycle and transmission**

*O. tsutsugamushi* is naturally transmitted in the mite population belonging to the genus *Leptotrombidium*. It can be transmitted by a female to its eggs through the process called transovarial transmission, and from the eggs to larvae and adults through the process of transstadial transmission. Thus, the bacterial life cycle is maintained entirely in mites. Infection to rodents and humans is an accidental transmission from the bite of mite larvae, and not required for reproduction or survival of the bacterium. In fact, in humans the transmission is stopped, and the bacterium meets a dead end.

**Figure 3** Genomes of *O. tsutsugamushi* strains. From outermost to innermost ring of each genome: repetitive regions in purple, core genes in green, repeat genes in red and pseudogenes in blue. The innermost line graph shows GC-content (1000bp windows) with above-median region in green, and below-median regions in red. *Batty et al., 2017 CC-BY 3.0*
 Cellular invasion

*O. tsutsugamushi* initially attacks the myelocytes (young white blood cells) in the area of inoculation, and then the endothelial cells lining the vasculature. The process of cellular invasion is shown in Figure 5. In the blood circulation, it targets professional phagocytes ("cell eaters", white blood cells) such as dendritic cells and macrophages in all organs as the secondary targets. The parasite first attaches itself to the target cells using surface proteoglycans present on the host cell and bacterial surface proteins such as type specific protein 56 (or type specific antigen, TSA56) and surface cell antigens (ScaA and ScaC, which are membrane transporter proteins). These proteins interact with the host fibronectin to induce phagocytosis (the process of ingesting the bacterium). The ability to actually enter the host cell depends on integrin-mediated signaling and reorganisation of the actin cytoskeleton. *O. tsutsugamushi* has a special adaptation for surviving in the host cell by evading the host immune reaction. Once it interacts with the host cells, it causes the host cell membrane to form a transportation bubble called a clathrin-coated vesicle by which it gets transported into the cytoplasm. Inside the cytoplasm, it makes an exit from the vesicle (now known as an endosome) before the endosome is destroyed (in the process of cell-eating called autophagy) by the lysosomes. It then moves towards the nucleus, specifically at the perinuclear region, where it starts to grow and multiply. Unlike other closely-related bacteria which use actin-mediated processes for movement in the cytoplasm (called intracellular trafficking or transport), *O. tsutsugamushi* is unusual in using microtubule-mediated processes similar to those employed by viruses such as adenoviruses and herpes simplex viruses. Further, the escape (exocytosis) from an infected host cell is also unusual. It forms another vesicle using the host cell membrane, gives rise to a small bud, and releases itself from the host cell surface while still enclosed in the vesicle. The membrane-bound bacterium is formed by interaction between cholesterol-rich lipid rafts as well as HtrA, a 47-kDa protein on the bacterial surface. However, the process of budding and importance of the membrane-bound bacterium are not yet understood.

Strains

*O. tsutsugamushi* is a diverse species of bacteria. Ida A. Bengtson of the United States Health Service was the first to note the existence of different strains using antigen-antibody interaction (complement fixation test)
in 1944.\(^{28}\) She observed that different strains had varying degree of virulence, and that the antibodies in the blood sera of patients cross-react to different strains. By 1946, she established that there were three principal strains (serotypes), namely Karp (from New Guinea), Gilliam (from India) and Seerangay (from British Malaya).\(^{29}\) Akira Shishido described the Kato strain, in addition to Gilliam and Karp, in Japan in 1958.\(^{30}\) Since then, six basic antigenic strains are recognised, namely Gilliam, Karp, Kato, Shimokoshi, Kawasaki, and Kuroki. Karp is the most abundant strain, accounting for about 50% of all infections.\(^{19}\) In Korea, the major strain is Boryong.\(^{33}\) As of 2009, more than 20 different strains have been established in humans based on antigenic variation using serological tests such as complement fixation and immunofluorescence assay.\(^{19}\) The number is much higher if the strains in rodents and mites are taken into account. For example, a study in Japan in 1994 reported 32 strains, 14 from human patients, 12 from wild rodents, and 6 from trombiculid mites. The different strains exert different levels of virulence, and the most virulent is KN-3, which is predominant among wild rodents.\(^{33}\) Another study in 1996 reported 40 strains.\(^{33}\) Genetic methods have revealed even greater complexity than had been previously described (for example, Gilliam is further divided into Gilliam and JG types). Due to immunological differences of the serotypes, simultaneous and repeated infection with different strains is possible.\(^{34,35}\)

### Antigenic variation

*O. tsutsugamushi* has four major surface-membrane proteins (antigens) having molecular weights 22 kDa, 47 kDa, 56 kDa and 110 kDa. A 56-kDa type specific antigen (TSA56) is the most important because it is not produced by any other bacteria, and is responsible for making the genetic diversity in different strains.\(^{36}\) It accounts for about 10–15% of the total cell proteins. The

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**Figure 5** Mechanism of cell invasion by *O. tsutsugamushi.* Chhandama, 2018 CC-BY 3.0
22-kDa, 47-kDa or 110-kDa antigens are not strain specific so that TSAg6 is the main target in sophisticated diagnostic tests such as immunoblotting, ELISA and DNA analysis. The protein assists the adhesion and entry of the bacterium into host cells, as well as evasion of the host’s immune reaction. It varies in size from 416 to 540 amino acid residues between different strains, and its gene is approximately 1,550 base pairs long. Its gene contains four hypervariable regions, indicating that it synthesises many antigenically different proteins. There are also 11-kDa and 60-kDa proteins inside the bacterium which are very similar to GroES and GroEL of the bacterium Escherichia coli, but not that of Rickettsia species. GroES and GroEL are heat shock proteins belonging to the family of molecular chaperones in bacteria. DNA analyses have shown that the GroES and GroEL genes are indeed present in O. tsutsugamushi with slight variation in different strains, and they produce the 11-kDa and 60-kDa proteins.

### Disease

*O. tsutsugamushi* causes a complex and potentially life-threatening disease known as scrub typhus. Infection starts when chiggers bite on the skin during their feeding. The bacteria are deposited at the site of feeding (inoculation), where they multiply. They cause progressive tissue damage (*necrosis*), which leads to formation of an *eschar* on the skin. Necrosis progresses to inflammation of the blood vessels, called *vasculitis*. This in turn causes inflammation of the lymph nodes, called *lymphadenopathy*. Within a few days, vasculitis extends to various organs including the liver, brain, kidney, *ménages* and lungs. The disease is responsible for nearly a quarter of all the febrile (high fever) illness in endemic areas. Mortality in severe cases or due to improper treatment or misdiagnosis may be as high as 30-70%. About 6% of infected people die untreated, and 1.4% of the patients die even with medical treatment. Moreover, the death rate can be as high as 14% with neurological problems and 24% with multi-organ dysfunction among treated patients. In cases of misdiagnosis and failure of treatment, systemic complications rapidly develop including acute respiratory distress syndrome, acute kidney failure, encephalitis, gastrointestinal bleeding, hepatitis, meningitis, myocarditis, pancreatitis, pneumonia, septic shock, subacute thyroiditis, and multi-organ dysfunctions. Harmful effects involving multiple organ failure and neurological impairment are difficult to treat, and can cause lifelong debilitation or be directly fatal. The central nervous system is often affected and results in various complications including cerebellitis, cranial nerve palsies, meningoencephalitis, plexopathy, transverse myelitis, and Guillain-Barré syndrome. Death rates due to complications can be up to 14% in brain infections, and 24% with multiple organ failure. In India, scrub typhus has become the major cause of acute encephalitis syndrome, which was earlier caused mainly by a viral infection, Japanese *encephalitis*.  

### Epidemiology

The *World Health Organization* in 1999 stated that:

*Scrub typhus is probably one of the most underdiagnosed and underreported febrile illnesses requiring hospitalization in the region. The absence of definitive signs and symptoms combined with a general dependence upon serological tests make the differentiation of scrub typhus from other common febrile diseases such as murine typhus, typhoid fever and leptospirosis quite difficult.*

Scrub typhus is historically endemic to the Asia-Pacific region, covering the Russian Far East and Korea in the north, to northern Australia in the south, and Afghanistan in the west, including islands of the western Pacific Oceans such as Japan, Taiwan, Philippines, Papua New Guinea, Indonesia, Sri Lanka, and the Indian Subcontinent. This geographic region is popularly called the Tsutsugamushi Triangle as shown in Figure 6. However, it has spread to Africa, Europe and South America. One billion people are estimated to be at risk of infection at any moment and an average of one million cases occur every year in the Tsutsugamushi Triangle. The burden of scrub typhus in rural areas of Asia is huge, accounting for up to 20% of febrile sickness in hospital, and seroprevalence (positive infection on blood test) over 50% of the population. More than one-fifth of the population carry the bacterial antibodies, i.e. they had been infected, in endemic areas. South Korea has the highest level incidence (with its highest of 59.7 infections out of 100,000 people in 2013), followed by Japan, Thailand, and China at top of the list. The age group of 60–69 years is at highest risk of infection. Higher infection (57.3%) is seen in females compared to males (42.7%). Farmers are most vulnerable, accounting for 70% of the cases in China. The disease is more prevalent in rural areas, but there is a rapid increase in urban areas. For example, in Korea, the annual incidence increased 21-fold between 2003 and 2013 in metropolitan areas.
Diagnosis

Symptom

The main symptom of *O. tsutsugamushi* infection is high (febrile) fever; however, the symptom is similar to other vector-borne tropical diseases such as *malaria*, *leptospirosis*, *typhoid*, *murine typhus*, *chikungunya*, and *dengue fever*. This makes precise clinical diagnosis difficult, which often leads to misdiagnosis. The initial indications are fever with chills, associated with headache, muscle pain (myalgia), sweating and vomiting. The appearance of symptoms (the incubation period) takes between 6 and 21 days. A simple visual diagnosis is the presence of an inflamed scar-like scab called eschar, which is regarded as “the most useful diagnostic clue in patients with acute febrile illness”. Eschar is formed on the skin where an infected mite bit, usually seen in the armpit, groin or any abdominal area (Figure 7). In rare cases, it can be seen on the cheek, ear lobe and dorsum of the feet. But, the problem is that eschar is not always present; at the highest record, only 55% of scrub typhus patients had eschar during an outbreak in south India. Also, eschar is not specific to scrub typhus, occurring in other rickettsial diseases such as Rocky Mountain spotted fever, Brazilian spotted fever and Indian tick typhus. Using DNA analysis by advanced polymerase chain reaction, different rickettsial infections can be identified from eschars.

Blood test

Suspected infections are confirmed with serological tests. *O. tsutsugamushi* is most often detected from blood serum using the Weil–Felix test. Weil–Felix is the simplest and most rapid test, but it is not sensitive or specific, as it detects any kind of rickettsial infection. More sensitive tests such as rapid immunochromatographic test...
(RICT), immunofluorescence assays (IFA), ELISA, and DNA analysis using polymerase chain reaction (PCR) are used. IFA is regarded as the gold standard test, as it gives a reliable result; however, it is expensive and not specific for different rickettsial bacteria. ELISA and PCR can detect O. tsutsugamushi-specific proteins such as the TSA56 and GroEL, so that they are highly specific and sensitive. On the other hand, they are highly sophisticated and expensive techniques.

Treatment

O. tsutsugamushi infection can be treated with antibiotics such as azithromycin, chloramphenicol, doxycycline, rifampicin, roxithromycin, and tetracyclin. Doxycycline is the most commonly used and is considered as the drug of choice because of its high efficacy and quick action. But, in pregnant women and babies, it is contraindicated, and azithromycin is the drug of choice. In Southeast Asia, where doxycycline and chloramphenicol resistance have been experienced, azithromycin is recommended for all patients.

A randomized controlled trial and systematic review showed that azithromycin is the safest medication.

Vaccine

No licensed O. tsutsugamushi vaccines are currently available. The first vaccines were developed in the late 1940s, but failed in clinical trials. Considered an ideal target, the unique TSA56 itself is highly variable in its chemical composition in different strains. An effective vaccine for one strain is not useful for another. An ideal vaccine should give protection to all the strains present locally. This complexity makes it difficult to produce a usable vaccine. A vaccine targeting the 47-kDa outer membrane protein (OMP) is a promising candidate with experimental success in mice against the Boryong strain. Combined targeting of TSA56 and ScaA is also a good candidate for mixed-strain infection.

Immunity

There is no lasting immunity to O. tsutsugamushi infection. Antigenic variation prevents the development of cross immunity to the various strains of O. tsutsugamushi. An infected individual may develop a short-term immunity but that disappears after a few months, and immunity to one strain does not confer immunity to another. An immunisation experiment was done in 1950 in which 16 volunteers still developed the infection after 11–25 months of primary infection. It is now known that the longevity of immunity depends on the strains of the bacterium. When reinfection occurs with the same strain as the previous infection, there can be immunity for 5–6 years in monkeys. But in humans, immunity declines after one year, and disappears within two years.

History

The earliest record of O. tsutsugamushi infection was in the 3rd century (313 C.E.) in China. Japanese were also familiar with the link between the infection and mites for centuries. They gave several names such as shima-mushi, akamushi (red mite) or kedani (hairy mite)
The aetiology of the disease was unknown until the early 20th century. In 1908, a mite theory of the transmission of tsutsugamushi disease was postulated by Taichi Kitashima and Mikinosuke Miyajima. In 1915, a British zoologist, Stanley Hirst, suggested that the larvae of mite *Leptotrombidium akamushi* (later renamed *Microtrombidium akamushi*) which he found on the ears of field mice could carry and transmit the infection. In 1917, Mataro Nagayo and colleagues gave the first complete description of the developmental stages such as egg, nymph, larva, and adult of the mite. They also asserted that only the larvae bites mammals, and are thus the only carriers of the parasites. But then, the actual infectious agent was not known, and it was generally attributed to either a virus or a protozoan.

The causative pathogen was first identified by Naosuke Hayashi in 1920. Confident that the organism was a protozoan, Hayashi concluded, stating, "I have reached the conclusion that the virus of the disease is the species of *Piroplasma* [protozoan] in question... I consider the organism in Tsutsugamushio disease as a hitherto undescribed species, and at the suggestion of Dr. Henry B. Ward designate it as *Theileria tsutsugamushi*."

Discovering the similarities with the bacterium *R. prowazekii*, Mataro Nagayo and colleagues gave a new classification with the name *Rickettsia orientalis* in 1930. ([79][80]) (*R. prowazekii* is a causative bacterium of epidemic typhus first discovered by American physicians Howard Taylor Ricketts and Russell M. Wilder in 1910, and described by a Brazilian physician Henrique da Rocha Lima in 1916.)

The taxonomic confusion worsened. In 1931, Norio Ogata gave the name *Rickettsia tsutsugamushio* ([83]) while Rinya Kawamura and Yoso Imagawa independently introduced the name *Rickettsia akamushio*. Kawamura and Imagawa discovered that the bacteria are stored in the salivary glands of mites, and that mites feed on body (lymph) fluid, thereby establishing the fact that mites transmit the parasites during feeding.([84])

For more than 60 years there was no consensus on the choice of name – both *R. orientalis* and *R. tsutsugamushio* were equally used. Akira Tamura and colleagues reported in 1991 the structural differences of the bacterium from *Rickettsia* species that warranted a separate genus, and proposed the name *Orientaltsutsugamushi*. Finally, in 1995, they made a new classification based on the morphological and biochemical properties, formally creating the new name *O. tsutsugamushi*.([1])

Acknowledgements

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References


"Scrub typhus" and "Orientia tsutsugamushi" are terms related to a bacterial disease. The disease is found in the Scrub typhus vector, which is a type of mite. The bacteria can cause disease in multiple species, including humans, which can result in a variety of symptoms.

References:
