WikiJournal Preprints/Orientia tsutsugamushi, the agent of scrub typhus

Author: Kholhring Lalchhandama , *et al.*

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 female to its eggs (transovarial transmission) and from the eggs to adults (transtadial transmission). With a genome of only 2.4– 2.7 Mb, it has the most repeated DNA sequences among bacteria. It is transmitted by mite larvae (chiggers) to humans and rodents through accidental bite. Naosuke Hayashi first described it in 1920, giving the name *Theileria tsutsugamushi*, but 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 56kDa type-specific antigen (TSA56), which renders the bacterium to exist in many strains (sub-types) such as Karp, Gilliam, Kato, Shimokoshi, Kuroki, and Kawasaki. Primarily indicated by undifferentiated febrile illnesses, the infection can be complicated and often fatal. Diagnosis is This article is an unpublished pre-print undergoing public peer review organised by the *WikiJournal of Medicine*.

First submitted: 14 September 2018

Last updated: 7 March 2019 **Reviewer comments**

Author info:

Department of Zoology, PUC Campus, Mizoram University, Aizawl 796001, India

chhandama@gmail.com

Suggested (provisional) citation format:

Lalchhandama, K; *et al.* (2019). "Orientia tsutsugamushi, the agent of scrub typhus". *WikiJournal Preprints*. https://en.wikiversity.org/wiki/WikiJournal_Preprints/Orientia_tsutsugamushi,_the_agent_of_scrub_typhu.s

difficult and requires laborious techniques such as Weil–Felix test, rapid immunochromatographic test, immunofluorescence assays, ELISA, or PCR. Eschar, if present on the skin, is a good indicator. One million infections are estimated to occur annually in the endemic region called 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.

Antibiotics such as azithromycin and doxycycline are the main prescription drugs. There is no working vaccine.

Keywords: *Orientia tsutsugamushi*, scrub typhus, *Leptotrombidium*, febrile illness, vaccine, immunity

Biology

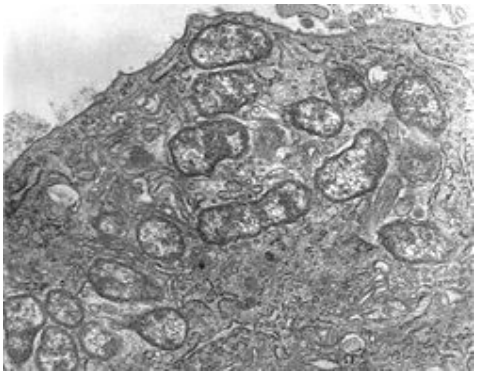

Figure 1 | A transmission electron micrograph of a mesothelial cell of a mouse containing numerous *O. tsutsugamushi.* CDC, CC-BY 3.0

O. tsutsugamushi belongs to Gram-negative bacteria 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 rod shaped and measures 0.5 to 0.8 µm wide and 1.2 to 3.0 µm long. Due to similarity, it was initially categorised in the genus *Rickettsia*, but later assigned a separate genus, *Orientia*,[1] which it shares (so far) only with *Candidatus Orientia chuto*.[2] It is larger and broader, but shorter than other rickettsial bacteria. 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 takes 9 to 18 hours.[3]

The bacterium is enclosed by a cell wall on the outside and cell membrane on the inside (Figure 2). The cell covering take up 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.[4] A capsule layer that forms a spherical halo in other bacteria is missing. The cell wall is soft and tender due to the absence of peptidoglycan, which is otherwise characteristic of the rigid cell walls of other bacteria. Classic bacterial lipophosphoglycans 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.[5] Its genome totally lacks the genes for lipophosphoglycan synthesis, but does contain those for peptidoglycan. In fact, peptidoglycan is synthesised in very small quantity that can hardly be detected and plays minor or no role in the cell wall. There are unique genes such as *PBP1*, *alr*, *dapF*, and *murl*, which are not known in other bacteria.[6] The cell membrane is also chemically different in its protein composition, and this difference gives rise to strain variations within the species itself.[7] The cytoplasm is clear and shows distinct DNA and ribosomes.

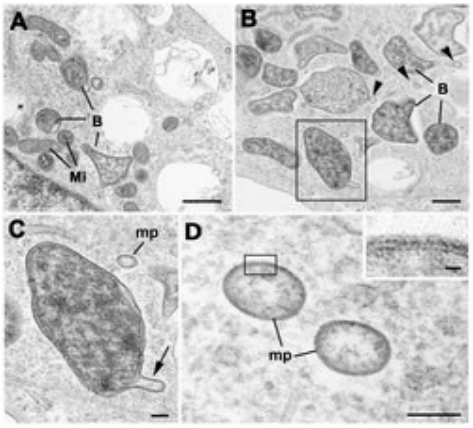

Figure 2 | *O. tsutsugamushi* in human (U937) cells. B = the bacteria; mp = microparticles formed by the outer cell wall leaflet.
Paris *et al.*, 2012 CC-BY 3.0

The bacterium is highly virulent such that its isolation and cell culture are done only in a laboratory with biosafety level 3 facility. Unlike other bacteria which can easily grow on different culture media, it can be grown only in the yolk sacs of developing chicken embryos and in cultured cell lines such as HeLa, BHK, Vero, and L929 cell lines.[8] In contrast to *Rickettsia* species which reside in the nucleus of the host cell, it mostly grow within the cytoplasm of the host cell.[9] Genetically, it differs from other *Rickettsia* by only 9%.[10] Even though adaptation to obligate intracellular parasitism among bacteria generally results in 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*,[11] the most well-known member. The entire genome is distributed in a single chromosome. Whole genome sequences are available only for Ikeda and Boryong strains, both from the Republic of Korea. The genome of Ikeda strain is 2,008,987 base pairs (bp) long, and contains 1,967 protein-coding genes.[12] The Boryong strain is larger with 2,127,051 bp and 2,179 protein-coding genes.[13]

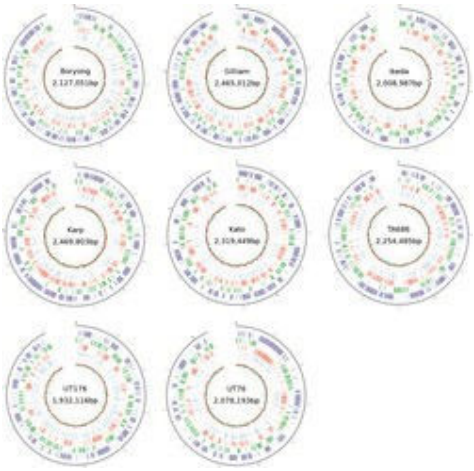

Figure 3 | Genomes of *O. tsutsugamushi* strains.
Batty *et al.*, 2017 CC-BY 3.0

Genome comparison shows only 657 core genes among the different strains.[14]

With about 42-47% of repetitive sequences, *O. tsutsugamushi* has the most highly repeated bacterial genome sequenced so far.[15] 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 rickettsial amplified genetic element (RAGE).[13] 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.[16]

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 transtadial 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 rodents and humans the transmission is stopped, and the bacterium meets a dead end.[7]

In rodent and human infections, *Leptotrombidium deliense* is the most universal vector of *O. tsutsugamushi*. *L. pallidum*, *L. fletcheri* and *L. scutellare* are also carriers in many countries. In addition, *L. akamushi* is an endemic carrier in Japan, *L. chiangraiensis* and *L. imphalum* in Thailand, *L. gaohuensis* in China, and *L. arenicola* in Malaysia and Indonesia.[17] In parts of India, a different mite species, *Schoengastiella ligula* is also a major vector.[18] The third-stage larvae, commonly referred to as chiggers (Figure 4), are the only ectoparasitic stage feeding on the body fluids of rodents and other opportunistic mammals. Thus, they are the only stage in the life of mites that cause infection. Wild rats of the genus *Rattus* are the principal natural hosts of the chiggers.[19] Chiggers feed only once on a mammalian host. The feeding usually takes 2 to 4 days. In contrast to most parasites, they do not feed on blood, but instead on the body fluid through the hair follicles or skin pores. They possess a special feeding apparatus called stylostome on their heads. Their saliva can dissolve the host tissue around the feeding site, so that they ingest the liquefied tissue. *O. tsutsugamushi* is present in the salivary glands of mites and is released into the host tissue during this feeding.[20]

Cellular invasion

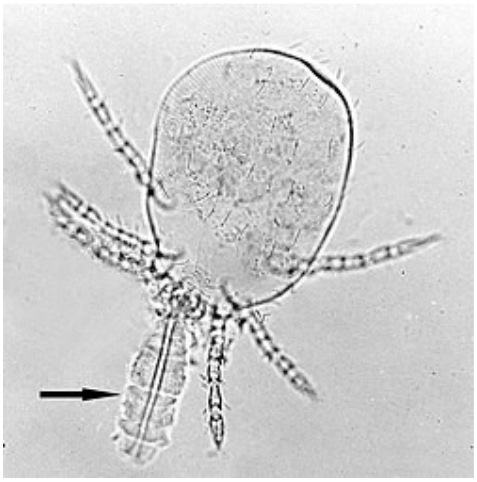

Figure 4 | Chigger with its feeding apparatus, the stylostome (arrowhead).

Walker, 2012 CC-BY 3.0

O. tsutsugamushi initially attacks the myelocyte (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 TSP56 (TSA56) and surface cell antigens (ScaA and ScaC).[21][22] These proteins interact with the host fibronectin to induce phagocytosis (the process of swallowing the bacterium). The ability to actually enter the host cell depends on integrin-mediated signaling and reorganisation of actin cytoskeleton.[23]

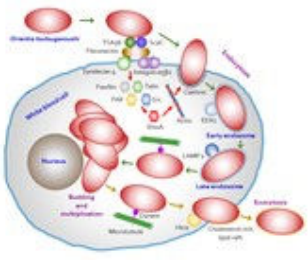

Figure 5 | Mechanism of cell invasion by *O. tsutsugamushi*.

Chhandama, 2018 CC-BY 3.0

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 in the cytoplasm. Inside the cytoplasm, it makes an exit from the vesicle (now known as an endosome) before it is destroyed (in the process of cell-eating called autophagy) by the lysosomes.[24] 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 membranebound bacterium is formed by interaction between cholesterol-rich lipid rafts as well as HtrA, a 47-kDa protein on the bacterial surface.[25] However, the process of budding and importance of the membrane-bound bacterium are not yet udnerstood.

Strains

O. tsutsugamushi is a diverse species of bacteria. Ida A. Bengtson of the United States Public Health Services was the first to note the existence of different strains using antigen-antibody interaction (complement fixation test) in 1944.[26] He observed that different strains had varying degree of virulence, and that the blood sera having different strains could cross react. By 1946, he established that there were three principal strains (serotypes), namely Karp (from New Guinea), Gilliam (from India) and Seerangay (from British Malaya).[27] Akira Shishido discovered Kato strain, in addition to Gilliam and Karp, in Japan in 1958.[28] 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.[17] But in Korea, the major strain is Boryong.[29] So far, more than 30 different strains have been established in humans.[15] 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.[30] Another study in 1996 reported 40 strains.[31] 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.[32][33]

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.[34] It accounts for about 10–15% of the total cell proteins. The 22kDa, 47-kDa or 110-kDa antigens are not normally detected by sophisticated diagnostic tests. But clinical tests easily detect the TSA56, making it the main target in diagnosis.[35] 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 516 to 540 amino acid residues between different strains, and its gene is about 1,550 base pairs long. It contains four hypervariable regions, indicating that it synthesise many antigenically different protein but of the same kind.[31] 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.[36] GroES and GroEL are heat shock proteins belonging to the family of molecular chaperones in bacteria. DNA analysis have shown that the *GroES* and *GroEL* genes are indeed present in

O. tsutsugamushi with slight variation in di erent strain and they produce the 11-kDa and 60-kDa proteins.[37]

Disease

O. tsutsugamushi causes a complex and dangerous infection 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). Necrosis progresses to inflammation of the blood vessels called vasculitis. This in turn causes imflammation of the lymph nodes, called lymphadenopathy. Within a few days, vasculitis extends to various organs including liver, brain, kidney, meninges and the lung.[38] The disease is responsible for nearly a quarter of all the febrile (high fever) illness in endemic areas. Mortality in severe case or due to improper treatment or misdiagnosis may be as high as 30-70%.[39] About 6% of

infected people die untreated, and 1.4% of the patients die even with medical treatment. Moreover, death rate can be as high as 13% where medical treatment is not properly handled.[40] 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.[41] Harmful symptoms involving multiple organ failure and neurological impairment are difficult to treat, and can be lifelong debilitation or directly fatal.[41] The central nervous system is often affected and result in various complications including cerebellitis, cranial nerve palsies , meningoencephalitis, plexopathy, transverse myelitis, neuroleptic malignant syndrome, and Guillan-Barré syndrome.[42] Death rates due to complications can be up to 14% in brain infections, and 24% with multiple organ failure.[40] It is the major cause of acute encephalitis syndrome in India, where viral infection Japanese encephalitis has been regarded as the main factor.[43]

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."[44]

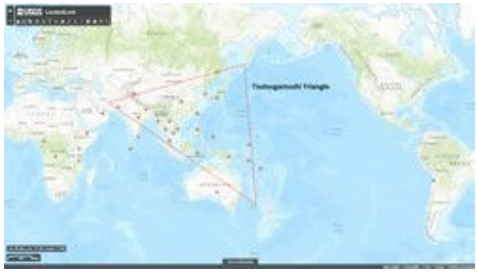

Figure 6 | Map showing the Tsutsugamushi Triangle.

Xu *et al*., 2017 CC-BY 3.0

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.[38] However, it has spread to Africa, Europe and South America.[45] 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. In the absence of proper medical care, the case fatality rate can go beyond 30% to as high as 70% in some areas.[20] 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.[46] 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 infection out of 100,000 people in 2013), followed by Japan, Thailand, and China at top of the list.[40]

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.[47] A randomized controlled trial and systematic review showed that azithromycin is the safest medication.[48][49]

Diagnosis

Symptom

The main symptom of *O. tsutsugamushi* infection is high (febrile) fever; however, the symptom is not unique and is similar to a group of acute undifferentiated fever, which includes those of malaria, leptospirosis, typhoid, murine typhus, chikungunya, and dengue fever.[50][51] 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.[38] 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.[52] 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.[53] Also that eschar is not specific to scrub typhus, as other rickettsial diseases such as Rocky Mountain spotted fever,[54] Brazilian spotted fever,[55] and Indian tick typhus.[56][57] Using DNA analysis by advanced polymerase chain reaction, different rickettsial infection can be identified from eschars.[58][59]

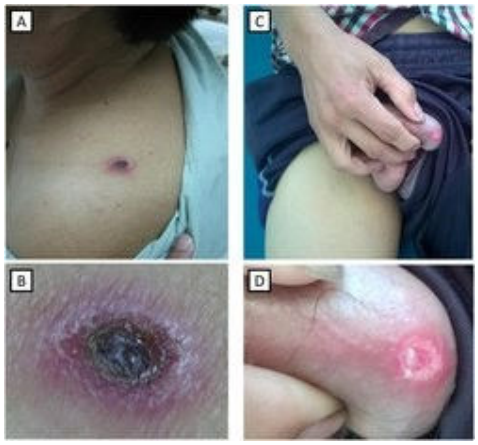

Figure 6 | Eschar due to *O. tsutsugamushi* infection on the shoulder (a, b) of a woman and on the penis (c, d) of a man.

Le Viet *et al*., 2017 CC-BY 3.0

Blood test

O. tsutsugamushi is most often detected from blood serum using Weil–Felix test. Weil–Felix test 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.[19][8] IFA is regarded as the gold standard test, as it gives reliable result. However, it is expensive and not specific for different rickettsial bacteria.[60] ELISA and PCR can detect *O. tsutsugamushi*-specific proteins such as the TSA56 and GroEL so that they are highly specific and sensitive.[61] On the other hand, they are highly sophisticated and expensive techniques.

Vaccine

No licensed *O. tsutsugamushi* vaccines are currently available. The first vaccines were developed in the late 1940s, but failed in clinical trials.[62][63] 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.[64] A vaccine targeting the 47-kDa outer membrane protein (OMP) is a promising candidate with experimental success in mice against Boryong strain.[65] Combined targetting of TSA56 and ScaA is also a good candidate for mixed-strain infection.[22]

Immunity

There is no complete immunity to *O. tsutsugamushi* infection. Enormous antigenic variation among *O. tsutsugamushi* strains makes the immune system unable to fully recognise them. An infected individual may develop a short-term immunity but that

disappears after a few month, and immunity to one strain does not confer immunity to another.[64] An immunisation experiment was done in 1950 in which 16 volunteers still developed the infection after 11–25 months of primary infection.[66] 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.[67] But in humans, immunity declines after one year, and disappears within two years.[68]

History

The earliest record of *O. tsutsugamushi* infection was in the 3rd century (313 C.E.) in China.[69] 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) disease of northern Japan, and most popularly as *tsutsugamushi* (from *tsutsuga* meaning fever or harm or illness, and *mushi* meaning bug or insect). Japanese physician Hakuju Hashimoto gave the first medical account from Niigata Prefecture in 1810. He recorded the prevalence of infection along the banks of the upper tributaries of Shinano River.[70] The first report to the Western world was made by Theobald Adrian Palm, a physician of the Edinburgh Medical Missionary Society at Niigata in 1878. Describing his first-hand experience, Palm wrote:

> "Last summer [i.e. 1877], I had the opportunity of observing a disease which, so far as I know, is peculiar to Japan, and has not yet been, described. It occurs, moreover, in certain well-marked districts, and at a particular season of the year, so that the opportunities of investigating it do not often occur. It is known here as the *shima-mushi*, or island-insect disease, and is so-named from the belief that it is caused by the bite or sting of some insect peculiar to certain islands in the river known as Shinagawa, which empties itself into the sea at Niigata."[71]

The aetiology of the disease was never apparent. In 1908, a mite theory of the transmission of tsutsugamushi disease was postulated by Taichi Kitashima and Mikinosuke Miyajima.[72] In 1915, a British zoologist Stanley Hirst suggested that the larvae of mite *Microtrombidium akamushi* (later renamed *Leptotrombidium akamushi*) which he found on the ears of field mice could carry and transmit the infection.[73] 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; and also asserted that only the larvae bites mammals, and are thus the only carriers of the parasites.[74] But then the actual infectious agent was not known, and it was generally attributed to either a virus or a protozoan.[75]

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 Tsutsugamushi disease as a hitherto undescribed species, and at the suggestion of Dr. Henry B. Ward designate it as *Theileria tsutsugamushi*." [76] Discovering the similarities with the bacterium *R. prowazekii*, Mataro Nagayo and colleagues gave a new classification with the name *Rickettsia orientalis* in 1930.[77][78] (*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.[79])

The taxonomic confusion worsened. In 1931, Norio Ogata gave the name *Rickettsia tsutsugamushi*,[80] while Rinya Kawamüra and Yoso Imagawa independently introduced the name *Rickettsia akamushi*.[81] Kawamüra 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.[82]

For more than 60 years there was no consensus on the choice of name – both *R. orientalis* and *R. tsutsugamushi* were equally used. Akira Tamura and colleagues reported in 1991 the structural differences of the bacterium from *Rickettsia* species that

warranted separate genus, and proposed the name *Orientia tsutsugamushi*.[9] Finally in 1995, they made a new classification based on the morphological and biochemical properties, formally creating the new name *O. tsutsugamushi*.[1]

References

1. Tamura, A.; Ohashi, N.; Urakami, H.; Miyamura, S. (1995). "Classification of *Rickettsia tsutsugamushi* in a new genus, *Orientia* gen. nov., as *Orientia tsutsugamushi* comb. nov.". *International Journal of Systematic Bacteriology* **45** (3): 589–591. doi:10.1099/00207713-45-3-589. PMID 8590688.
2. Izzard, L (2010). "Isolation of a novel *Orientia* species (*O. chuto* sp. nov.) from a patient infected in Dubai. "*Journal of Clinical Microbiology* **48** (12): 4404–4409. doi:10.1128/JCM.01526-10. PMID 20926708. PMC 3008486. //www.ncbi.nlm.nih.gov/pmc/articles/PMC3008486/.
3. Moree, M.F.; Hanson, B. (1992). "Growth characteristics and proteins of plaque-purified strains of *Rickettsia tsutsugamushi*". *Infection and Immunity* **60** (8): 3405-3415. PMID 1379212. PMC 257328. //www.ncbi.nlm.nih.gov/pmc/articles/PMC257328/.
4. Silverman, D.J.; Wisseman, C.L. J.r (1978). "Comparative ultrastructural study on the cell envelopes of *Rickettsia prowazekii*, *Rickettsia rickettsii*, and *Rickettsia tsutsugamushi*". *Infection and Immunity* **21** (3): 1020-1023. PMID 101465. PMC 422098. //www.ncbi.nlm.nih.gov/pmc/articles/PMC422098/.
5. Amano, K.; Tamura, A.; Ohashi, N.; Urakami ,H.; Kaya, S.; Fukushi, K. (1987)."Deficiency of peptidoglycan and lipopolysaccharide components in *Rickettsia tsutsugamushi*". *Infection and Immunity* **55** (9): 2290-2292. PMID 3114150. PMC 260693. //www.ncbi.nlm.nih.gov/pmc/articles/PMC260693/.
6. Atwal, S.; Giengkam, S.; Chaemchuen, S.; Dorling, J.; Kosaisawe, N.; aVnNieuwenhze, M.; Sampattavanich, S.; Schumann, P. *et al*. (2017). "Evidence for a peptidoglycan-like structure in *Orientia tsutsugamushi*". *Molecular Microbiology* **105** (3): 440–452. doi:10.1111/mmi.13709. PMID 28513097. PMC 5523937. //www.ncbi.nlm.nih.gov/pmc/articles/PMC5523937/.
7. Lalchhandama, K. (2017)."Rickettsiosis as a critical emerging infectious disease in India". *Science Vision* **17** (4): 250–259. doi:10.33493/scivis.17.04.09. https://www.sciencevision.org/issue/2/article4/6.
8. Koh, G.C.; Maude, R.J.; Paris, D.H.; Newton, P.N.; Blacksell, S.D. (2010)."Diagnosis of scrub typhus". *The American Journal of Tropical Medicine and Hygiene* **82** (3): 368–370. doi:10.4269/ajtmh.2010.09-0233. PMID 20207857. PMC 2829893. //www.ncbi.nlm.nih.gov/pmc/articles/PMC2829893/.
9. Tamura, A.; Urakami, H.; Ohashi, N. (1991). A" comparative view of *Rickettsia tsutsugamushi* and the other groups of Rickettsiae". *European Journal of Epidemiology* **7** (3): 259-269. doi:10.1007/BF00145675. PMID 1909244.
10. Ohashi, N.; Fukuhara, M.; Shimada, M.; Tamura, A. (1995). "Phylogenetic position of *Rickettsia tsutsugamushi* and the relationship among its antigenic variants by analyses of 16S rRNA gene sequences". *FEMS Microbiology Letters* **125** (2-3): 299-304. doi:10.1111/j.1574-6968.1995.tb07372.x. PMID 7875578.
11. Bishop-Lilly, K.A.; Ge, H.; Butani, A.; Osborne, B.; Verratti, K.; Mokashi, V.; Nagarajan, N.; Pop, M. *et al*. (2013). "Genome sequencing of four strains of *Rickettsia prowazekii*, the causative agent of epidemic typhus, including one flying squirrel isolate". *Genome Announcements* **1** (3): e00399-13. doi:10.1128/genomeA.00399-13. PMID 23814035. PMC 3695431. //www.ncbi.nlm.nih.gov/pmc/articles/PMC3695431/.
12. Nakayama, K.; Yamashita, A.; Kurokawa, K. ;Morimoto, T.; Ogawa, M.; Fukuhara, M.; Urakami, H.; Ohnishi, M. *et al*. (2008). "The whole-genome sequencing of the obligate intracellular bacterium *Orientia tsutsugamushi* revealed massive gene amplification during reductive genome evolution". *DNA Research* **15** (4): 185–199. doi:10.1093/dnares/dsn011. PMID 18508905. PMC 2575882. //www.ncbi.nlm.nih.gov/pmc/articles/PMC2575882/.
13. Cho, N.-H.; Kim, H.-R.; Lee, J.-H.; Kim, S.-Y.; Kim, J.; Cha, S.; Kim, S.-Y.; Darby, A. C. *et al*. (2007). "The *Orientia tsutsugamushi* genome reveals massive proliferation of conjugative yTpe IV secretion system and host-cell interaction genes". *Proceedings of the National Academy of Sciences* **104** (19): 7981–7986. doi:10.1073/pnas.0611553104. PMID 17483455. PMC 1876558. //www.ncbi.nlm.nih.gov/pmc/articles/PMC1876558/.
14. Batty, E.M.; Chaemchuen, S.; Blacksell, S.; Richards, A.L.; Paris, D.; Bowden, R.; Chan, C.; Lachumanan, R. *et al*. (2018). "Long-read whole genome sequencing and comparative analysis of six strains of the human pathogen *Orientia tsutsugamushi*". *PLOS Neglected Tropical Diseases* **12** (6): e0006566. doi:10.1371/journal.pntd.0006566. PMID 29874223. PMC 6005640. //www.ncbi.nlm.nih.gov/pmc/articles/PMC6005640/.
15. Viswanathan, S.; Muthu, V.; Iqbal, N.; Remalayam, B.; George, T (2013). "Scrub typhus meningitis in South India—a retrospective study". *PLOS One* **8** (6): e66595. doi:10.1371/journal.pone.0066595. PMID 23799119. PMC 3682970. //www.ncbi.nlm.nih.gov/pmc/articles/PMC3682970/.
16. Salje, J.; Kline, K.A. (2017)." *Orientia tsutsugamushi*: A neglected but fascinating obligate intracellular bacterial pathogen". *PLOS Pathogens* **13** (12): e1006657. doi:10.1371/journal.ppat.1006657. PMID 29216334. PMC 5720522. //www.ncbi.nlm.nih.gov/pmc/articles/PMC5720522/.
17. Kelly, D.J.; Fuerst, P.A.; Ching, W.M.; Richards, A.L. (2009). "Scrub typhus: the geographci distribution of phenotypic and genotypic variants of *Orientia tsutsugamushi*". *Clinical Infectious Diseases* **48 Suppl** (Suppl): S203–S230. doi:10.1086/596576. PMID 19220144.

18. Tilak, R.; Wankhade, U.; Kunwar, R.; Tilak, V.W. (2011). "Emergence of *Schoengastiella ligula* as the vector of scrub typhus outbreak in Darjeeling: Has *Leptotrombidium deliense* been replaced?". *Indian Journal of Public Health* **55** (2): 92–99. doi:10.4103/0019-557X.85239. PMID 21941043.
19. Luce-Fedrow, A.; Lehman, M.; Kelly, D.; Mullins, K.; Maina, A.; Stewart, R.; Ge, H.; JohnH, . *et al*. (2018). "A review of scrub typhus (*Orientia tsutsugamushi* and related organisms): then, now, and tomorrow". *Tropical Medicine and Infectious Disease* **3** (1): 1–8. doi:10.3390/tropicalmed30100 8.
20. Xu, G.; Walker, D.H.; Jupiter, D.; Melby, P.C.; Arcari, C.M.; Day, N.P. (2017). "A review of the global epidemiology of scrub typhus". *PLOS Neglected Tropical Diseases* **11** (11): e0006062. doi:10.1371/journal.pntd.0006062. PMID 29099844. PMC 5687757. //www.ncbi.nlm.nih.gov/pmc/articles/PMC5687757/.
21. Ge, Y.; Rikihisa, Y. (2011). "Subversion of host cell signaling by *Orientia tsutsugamushi*". *Microbes and Infection* **13** (7): 638–648. doi:10.1016/j.micinf.2011.03.003. PMID 21458586.
22. Ha, N.Y.; Sharma, P.; Kim, G.; Kim, Y.; Min, C.K.; Choi, M.S.; Kim, I.S.; Cho, N.H. (2015)"Immunization with an. autotransporter protein of *Orientia tsutsugamushi* provides protective immunity against scrub typhus. "*PLoS Neglected Tropical Diseases* **9** (3): e0003585. doi:10.1371/journal.pntd.0003585. PMID 25768004. PMC 4359152. //www.ncbi.nlm.nih.gov/pmc/articles/PMC4359152/.
23. Cho, B. A.; Cho, N. H.; Seong, S. Y.; Choi, M. S.; Kim, I. S. (2010)."Intracellular invasion by *Orientia tsutsugamushi* is mediated by integrin signaling and actin cytoskeleton rearrangements". *Infection and Immunity* **78** (5): 1915–1923. doi:10.1128/IAI.01316-09. PMID 20160019. PMC 2863532. //www.ncbi.nlm.nih.gov/pmc/articles/PMC2863532/.
24. Ko, Y.; Choi, J.H.; Ha, N.Y; Kim, I.S.; Cho, N.H.; Choi, M.S.; Bäumle,r A. J. (2013). "Active escape of *Orientia tsutsugamushi* from cellular autophagy". *Infection and Immunity* **81** (2): 552–559. doi:10.1128/IAI.00861-12. PMID 23230293. PMC 3553808. //www.ncbi.nlm.nih.gov/pmc/articles/PMC3553808/.
25. Kim, M.J.; Kim, M.K.; Kang, J.S. (2013). "Involvement of lipid rafts in the budding-like exit of *Orientia tsutsugamushi*". *Microbial Pathogenesis* **63**: 37–43. doi:10.1016/j.micpath.2013.06.002. PMID 23791848.
26. Bengston, I.A. (1945). "Apparent serological heterogeneity among strains ofs Tutsugamushi disease (scrub typhus)". *Public Health Reports* **60** (50): 1483-1488. PMID 21004496.
27. Bengston, I.A. (1946). "A serological study of 37 cases of tsutsugamushi disease (scrub typhus) occurring in Burma and the Philippine Islands". *Public Health Reports* **61** (24): 887-894. PMID 20987857.
28. Shishido, A.; Ohtawara, M.; Tateno, S.; Mizuno, S.; Ogura, M.; Kitaoka, M. (1958). "The nature of immunity against scrub typhus in mice i. the resistance of mice, surviving subcutaneous infection of scrub typhus rickettsia, to intraperitoneal reinfection of the same agent". *Japanese Journal of Medical Science and Biology* **11** (5): 383-399. doi:10.7883/yoken1952.11.383.
29. Jang, M.S.; Neupane, G.P.; Lee, Y.M.; Kim, D.M.; Lee, S.H. (2011)."Phylogenetic analysis of the 56 kDa protei n genes of *Orientia tsutsugamushi* in southwest area of Korea". *The American Journal of Tropical Medicine and Hygiene* **84** (2): 250–254. doi:10.4269/ajtmh.2011.09-0601. PMID 21292894. PMC 3029177. //www.ncbi.nlm.nih.gov/pmc/articles/PMC3029177/.
30. Yamashita, T.; Kasuya, S.; Noda, N.; Nagano, I.; Kang, J.S. (1994)"Transmission of . *Rickettsia tsutsugamushi* strains among humans, wild rodents, and trombiculid mites in an area of Japan in which tsutsugamushi disease is newly endemic". *Journal of Clinical Microbiology* **32** (11): 2780–2785. PMID 7852572. PMC 264159. //www.ncbi.nlm.nih.gov/pmc/articles/PMC264159/.
31. Ohashi, N.; Koyama, Y.; Urakami, H.; Fukuhara, M.; Tamura, A.; Kawamori, F.; Yamamoto, S.; Kasuya, S. *et al*. (1996). "Demonstration of antigenic and genotypic variation in *Orientia tsutsugamushi* which were isolated in Japan, and their classification into type and subtype". *Microbiology and Immunology* **40** (9): 627–638. doi:10.1111/j.13480421.1996.tb01120.x. PMID 8908607.
32. Bakshi, D.; Singhal, P.; Mahajan, S.K.; Subramaniam, P.; Tuteja, U.; Batra, H.V. (2007). "Development of a real-time PCR assay for the diagnosis of scrub typhus cases in India and evidence of the prevalence of new genotype of *O. tsutsugamushi*". *Acta Tropica* **104** (1): 63–71. doi:10.1016/j.actatropica.2007.07.013. PMID 17870041.
33. Parola, P.; Blacksell, S.D.; Phetsouvanh, R.; Phongman,y S.; Rolain, J.M.; Day, N.P.; Newton, P.N.; Raoult, D. (2008). "Genotyping of *Orientia tsutsugamushi* from humans with scrub typhus, Laos. "*Emerging Infectious Diseases* **14** (9): 1483–1485. doi:10.3201/eid1409.071259. PMID 18760027. PMC 2603112. //www.ncbi.nlm.nih.gov/pmc/articles/PMC2603112/.
34. Tamura, A; Ohashi, N; Urakami, H; Takahashi, K; Oyanagi, M (1985)."Analysis of polypeptide composition an d antigenic components of *Rickettsia tsutsugamushi* by polyacrylamide gel electrophoresis and immunoblotting". *Infection and Immunity* **48** (3): 671–675. PMID 3922893. PMC 261225. //www.ncbi.nlm.nih.gov/pmc/articles/PMC261225/.
35. Stover, CK; Marana, DP; Carter, JM; Roe, BA; Mardis, E; Oaks, EV (1990). "The 56-kilodalton major protein antigen of *Rickettsia tsutsugamushi*: molecular cloning and sequence analysis of the sta56 gene and precise identification of a strain-specific epitope". *Infection and Immunity* **58** (7): 2076–2084. PMID 1694818. PMC 258779. //www.ncbi.nlm.nih.gov/pmc/articles/PMC258779/.
36. Stover, C.K.; Marana, D.P.; Dasch, G.A.; Oaks, E.V. (1990). "Molecular cloning and sequence analysis of the Sta58 major antigen gene of *Rickettsia tsutsugamushi*: sequence homology and antigenic comparison of Sta58 to the 60kilodalton family of stress proteins. "*Infection and Immunity* **58** (5): 1360-1368. PMID 2108930. PMC 258633. //www.ncbi.nlm.nih.gov/pmc/articles/PMC258633/.
37. Arai, S.; Tabara, K.; Yamamoto, N.; Fujita, H.; Itagaki, A.; Kon, M.; Satoh, H.; Araki, K. *et al*. (2013). "Molecular phylogenetic analysis of *Orientia tsutsugamushi* based on the *groES* and *groEL* genes". *Vector Borne and Zoonotic Diseases* **13** (11): 825-829. doi:10.1089/vbz.2012.1155. PMID 24107204. PMC 3822374. //www.ncbi.nlm.nih.gov/pmc/articles/PMC3822374/.

38. Peter, J.V.; Sudarsan, T.I.; Prakash, J.A.J.; Varghese, G.M. (2015). "Severe scrub typhus infection: Clinical features, diagnostic challenges and management. "*World Journal of Critical Care Medicine* **4** (3): 244–250. doi:10.5492/wjccm.v4.i3.244. PMID 26261776. PMC 4524821. //www.ncbi.nlm.nih.gov/pmc/articles/PMC4524821/.
39. Taylor, A.J.; Paris, D.H.; Newton, P.N.; Walker, D.H. (2015). "A systematic review of mortality from untreated scrub typhus (*Orientia tsutsugamushi*)". *PLOS Neglected Tropical Diseases* **9** (8): e0003971. doi:10.1371/journal.pntd.0003971. PMID 26274584. PMC 4537241. //www.ncbi.nlm.nih.gov/pmc/articles/PMC4537241/.
40. Bonell, A.; Lubell, Y.; Newton, P.N.; Crump, J.A.; Paris, D.H. (2017)."Estimating the burden of scrub typhus: A systematic review.". *PLoS Neglected Tropical Diseases* **11** (9): e0005838. doi:10.1371/journal.pntd.0005838. PMID 28945755. PMC 5634655. //www.ncbi.nlm.nih.gov/pmc/articles/PMC5634655/.
41. Rajapakse, S.; Weeratunga, P.; Sivayoganathan, S.; Fernando, S.D. (2017). "Clinical manifestations of scrub typhus". *Transactions of the Royal Society of Tropical Medicine and Hygiene* **111** (2): 43–54. doi:10.1093/trstmh/trx017. PMID 28449088.
42. Mahajan, S.K.; Mahajan, S.K. (2017)."Neuropsychiatric manifestations of scrub typhus . "*Journal of Neurosciences in Rural Practice* **8** (3): 421–426. doi:10.4103/jnrp.jnrp_44_17. PMID 28694624. PMC 5488565. //www.ncbi.nlm.nih.gov/pmc/articles/PMC5488565/.
43. Jain, P.; Prakash, S.; Tripathi, P.K.; Chauhan, A.; Gupta, S.; Sharma, U.; Jaiswal, A.K.; Sharma, D. *et al*. (2018). "Emergence of *Orientia tsutsugamushi* as an important cause of acute encephalitis syndrome in India". *PLoS Neglected Tropical Diseases* **12** (3): e0006346. doi:10.1371/journal.pntd.0006346. PMID 29590177. PMC 5891077. //www.ncbi.nlm.nih.gov/pmc/articles/PMC5891077/.
44. WHO (1999). "WHO Recommended Surveillance Standards (Second edition.)"*WHO/CDS/CSR/ISR/99.2*. World Health Organization, Geneva. p. 124.
45. Jiang, J.; Richards, A.L. (2018)."Scrub typhus: No longer restricted to the Tsutsugamushi Triangle" . *Tropical Medicine and Infectious Disease* **3** (1): 11. doi:10.3390/tropicalmed3010011. PMID 30274409. PMC 6136623. //www.ncbi.nlm.nih.gov/pmc/articles/PMC6136623/.
46. Walker, D.H.; Paris, D.H.; Day, N.P.; Shelite, T.R. (2013). "Unresolved problems related to scrub typhus: A seriously neglected life-threatening disease. "*The American Journal of Tropical Medicine and Hygiene* **89** (2): 301–307. doi:10.4269/ajtmh.13-0064. PMID 23926142. PMC 3741252. //www.ncbi.nlm.nih.gov/pmc/articles/PMC3741252/.
47. Rahi, M.; Gupte, M.D.; Bhargava, A.; Varghese, G.Mm; Arora, R. (2015)."DHR-ICMR Guidelines for diagnosis & management of rickettsial diseases in India. "*Indian Journal of Medical Research* **141** (4): 417-22. doi:10.4103/09715916.159279. PMID 26112842. PMC 4510721. //www.ncbi.nlm.nih.gov/pmc/articles/PMC4510721/.
48. Chanta, C.; Phloenchaiwanit, P. (2015). "Randomized Controlled trial of azithromycin versus doxycycline or chloramphenicol for treatment of uncomplicated pediatric scrub typhus". *Journal of the Medical Association of Thailand* **98** (8): 756–760. PMID 26437532.
49. Lee, S.C.; Cheng, Y.J.; Lin, C.H.; Lei, W.T.; Chang, H.Y.; Lee, M.D.; Liu, J.M.; Hsu, R.J. *et al*. (2017). "Comparative effectiveness of azithromycin for treating scrub typhus". *Medicine* **96** (36): e7992. doi:10.1097/MD.0000000000007992. PMID 28885357.
50. Mørch, K.; Manoharan, A.; Chandy, S.; Chacko, N.; Alvarez-Uria, G.; Patil, S.; Henr,y A.; Nesaraj, J. *et al*. (2017). "Acute undifferentiated fever in India: a multicentre study of aetiology and diagnostic accuracy. "*BMC Infectious Diseases* **17** (1): 665. doi:10.1186/s12879-017-2764-3. PMID 28978319. PMC 5628453. //www.ncbi.nlm.nih.gov/pmc/articles/PMC5628453/.
51. Wangrangsimakul, T.; Althaus, T.; Mukaka, M.; Kantipong, P.; Wuthiekanun, V.; Chierakul, W.; Blacksell, S.D.; Day, N.P. *et al*. (2018). "Causes of acute undi erentiated fever and the utility of biomarkers in Chiangrai, northern Thailand". *PLoS Neglected Tropical Diseases* **12** (5): e0006477. doi:10.1371/journal.pntd.0006477. PMID 29852003. PMC 5978881. //www.ncbi.nlm.nih.gov/pmc/articles/PMC5978881/.
52. Kundavaram, A.P.; Jonathan, A.J.; Nathaniel, S.D.; Varghese, G.M. (2013). "Eschar in scrub typhus: a valuable clue to the diagnosis". *Journal of Postgraduate Medicine* **59** (3): 177-178. doi:10.4103/0022-3859.118033. PMID 24029193.
53. Varghese, G.M.; Janardhanan, J.; Trowbridge, P.; Peter, J.V.; Prakash, J.A.; Sathyendra, S.; Thomas, K.; David, .TS. *et al*. (2013). "Scrub typhus in South India: clinical and laboratory manifestations, genetic variabil,i taynd outcome". *International Journal of Infectious Diseases* **17** (11): e981-987. doi:10.1016/j.ijid.2013.05.017. PMID 23891643.
54. Kelman, P.; Thompson, C.W.; Hynes, W.; Bergman, C.; Lenahan, C.; Brenne,r J.S.; Brenner, M.G.; Goodman, B. *et al*. (2018). "*Rickettsia parkeri* infections diagnosed by eschar biopsy, Virginia, USA". *Infection* **46** (4): 559-563. doi:10.1007/s15010-018-1120-x. PMID 29383651.
55. Silva, N.; Eremeeva, M.E.; Rozental, T.; Ribeiro, G.S.; Paddock, C.D.; Ramos, E.A.; Favacho, A.R.; Reis, M.G. *et al*. (2011). "Eschar-associated spotted fever rickettsiosis, Bahia, Brazi. l"*Emerging Infectious Diseases* **17** (2): 275-278. doi:10.3201/eid1702.100859. PMID 21291605. PMC 3204763. //www.ncbi.nlm.nih.gov/pmc/articles/PMC3204763/.
56. Hulmani, M.; Alekya, P.; Kumar, V.J. (2017). "Indian tick typhus presenting as purpura fulminans with review on rickettsial infections". *Indian Journal of Dermatology* **62** (1): 1-6. doi:10.4103/0019-5154.198030. PMID 28216718. PMC 5286740. //www.ncbi.nlm.nih.gov/pmc/articles/PMC5286740/.
57. Walker, D.H. (1989). "Rickettsioses of the spotted fever group around the world". *The Journal of Dermatology* **16** (3): 169–177. doi:10.1111/j.1346-8138.1989.tb01244.x. PMID 2677080.
58. Denison, A.M.; Amin, B.D.; Nicholson, W.L.; Paddock, C.D. (2014). "Detection of *Rickettsia rickettsii*, *Rickettsia parkeri*, and *Rickettsia akari* in skin biopsy specimens using a multiplex real-time polymerase chain reaction assa.y" *Clinical Infectious Diseases* **59** (5): 635-642. doi:10.1093/cid/ciu358. PMID 24829214. PMC 4568984. //www.ncbi.nlm.nih.gov/pmc/articles/PMC4568984/.

59. Le Viet, N.; Laroche, M.; Thi Pham, H.L.; Viet, N.L.; Mediannikov, O.; Raoult, D.; Parola, P. (2017). "Use of eschar swabbing for the molecular diagnosis and genotyping of *Orientia tsutsugamushi* causing scrub typhus in Quang Nam province, Vietnam". *PLoS Neglected Tropical Diseases* **11** (2): e0005397. doi:10.1371/journal.pntd.0005397. PMID 28241043. PMC 5344524. //www.ncbi.nlm.nih.gov/pmc/articles/PMC5344524/.
60. Koraluru, M.; Bairy, I.; Varma, M.; Vidyasagar, S. (2015). "Diagnostic validation of selected serological tests for detecting scrub typhus". *Microbiology and Immunology* **59** (7): 371–374. doi:10.1111/1348-0421.12268. PMID 26011315.
61. Patricia, K.A.; Hoti, S.L.; Kanungo, R.; Jambulingam, .P; Shashikala, N.; Naik, A.C. (2017)."Improving the diagnosi s of scrub typhus by combining *groEL*-based polymerase chain reaction and IgM ELISA. "*Journal of Clinical and Diagnostic Research* **11** (8): DC27–DC31. doi:10.7860/JCDR/2017/26523.10519. PMID 28969124. PMC 5620764. //www.ncbi.nlm.nih.gov/pmc/articles/PMC5620764/.
62. Card, W.I.; Walker, J.M. (1947). "Scrub-typhus vaccine; field trial in South-east Asia". *Lancet* **1** (6450): 481–483. doi:10.1016/S0140-6736(47)91989-2. PMID 20294827.
63. Berge, T.O.; Gauld, R.L.; Kitaoka, M. (1949)." A field trial of a vaccine prepared from the Volner strain of *Rickettsia tsutsugamushi*". *American Journal of Hygiene* **50** (3): 337–342. doi:10.1093/oxfordjournals.aje.a119366. PMID 15391985.
64. Valbuena, G.; Walker, D. H. (2013). "Approaches to vaccines against *Orientia tsutsugamushi*". *Frontiers in Cellular and Infection Microbiology* **2**: 127. doi:10.3389/fcimb.2012.00170. PMID 23316486. PMC 3539663. //www.ncbi.nlm.nih.gov/pmc/articles/PMC3539663/.
65. Choi, S.; Jeong, H.J.; Hwang, K.J.; Gill, B.; Ju, Y.R.; Lee, Y.S.; Lee, J. (2017). "A recombinant 47-kDa outer membrane protein induces an immune response against *Orientia tsutsugamushi* strain Boryong". *The American Journal of Tropical Medicine and Hygiene* **97** (1): 30–37. doi:10.4269/ajtmh.15-0771. PMID 28719308. PMC 5508880. //www.ncbi.nlm.nih.gov/pmc/articles/PMC5508880/.
66. Smadel, JE; Ley, H.L.Jr.; Diercks, F.H.; Traub, R. (1950). "Immunity in scrub typhus: resisatnce to induced reinfection". *A.M.A. Archives of Pathology* **50** (6): 847–861. PMID 14789327.
67. MacMillan, J.G.; Rice, R.M.; Jerrells, T.R. (1985). "Development of antigen-specific cell-mediated immune responses after infection of cynomolgus monkeys (*Macaca fascicularis*) with *Rickettsia tsutsugamushi*". *The Journal of Infectious Diseases* **152** (4): 739–749. doi:10.1093/infdis/152.4.739. PMID 2413138.
68. Ha, N.Y.; Kim, Y.; Min, C.K.; Kim, H.I.; Yen, N.T.H.; Choi, M.S.; Kang, J.S.; Kim, Y.S. *et al*. (2017). "Longevity of antibody and T-cell responses against outer membrane antigens of *Orientia tsutsugamushi* in scrub typhus patients". *Emerging Microbes & Infections* **6** (12): e116. doi:10.1038/emi.2017.106. PMID 29259327.
69. Fan, M.Y.; Walker, D.H.; Yu, S.R.; Liu, Q.H. (1987). "Epidemiology and ecology of rickettsial diseases in the People's Republic of China". *Reviews of Infectious Diseases* **9** (4): 823-840. PMID 3326129.
70. Kawamura, R. (1926). *Studies on tsutsugamushi disease (Japanese Blood Fever)*. Cincinnati, OH (USA): Spokesman Printing Company. p. 2.
71. Palm, T.A. (1878). "Some account of a disease called "shima-mushi," or "island-insect disease," by the natives of Japan; peculiar, it is believed, to that country, and hitherto not described". *Edinburgh Medical Journal* **24** (2): 128– 132. PMID 29640208. PMC 5317505. //www.ncbi.nlm.nih.gov/pmc/articles/PMC5317505/.
72. Miyajima, M.; Okumura, T. (1917). "On the life cycle of the "Akamushi" carrier of Nippon river fever". *Kitasato Archives of Experimental Medicine* **1** (1): 1–14.
73. Hirst, S. (1915). "On the Tsutsugamushi (*Microtrombidium akamushi*, Brumpt), carrier of Japanese river fever". *Journal of Economic Biology* **10** (4): 79–82.
74. Nagayo, M. (1917). "On the nymph and prosopon of the tsutsugamushi, *Leptotrombidium akamushi, N. Sp.* (*Trombidium akamushi* Brumpt), carrier of tsutsugamushi disease". *Journal of Experimental Medicine* **25** (2): 255– 272. doi:10.1084/jem.25.2.255.
75. Lalchhandama, K. (2018)."The saga of scrub typhus with a note on the outbreaks in Mizoram . "*Science Vision* **18** (2): 50–57. doi:10.33493/scivis.18.02.01. https://www.sciencevision.org/issue/4/article1/1.
76. Hayashi, N. (1920). "Etiology of tsutsugamushi disease". *The Journal of Parasitology* **7** (2): 52–68. doi:10.2307/3270957.
77. Nagayo, M.; Tamiya, T.; Mitamura, T.; Sato, K. (1930). "On the virus of tsutsugamushi disease and its demonstration by a new method". *Jikken Igaku Zasshi (Japanese Journal of Experimental Medicine)* **8** (4): 309–318.
78. Nagayo, M.; Tamiya, T.; Mitamura, T.; Sato, K. (1930). "Sur le virus de la maladie de Tsutsugamushi [On the virus of tsutsugamushi]". *Compte Rendu des Séances de la Société de Biologie* **104**: 637–641.
79. da Rocha Lima, H. (1916). "Untersuchungen über fleckfleber [Reseraches on typhus]". *Münchener medizinische Wochenschrift* **63** (39): 1381-1384.
80. Ogata, N. (1931). "Aetiologie der Tsutsugamushi-krankheit: *Rickettsia tsutsugamushi* [Aetiology of the tsstsugamushi disease: *Rickettsia tsutsugamushi*". *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* **122**: 249–253.
81. Kawamüra, R.; Imagawa, Y. (1931). "Ueber die Proliferation der pathogenen *Rickettsia* im tierischen organismus bei der tsutsugamushi-krankheit [The multiplication of the *Rickettsia* pathogen of tsutsugamushi disease in animals]". *Transactions of the Japanese Society of Pathology* **21**: 455-461.
82. Kawamüra, R.; Imagawa, Y. (1931). "Die feststellung des erregers bei der tsutsugamushikrankheit [Confirmation of the infective agent in tsutsugamushi disease]". *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* **122** (4/5): 253-261.