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Classification of *Rickettsia tsutsugamushi* in a New Genus, *Orientia* gen. nov., as *Orientia tsutsugamushi* comb. nov.

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Recent studies of *Rickettsia tsutsugamushi* have demonstrated clearly the phenotypic and genotypic differences between this microorganism and other species belonging to the genus *Rickettsia*. Therefore, classification of *R. tsutsugamushi* in a new genus, *Orientia* gen. nov., is proposed.

The genus *Rickettsia* includes etiological agents of human diseases, including typhus, spotted fever, and scrub typhus. All of the microbes belonging to this genus are similar in the following respects: they exhibit obligate intracellular parasitism, they are morphologically similar to gram-negative bacteria, they survive in both vertebrate and arthropod hosts, and human infection is mediated by arthropods. However, recent studies of *Rickettsia tsutsugamushi*, which is the only species in the scrub typhus group, have revealed some differences between this species and other species belonging to the genus *Rickettsia*. The most striking difference is in the structure of the outer envelope; as revealed by electron microscopy, the outer leaflet of the cell wall of *R. tsutsugamushi* is considerably thicker than the inner leaflet, while the opposite is true of the other *Rickettsia* species (36). In addition, chemically, *R. tsutsugamushi* lacks constitutional components of peptidoglycan and lipopolysaccharide, such as muramic acid, glucosamine, hydroxy fatty acids, and 2-keto-3-deoxyoctonic acid, suggesting that neither peptidoglycan nor lipopolysaccharide is present in *R. tsutsugamushi* (4), while these substances are generally found in the other species belonging to the genus *Rickettsia* (1, 8, 15, 24, 35). *R. tsutsugamushi* is very soft and fragile (39, 44), which reflects the lack of peptidoglycan in this microorganism, and the growth of *R. tsutsugamushi* is more resistant to penicillin than the growth of other rickettsiae (21, 32).

The protein composition of *R. tsutsugamushi* as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis is very different from the protein compositions of the other rickettsiae, especially in the envelopes (43). In *R. tsutsugamushi*, a 54- to 58-kDa protein (designated the 56-kDa protein) is most abundant and is located on the cell surface (16, 41). Other major proteins (80, 46, 43, 39, 35, 28, and 25 kDa) are also located on the rickettsial surface (41); three of these proteins, the 25-, 28-, and also 56-kDa proteins, are heat modifiable (16, 22, 46). Another abundant protein, the 60-kDa protein, which is located inside the rickettsial cells, has been shown to exhibit homology to the GroEL family of proteins (37). On the other hand, rickettsiae belonging to the typhus and spotted fever groups resemble each other in their protein compositions. The major antigenic proteins located on the surfaces of these organisms have been estimated to have the molecular sizes of 150 to 180, 110 to 130, 49, 32, 27.5, and 16.5 to 17.5 kDa (51, 53). The two proteins larger than 110 kDa are immunodominant antigens and have both heat-labile and heat-stable antigenic sites (5–7, 12, 15). The 16.5- to 17.5-kDa antigen is a lipoprotein and exhibits common antigenicity among the species (9, 10). Three proteins that range in size from 20 to 32 kDa and 120-kDa proteins are heat modifiable (8, 12, 13).

R. tsutsugamushi can be cultivated in the yolk sacs of developing chicken embryos and can be grown in culture in various cell lines, including the HeLa, BHK, Vero, and L929 cell lines. Most of the microorganisms grow in the perinuclear cytoplasm of host cells, and they release themselves by pushing out the host cytoplasmic membrane from the inside like the budding process observed with enveloped viruses (49, 50). The budding rickettsiae accumulate at a high density on the host cell surface. Such budding forms are not commonly seen in the other *Rickettsia* species. In addition, the electron-lucent halo zones which are commonly observed around growing cells of the other *Rickettsia* species are not observed with *R. tsutsugamushi*. The doubling time of *R. tsutsugamushi* is about 9 to 18 h in well-adapted cells (22, 50).

Various antigenic variants are recognized among the strains of *R. tsutsugamushi*. The strains of the Gilliam, Karp, and Kato types are well known, but other antigenic types, such as Shimokoshi, Kawasaki, Kuroki and others, have also been described (14, 31, 42, 55). These antigenic types are distinguishable immunologically, especially with strain- or type-specific monoclonal antibodies. The main type-specific antigen is a 56-kDa protein located on the rickettsial cell surface (17, 23, 41), although this antigen also has epitopes common among the antigenic variants (17, 26). The deduced primary amino acid sequences of this protein from six variants were determined by a genomic sequence analysis (29, 30, 38), which demonstrated that these molecules exhibit levels of homology ranging from 60 to 82% between the variants and have four variable domains (30). The 22-, 47-, and 110-kDa proteins also exhibit both common and type-specific antigenicities (17, 26). Our recent observations indicate that each antigenic type strain can be further classified into several subtypes on the basis of the results of analyses performed with monoclonal antibodies and restriction fragment length polymorphism analysis of 56-kDa protein genes amplified by a PCR (unpublished data), suggesting that many other variants with various levels of relationship to prototype strains may be found in future studies. Furthermore, differences in virulence for mice have been observed among the strains of *R. tsutsugamushi* (19, 22, 43); most strains of the Shimokoshi, Kawasaki, and Kuroki types are avirulent in mice. The numerous differences in antigenic and/or genetic structure and in virulence for mice among the strains are unique to *R. tsutsugamushi.*

R. tsutsugamushi is transmitted to human and other vertebrate hosts by mites. The microbes in the infected mites are maintained for long periods by transovarial transmission (33, 34, 40, 45, 47, 48, 54). Ticks, lice, and fleas, which serve as vectors or reservoirs of the typhus and spotted fever group rickettsiae, do not participate in *R. tsutsugamushi* transmission.

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In the Weil-Felix reaction, sera of scrub typhus patients agglutinate *Proteus mirabilis* OXK antigen (3), but not *Proteus vulgaris* OX19 and OX2 antigens. The antigenic component in *R. tsutsugamushi* shared with OXK antigen has not been identified, although lipopolysaccharide antigens have been shown to be involved in other rickettsiae (2).

In addition to determining the phenotypic differences between *R. tsutsugamushi* and other species belonging to the genus *Rickettsia*, we recently analyzed the 16S rRNA sequence of *R. tsutsugamushi* and determined the position of this rickettsia on a phylogenetic tree containing 38 species belonging to the *Proteobacteria* (28). Our results indicated that Gilliam and Kato strains of *R. tsutsugamushi* clustered together at a position that was far from the subcluster containing the other members of genus *Rickettsia* (*Rickettsia prowazekii*, *Rickettsia typhi*, *Rickettsia rickettsii*, and *Rickettsia sibirica*). The levels of similarity among *R. prowazekii*, *R. rickettsii*, and *R. sibirica* were more than 98.1% (evolutionary distances, less than 0.017485), but the levels of similarity between these three species and Gilliam and Kato strains of *R. tsutsugamushi* were 90.2 to 90.6% (evolutionary distances, 0.097480 to 0.106272) and the levels of similarity between Gilliam and Kato strains and selected members of the genera *Ehrlichia* and *Bartonella* (*Rochalimaea*) were 81.0 to 82.9% (evolutionary distances, 0.180613 to 0.207974). The levels of similarity among the antigenic variants of *R. tsutsugamushi*, including Gilliam, Kato, Karp, Kawasaki, Kuroki, and Shimokoshi strains, were more than 98.5% (evolutionary distances, less than 0.01529).

Thus, on the basis of 16S rRNA sequences, *R. tsutsugamushi* was found to be distinct from other species belonging to the genus *Rickettsia* and also to be not specifically related to other members of the order *Rickettsiales*. In addition, the evolutionary distances between *R. tsutsugamushi* and other species belonging to the genus *Rickettsia* were nearly equal to the evolutionary distances that separate other genera belonging to the order *Rickettsiales*. Since a working taxonomy is generally considered from both phenotypic and phylogenetic aspects, the lines of evidence regarding *R. tsutsugamushi* described above may provide sufficient reason to separate *R. tsutsugamushi* from the genus *Rickettsia*. Therefore, we propose that *R. tsutsugamushi* should be removed from the genus *Rickettsia* and placed in a new genus that is separated from the genus *Rickettsia.*

The causative agent of scrub typhus was discovered by several Japanese workers, and the following designations have been proposed for this organism: *Theileria tsutsugamushi*, a member of the *Protozoa*, proposed by Hayashi in 1920 (18); *Rickettsia orientalis*, proposed by Nagayo et al. in 1930 (25); and *Rickettsia tsutsugamushi*, proposed by Ogata in 1931 (27). Since the publication of *Bergey's Manual of Determinative Bacteriology*, 6th ed. (10a), the name *Rickettsia tsutsugamushi* (Hayashi 1920) Ogata 1931 has been accepted. In this paper, we propose a new genus name for this organism, *Orientia*, as well as the species name *Orientia tsutsugamushi*, in honor of both Nagayo et al. and Ogata. This nomenclature is appropriate because this microorganism is widely distributed in eastern Asia.

Description of *Orientia* gen. nov. *Orientia* (O. ri. en'ti. a. M. L. fem. n. *Orientia*, the Orient, the area where the organism is widely distributed). Cells are short rods that are 0.5 to 0.8 μm in diameter and 1.2 to 3.0 μm long. Obligately intracellular parasite. Gram negative. Each cell is surrounded by a very soft cell wall and cell membrane. Muramic acid, glucosamine, 2-keto-3-deoxyoctonic acid, and hydroxy fatty acids are not present in the cell walls, suggesting that the cells lack peptidoglycan and lipopolysaccharide. As determined by electron microscopy, the outer leaflet of the cell wall is considerably thicker than the inner leaflet. Flagella and endospores are not formed. A slime layer is not recognized. The cells, which can be stained by Giemsa or Gimenez stain, grow in the yolk sacs of developing chicken embryos and in various cultured cell lines. The microbes are taken into the host cells by phagocytosis, enter the cytoplasm, and grow mainly in the perinuclear cytoplasm. There is no electron-lucent halo zone around the microbes growing in host cells. The microbes are released from the host cells by being covered with the host cell membrane, like the budding form of enveloped viruses. The doubling time is about 9 to 18 h. Very resistant to penicillin. The most abundant constitutional protein has a molecular mass of 54 to 58 kDa. This protein and 25- and 28-kDa proteins, all of which are located on the cell surface, are heat modifiable. Another abundant protein, the 60-kDa protein, is located inside cells and belongs to the GroEL protein family. Sequence analyses of the 16S rRNA reveal that the members of this genus form a cluster on phylogenetic trees and that the levels of similarity are more than 98.5% (evolutionary distances, less than 0.015129). The G+C contents are 28.1 to 30.5 mol% (as determined by high-performance liquid chromatography) (20).

The members of this genus are classified in one species at the present time, but they exhibit antigenic variety and differ in their virulence for mice. These organisms are found only in the eastern hemisphere. Mites, including several species belonging to the genus *Leptotrombidium*, are vectors for transmission of the microbes to humans and other vertebrate hosts. In these mites, the microorganisms are transmitted transovarially from female adult mites to their progeny. Sera of patients infected with these microbes agglutinate OXK antigen in the Weil-Felix test.

The genus *Orientia* belongs to the family *Rickettsiaceae* together with the genus *Rickettsia*. On the basis of the results of recent 16S rRNA sequences analyses (11, 28), retention of the tribe *Rickettsieae* may not be required in this family. The type species is *Orientia tsutsugamushi* comb. nov. (basonym, *Theileria tsutsugamushi* Hayashi [18]).

Description of *Orientia tsutsugamushi* comb. nov. Only one species, *Orientia tsutsugamushi*, belongs in the genus *Orientia*, and characteristics of this species are described above. The description given by Weiss and Moulder (52) can be used as a supplemental description. The type strain is strain Karp (= ATCC VR 150).

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