Infect Chemother. 2024 Dec;56(4):440-452
https://doi.org/10.3947/ic.2024.0095
pISSN 2093-2340·eISSN 2092-6448

Infection & Chemotherapy

Special Article

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Historical Overview of Tsutsugamushi Disease in Japan before World War II

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ABSTRACT

Tsutsugamushi disease is a febrile mite-borne disease caused by *Orientia tsutsugamushi*. Before 1945, this disease had been prevalent in Niigata, Akita, and Yamagata prefectures for centuries, occurring in areas along major rivers in these prefectures every summer about a month after floods. The patients affected were farmers, possibly new settlers on reclaimed lands, who contracted the disease following bites of tiny red bugs. From the perspective of Western medicine, the disease was first identified by Nagino, Palm, Baelz, and Kawakami in 1878–79. In 1888, the Niigata Prefectural Government mandated the reporting of tsutsugamushi disease cases. In 1892, Tanaka associated the disease and eschars with mite bites. In 1917, Kitashima, Miyajima, and Okumura confirmed its transmission only by larval mites. Ishiwara and Ogata successfully maintained the bacteria in the laboratory through serial intratesticular passage in rabbits starting in 1927. In 1930–31, the causative organism was identified by Nagayo (*Rickettsia orientalis*), Ogata (*R. tsutsugamushi*), and Kawamura ("*R. akamushi*"). From 1932 onwards, the incidence of the disease began to decline slowly, possibly due to reduced human activity in riverside areas.

Keywords: History; Japan; Mites; *Orientia tsutsugamushi*; Scrub typhus

INTRODUCTION

We have reviewed the records of tsutsugamushi disease in ancient and medieval China [1]. Unlike research conducted in China, the discovery and research in Japan were approached from the perspective of Western medicine. During the Edo period (1603–1868), Japan embraced Western science, acquired from the Netherlands beginning in the 17th century, and this field of science was called Rangaku. At the onset of the Meiji era in 1868, the Japanese government introduced German medicine and transitioned the medical system from Rangaku or Oriental medicine to Western medicine. In 1877, the University of Tokyo Faculty of Medicine, the first Western-style medical school in Asia, was established. In this context, the first clinical identification of tsutsugamushi disease

Received: Aug 18, 2024
Accepted: Sep 10, 2024
Published online: Nov 1, 2024

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in 1878 stimulated further research into its etiology and transmission, drawing upon modern bacteriological and entomological knowledge. Various authors have reviewed the occurrence of tsutsugamushi disease and related factors in Niigata-ken (Niigata Prefecture) and Akita-ken [2-5]. However, most of these reviews and monographs except one were written five decades ago and do not encompass recent studies that offer new information on the occurrence of the disease prior to 1945. This article aims to review tsutsugamushi disease in Japan before World War II on the basis of currently available evidence, and to elucidate the factors that influenced the occurrence of the disease from the 18th century until 1945.

1) Background information on tsutsugamushi disease before its scientific discovery

Tsutsugamushi disease was endemic in Niigata-ken and Akita-ken, with outbreaks in Niigata-ken dating back to 1754. Its occurrence in Yamagata-ken was confirmed later than in the above two prefectures. This disease was well-known as a dreaded one among the indigenous farmers of these prefectures, and some practitioners of traditional Oriental medicine knew about it from Chinese medical literature. However, the disease was unfamiliar to medical personnel trained in Western medicine.

At the time, tsutsugamushi disease was found in Japan only along the rivers of Agano, Shinano, Uono (Niigata-ken), Omono (Akita-ken), and Mogami (Yamagata-ken). These prefectures were famous for heavy snowfall in winter and the rivers were prone to flooding due to their steep slopes when the deep snow in the Echigo Mountains to the east of the prefectures melted. As a result, the rivers overflowed every year in late spring, leaving behind large floodplains after several days of flooding. Additional flooding was often caused by typhoons or heavy rains during the summer months. Farmers in these areas could not cultivate rice in the river basins because of the flooding, leading to these fertile areas being left unused and neglected. About a month after the floods, weeds and undergrowth became overgrown and red mites began to appear. Since these areas were unclaimed, anyone could enter and desultorily plant fast-growing crops, such as soybeans and hemp. Farmers, many of whom were poor and possibly immigrants from other prefectures, were bitten by the red mites and contracted tsutsugamushi disease when they entered these areas to harvest hemp, collect mulberry leaves, feed cattle on grass, or gather tall grasses for thatching. Since thiamine deficiency and beriberi were well known in Japan and vitamin deficiency in laboratory animals increased their susceptibility to rickettsial infections [6], nutritional deficiencies may have contributed to the high mortality of patients with tsutsugamushi disease and might have made the disease more noticeable to medical personnel. Areas where tsutsugamushi disease occurred in humans were called *yudokuchi* (noxious or poisonous place): *yu*, present or existing; *doku*, poison/poisoning or toxin; *chi*, area or place; *yudokuchi* literally means a place where poison exists. This term has long been used by Japanese researchers and was first introduced to the Western medical community by Nagayo as "some small circumscribed areas" [7]. Yudokuchi is similar in concept to the "mite island of Audy," which represents a patch where a chigger mite colony is established [4]. The noxious areas in Niigata-ken were characterized by riverside areas 1–2 m above the water level, flooded only during major floods, well-drained sandy areas, and the growth of tall grasses such as yoshi, which provided food and shelter for voles [8]. Sometimes, children or the elderly who had not been in the noxious area contracted the disease possibly because they had handled the grasses collected from the noxious area and may have been bitten by the red mites on the grasses. In areas outside the river banks, rice was grown every year, and strangely enough, there were no cases of the disease [9].

To prevent flooding, the prefectures began reinforcing natural levees in the early 1600s. During the Meiji era (1868–1912) they further renovated the river banks using Western construction techniques. The construction of a diversion canal downstream and dams upstream of the rivers also helped to prevent flooding. Subsequently, several drainage channels were created to make these wetlands suitable for cultivation. As a result, arable lands in these prefectures gradually expanded over three centuries, leading to an influx of farmers from other prefectures to the newly developed lands where the disease began to emerge. For example, tsutsugamushi disease was first observed in Yamagata-ken in 1913 as *Shinkaibyo* (the disease of newly reclaimed land). Retrospectively, records from 1804 to 1835 indicated outbreaks of suspected tsutsugamushi disease on the east bank of the Mogami River, resulting in many fatalities. The disease disappeared from 1836 to 1904 when the river bank was renovated and the river was widened. However, in 1905, a typhoid fever-like illness emerged in the newly reclaimed areas. In 1913, the Yamagata Prefectural Government consulted Tanaka in Akita-ken for an accurate diagnosis of this mysterious disease, which

https://doi.org/10.3947/ic.2024.0095

was confirmed to be tsutsugamushi disease [5].

The newly settled farmers were young to middle-aged adults along with their children. They may have had little immunity to the strain of *O. tsutsugamushi* prevalent in these prefectures, later identified as the Kato serotype, as opposed to the Karp, Gilliam or Japanese Gilliam, Kuroki, and Kawasaki genotypes or serotypes in the other prefectures of Japan [10-14]. The geographic distribution and chigger species responsible for the Shimokoshi genotype require further investigation. Since resistance to reinfection with heterologous strains of *O. tsutsugamushi* only lasts 11 to 25 months after recovery from the previous infection [15], the newcomers, even if they had immunity to non-Kato serotypes, would have suffered from tsutsugamushi disease caused by the Kato serotype. *Leptotrombidium akamushi* was identified as the primary vector of tsutsugamushi disease in these areas during the summer and was later identified to be associated with the Kato serotype, although this association requires further investigation [14]. These prefectures experience hot summer months because of the foehn wind blowing from the Echigo Mountains. The hot and humid environmental conditions are conducive to the growth of *L. akamushi*, leading to the disease affecting individuals working in tall grasses near river water during the daytime in July and August [16]. Experienced indigenous farmers avoided entering these areas during these months to prevent mite bites. Furthermore, *L. akamushi* can survive the winter months as temperatures rarely drop below freezing point in these regions. *L. pallidum* was identified in Yamagata-ken mainly in spring and autumn and was not associated with human disease at that time [17]. However, this mite species has been identified as the primary vector of tsutsugamushi disease in northern prefectures of Japan [18]. *Microtus montebelli*, the Japanese grass vole, was the main mammal in these areas feeding on the weeds and undergrowth.

Indigenous farmers in these areas knew that the disease was transmitted through the bites of tiny red "insects" and may have been concerned about getting bitten while working in the fields. The bugs were variously called aka-mushi (red insect), shima-mushi (island insect), ogi-mushi (silver grass insect), kaya-mushi (daylily insect), tsutsuga-mushi (disease or worry insect), or tsutsuga-no-mushi (insect of disease or worry) in Niigata-ken, and ke-dani (hairy mite) in Akita-ken and Yamagata-ken.

Regarding the origin of the term *tsutsugamushi*, the Japanese use the Chinese characters y*ang-chung-bing* when writing the disease name but pronounce it *tsutsuga-mushi-byo*. The reason for the use and association of *yang* and *tsutsuga* is unclear. In China, *yang* was first described as worry, disaster, accident, or grief in *Shiji* (around the 1st century BC) and in *Erya* (202 BC–220 AD). At that time, *yang* was not used to mean disease or insect. According to *Kuang Miu Zheng Su* (Tang Dynasty: 619–909) and *Yi Zhuan* (1099), *yang* is described as a biting bug that easily eats the human heart. In *Guangyun* (1007–08), *yang* is described as meaning worry, disease, and a biting insect. Thus, from this time onwards, *yang* began to include the meaning of disease and a biting insect. Additionally, there is a homonym of *yang* that is created by attaching the word *quan* (literally dog) to the original word. This term, also pronounced *tsutsuga* in Japan, refers to a beast that resembles a lion and eats humans, adding a more fearsome connotation to the original word *yang*. The etymology of *tsutsuga* is uncertain. In the letter of Prince Shotoku (607), *yang* was used to mean 'accident-absent', although its pronunciation was not known. The oldest anthology of Japanese poetry, *Manyoshu* (770), used *tsutsumi-naku* to refer to *tsutsuga-naku* (accident-absent). *Genji monogatari* (early 11th century) used *tsutsuga* to mean disease. According to *Nihon Shakumyo* (1700), a book on the etymology of Japanese words, *tsutsu* is derived from *tsutsi*, meaning soil or earth, while *ga* and *mushi* refer to bite and bug, respectively; thus, *tsutsu-ga-mushi* means the biting bug that comes from the soil [19]. It is believed that this is how the terms *yang* and *tsutsuga* came to refer to an insect-related disease. In the 1970s, Suto argued that *tsutsuga* is a Japanese proper noun, and therefore it is more reasonable to use *tsutsuga* in Japanese characters while using *mushi-byo* in Kanji characters. As a result, recent Japanese medical articles have named the disease accordingly.

At the time, *tsutsuga-no-mushi* was more commonly used than *tsutsugamushi* in Niigata-ken. In *Seishinhu* (1754), the yearbook of Shibata-han (Shibata Domain) (now merged with Niigata-ken) (1801), and articles by Nagino (1878) and Kawakami (1879), the bugs were described as *tsutsuganomushi* [20, 21]. The word *no* is possessive and is used to connect two common nouns. Since *tsutsugamushi* is now a specific noun referring to the vector of tsutsugamushi disease or the larvae of trombiculid mites, *no* is dropped from *tsutsuganomushi*. Additionally, farmers in the affected areas used the name *tsutsuga* not only for the insect but also for the disease, and later these two meanings were distinguished by adding the suffix *mushi* or *byo* (literally disease): *tsutsugamushi*

https://doi.org/10.3947/ic.2024.0095

for the insect and *tsutsugamushibyo* for tsutsugamushi disease. Regarding *shima-mushi*, *shima* means island in general, but in tsutsugamushi disease, it refers to a river islet or island formed after flooding. In the past, the river changed its course following each flood. Islets would be created after one flood only to disappear after the next, with new ones emerging elsewhere. Farmers waded or paddled the shallow river to access these islets, often getting bitten by red insects. The term *shima-mushi* was used only in Nagaoka, Niigata-ken. After the river banks were renovated, especially during the Meiji era, the course of the rivers stopped changing with each flood. The islets that once formed were expanded into river islands, where people began to settle and establish villages, usually called Nakanoshima. Some traditional doctors, such as Nakatoku Haga, were skilled in removing mites from the skin, earning them the nickname "bug doctors." Evidence of tsutsugamushi disease before its scientific discovery can be found in shrines and statues built by farmers in the early 1800s to pray for the protection from the disease [4, 5]. In Tsuruoka, Yamagata-ken, people made mite-like effigies out of rope and placed them under their eaves each January for the same purpose. Various ceremonies were practiced in endemic regions to pray for the prevention of the disease, though many have now been discontinued or lost their original significance due to the decline in disease incidence. The Shoreisai Festival on Mt. Haguro in Yamagata-ken is one such ceremony [4]. Another is Mushi-okuri (*mushi*, bug; *okuri*, sending; *mushi-okuri* literally means sending off the bugs), held in Niigata-ken [5].

After working in the noxious areas, the farmers thoroughly inspected their bodies. If they found any bugs or felt a prick sensation, they would go to the doctor before the onset of fever. For example, Kawakami often removed attached mites from patients with tsutsugamushi disease [22], which suggests that hospitalization should occur within three to four days of the mite bite. This practice was unique in Japan and provided useful information about the risk of developing the disease after mite bites. Out of 274 individuals who entered the noxious area, 212 were bitten by mites, and 17 of those individuals developed the disease [23]. Walch in Sumatra also described that only one out of 195 laborers infested with *T. deliensis* developed Sumatran mite fever [24]. Building on this background, the sequential changes of chigger bite sites are well documented in Japan [1]. In Japan, there were several colloquial names for eschar: *bouguchi*, *sekiguchi*, *sashiguchi*, and *nomiguchi* all refer to a small round opening caused by an insect bite. Among these, *sashiguchi* (*sashi*, sting or prick; *guchi*, mouth or small opening; *sashiguchi* literally means a small opening following the sting) has become the most widely used term in Japan. In contrast, non-Japanese terms like eschar and *tache noire* are not as appropriate for describing a skin lesion without a black crust. The term inoculation lesion refers to the initial lesion caused by something possibly introduced by an insect, without providing details about the morphology of the skin lesion. The terms primary lesion or initial lesion refer to the first lesion of tsutsugamushi disease, but do not provide information about its relationship to the insect bite or the morphology of the skin lesion. The terms primary sore or primary ulcer describe the morphology of the initial lesion, but do not imply its relationship to the insect bite.

2) Identification of tsutsugamushi disease

Tadashi Nagino grew up with a samurai physician in Nagaoka. He studied Rangaku for five years in private schools and a few more years at a medical institute in Nagasaki, followed by a brief period studying German medicine in Tokyo. In 1873, he was invited to become the director of the newly built Nagaoka Hospital in Niigata-ken [25]. He transitioned from a samurai physician to a military doctor as Japan underwent political and social reforms from the Tokugawa Shogunate to the Meiji era. In Nagaoka, patients with tsutsugamushi disease sought treatment at the new hospital, leading Nagino to recognize it as a new disease. In December 1877, he proposed an investigation into the disease to the Surgeon General, who accepted the proposal. The Niigata Prefectural Government provided research funds and auxiliary personnel to establish a temporary facility in a temple, where patients could receive care free of charge. Seisai Kawakami, the son of another samurai physician in Nagaoka and a student at the University of Tokyo Faculty of Medicine, served as a house surgeon at the facility. He may have recorded detailed clinical manifestations of the disease. Through microscopic examination, they observed small, six-legged red bugs extracted from the patients, some of which matured into eight-legged bugs in a laboratory room at the facility. They described eschars and referred to them as *sekiguchi*. These observations were published in a Japanese medical journal in 1878 (**Table 1**) [20, 22]. The two Japanese authors hypothesized that the poisonous insect was likely the cause of the disease [21, 22, 26]. At that time, the term "poison" was used to describe both poison and infection, much like how we still use the term "food poisoning" today to refer

https://doi.org/10.3947/ic.2024.0095

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Table 1. First scientific descriptions of tsutsugamushi disease in Japan

Author (year)	Disease name or etiology
Nagino (1878)	Poisonous insect
Palm (1878)	Shima-mushi (Island-insect) disease
Bälz & Kawakami (1879)	Japanese river or flood fever based on the miasma theory
Kawakami (1879)	Poisonous insect

to either food poisoning caused by microbial toxins or infectious enteritis.

Theobald Adrian Palm, a medical missionary in Niigata City, visited the facility in 1877 and observed patients who had been admitted. In 1878, he published a short article in the Edinburgh Medical Journal entitled "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." A boil-like papule and regional lymphadenopathy were described in a few patients with early disease. He noted the similarities between tsutsugamushi disease and syphilis: a putative organism enters humans through an entry site (i.e., eschar for the former and chancre for syphilis) and then spreads systemically (fever, lymphadenopathy, and rash for both tsutsugamushi disease and secondary syphilis). He described his plan, if allowed, to prevent the disease the following year using insect repellent. He received an insect specimen slide from Kawakami and thought it resembled a scabies mite, although the slide was poorly prepared [27].

Erwin Bälz, a professor at the University of Tokyo Faculty of Medicine, visited Nagaoka in 1878 to observe the patients at the facility for three weeks. In 1879, Bälz and Kawakami published a detailed article on their findings. Bälz proposed that the bad air from the flooded land caused tsutsugamushi disease, based on the miasma theory, a prevailing theory to explain several epidemic diseases including typhus. Local farmers, however, said that the disease followed the bites of red mites, and Kawakami sent him a mite extracted from a patient. However, Bälz dismissed these claims because only a small number of people bitten by the red mites actually developed the disease, and the mite in question was identified as *Leptus autumnalis* (now *Neotrombicula autumnalis*), a mite known to cause scrub itch [28, 29]. In this context, Bälz referred to the disease as Japanese river or flood fever rather than calling it an insect-related disease. The morphological similarity of *L. autumnalis* to *L. akamushi* was recognized much later. In 1879, Kawakami published his article [21]. These articles, except Bälz's, were not recognized by other Japanese researchers until the late 1950s, and Palm's report was only occasionally referenced in English-speaking countries.

In 1888, the Niigata Prefectural Government announced that cases of tsutsugamushi disease should be notified to the prefectural government within 24 hours of diagnosis. Subsequently, the number of officially reported cases of tsutsugamushi disease has been recorded in Niigata-ken since 1889, and Akita-ken adopted this reporting system in 1894, followed by Yamagata-ken in 1914 (**Fig. 1**). In this figure, although the reported numbers from 1914 to 1949 are only from the three prefectures of Japan, these data can be considered nationwide statistics because no cases were reported from the other prefectures [30]. As a result, Japan has 130 years statistics on tsutsugamushi disease, providing valuable information

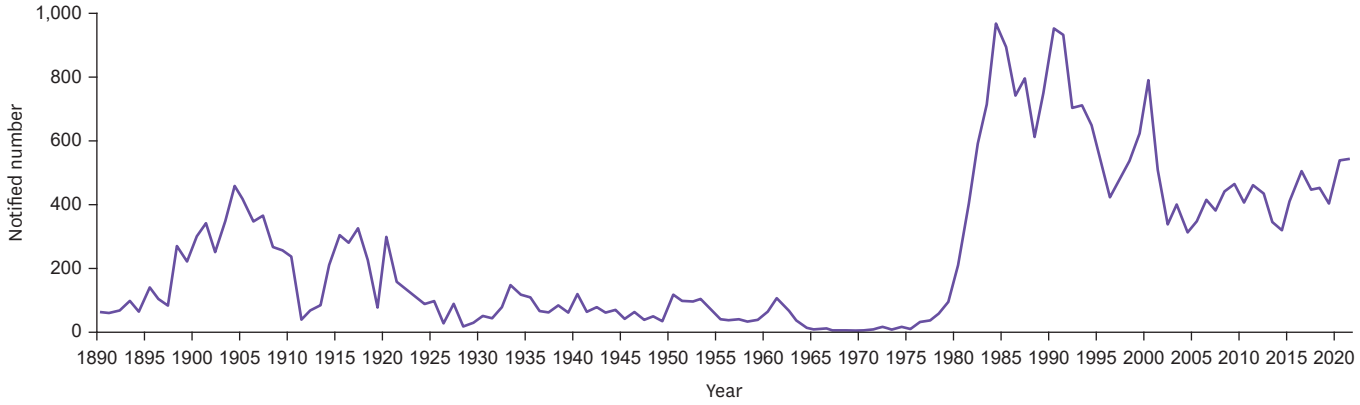

Figure 1. The number of reported cases of tsutsugamushi disease in Japan from 1890 to 2021. The data from 1914 to 1949 represent three northwestern prefectures of Japan, according to Sasa's monograph (1956), *Tsutsugamushi and Tsutsugamushibyo* [30]. From 1950 onwards, the data are nationwide and sourced from the National Epidemiological Surveillance of Infectious Diseases (in Japan). The rise in cases between 1951 and 1952 is attributed to Shichito fever.

https://doi.org/10.3947/ic.2024.0095

on how the epidemiology of the disease has evolved over time. If the factors contributing to these temporal trends are accurately identified, they can be used to predict future changes in the epidemiology of *O. tsutsugamushi* infection in other regions or countries. For example, the incidence of the disease in Korea from 1994 to 2016 was similar to that in Japan from 1975 to 1995 [31].

3) Discovery of the etiologic agent of tsutsugamushi disease

After the aforementioned studies revealed the occurrence of a novel disease in Niigata-ken, many Japanese researchers commenced investigations into the disease. Several physicians in Niigata-ken, under the auspices of the prefectural government, had studied the clinical and epidemiological characteristics of tsutsugamushi disease. However, they were unable to identify its vector and causative agent [32].

Keisuke Tanaka, the director of Yokote Municipal Hospital in Akita-ken, established a laboratory in the yard of his house in 1890 to study the disease. Throughout his life, he closely observed many patients with the disease from the time of mite bites. He described the disease in detail and, contrary to the miasma theory proposed by Bälz, emphasized the importance of mite bites in causing the disease [33, 34]. He noted the existence of two types of mites, "akamushi" detached from humans and "yasodani" detached from field rodents, and depicted their detailed morphology. Émile Brumpt in France, an orthodox medical entomologist at the time, used this depiction to name the former mite as *Trombicula akamushi* (Brumpt) in his book *Précis de parasitologie* (1910). However, the figure lacked descriptions of important points for determining the mite species. Hirst (1915) examined chiggers sent by Miyajima, collected from the ears of field mice in Niigata-ken. He described the morphology of one of the chiggers and renamed it *Microtrombidium akamushi*, Brumpt. Hirst noted that *M. akamushi* differed slightly from Tanaka's description and identified four other chigger species in Japan [35]. Later, Manabu Sasa suggested that Tanaka's depiction was more likely *Neotrombicula japonica* than *T. akamushi* [3]. Since attached chiggers are rarely observed in patients with tsutsugamushi disease at the onset of fever, Tanaka used to remove chiggers from healthy individuals who had just passed through the noxious areas, most of whom did not develop the disease later. Therefore, he may have depicted a chigger removed from one of these people, which would be a non-*L. akamushi* chigger. Tanaka described that eight patients experienced multiple episodes of tsutsugamushi disease: five patients had two attacks, and three patients had three attacks. The reinfection episodes were usually mild, but one of the eight patients experienced a more severe episode, suggesting that immunity to tsutsugamushi disease is short-lived [36]. In 1908 and 1925, Tanaka compiled old Chinese and Japanese literature on tsutsugamushi disease [1]. From 1894 onwards, he had collected case records of tsutsugamushi disease in Akita-ken and submitted them to the prefectural government each year. Between 1896 and 1910, he was the only one to submit the records, and the case fatality rate was 12.3%. Especially between 1904 and 1910, the case fatality rate ranged from 4.4 to 8.2%, which contrasted with the general knowledge that the case fatality rate of tsutsugamushi disease in Japan before 1950 was high (20.2 to 45.8%) [37]. These unexpectedly low case fatality rates may have been due to the inclusion of patients with benign or atypical tsutsugamushi disease, since he could diagnose the disease based on the presence of a papule or bulla, as well as a typical eschar. For etiology, he initially described the presence of intraerythrocytic bodies in the blood, but later dismissed this finding (**Table 2**). Subsequently, when *Proteus* strains were isolated from a few patients with tsutsugamushi disease, he proposed *Proteus* as the cause of the disease in 1899, as did Anigstein in 1933. Teramura, another local physician in Akita-ken, also studied tsutsugamushi disease. He learned about the disease from Tanaka, and every summer since 1925, he provided accommodation and clinical specimens to several visiting researchers, including Norio Ogata. In 1927, Teramura inoculated the blood of a patient into rabbit testicles. Ishiwara and Ogata independently maintained

Table 2. Proposed etiologic agents of tsutsugamushi disease

Author (year)	Etiology
Based on microscopic finding	
Tanaka (1892)	Intraerythrocytic bodies; (1899) *Proteus* spp.; (1906) Insect toxin
Kitasato (1893)	Intraerythrocytic protozoan
Ogata M & Ishiwara (1910)	Protozoan, "*Gregarina tsutsugamushi*"; (1912) Schizomycetes
Miyajima (1918)	"Akamushi corpuscles"
Hayashi (1920)	Protozoan, "*Theileria tsutsugamushi*"; (1922) Rickettsia (?)
Nagayo et al. (1924)	Intracellular rickettsia-like bodies
Based on culture result	
Sellards (1923)	"*Rickettsia nipponica*"
Ishiwara & Ogata N (1928)	Rickettsia
Nagayo et al. (1930)	*Rickettsia orientalis*
Ogata N (1931)	*Rickettsia tsutsugamushi*
Kawamura & Imagawa (1931)	"*Rickettsia akamushi*"

https://doi.org/10.3947/ic.2024.0095

the bacteria in their laboratories by serial passage of the rabbit testicular tissue. Finally, in 1931, Ogata identified the causative agent of tsutsugamushi disease.

In 1892–93, Kitasato studied the etiology of tsutsugamushi disease in Niigata-ken. He first reproduced the disease in monkeys by inoculating the blood of patients and documented that the disease was caused by an infection, a proliferating agent, rather than a poison, a non-proliferating agent. He claimed that plasmodium-like intraerythrocytic bodies were observed in the blood smears of both the patients and the monkeys. He emphasized the presence of eschar and the development of lymphadenopathy before the onset of fever in the disease [38]. The results were not only published individually in 1893–95, but were also reprinted in the Gazette in 1894, making the disease known to many non-medical people. He may have considered tsutsugamushi disease as a vector-borne intraerythrocytic protozoan disease, but could not formalize this idea until the report of Smith and Kilbourne (1893) on Texas cattle fever (tick-borne piroplasmosis), which first demonstrated the transmission of an intraerythrocytic protozoan by an arthropod. The prefectural government was satisfied with this result, and further funding for the study was discontinued. Based on these results, from 1894 to 1897, the prefectural government sprayed carbolic acid on the noxious areas and distributed gasoline to the farmers as an insect repellent. Meanwhile, as other researchers failed to observe intraerythrocytic bodies and the disease continued to occur, the Niigata Medical Society proposed to the prefectural government to continue the research. The government accepted this proposal, and consulted research centers outside the prefecture as there was no specialized research institution in Niigata-ken at that time.

The Institute for Researches of Infectious Diseases, later renamed the Kitasato Institute, began studying the disease at the request of the Niigata Government. Because Kitasato was studying plague in Hong Kong, other colleagues, Asakawa and Miyajima, and later Kitashima and Miyajima, summarized the epidemiological and clinical features of tsutsugamushi disease. They conducted extensive entomological and experimental animal studies from 1904 to 1914 [39, 40]. Monkeys were exposed to the noxious area for several days, and many of them contracted tsutsugamushi disease. However, only a small percentage of the mites attached to the monkeys induced eschars, suggesting that the infectious mites were quite few in number. The organism was maintained in the laboratory through serial passage in monkeys, and later by alternating passage between guinea pigs and monkeys. Guinea pigs, while asymptomatic, retained the bacteria for approximately two weeks after infection, and the bacteria were virulent enough to infect a series of guinea pigs. When blood from the infected guinea pigs was inoculated into monkeys, the monkeys developed fever. They also reared chiggers collected from field rodents, successfully developing them into nymphs (in 1909) and adult mites (in 1917) [41, 42]. The nymphs and adult mites did not seek out animals, and the adult female mites did not ovulate when they were fed only vegetable juice [41]. Wharton and Jayewickreme independently discovered in 1946 that adult female mites could ovulate when fed animal proteins, such as mosquito or dragonfly eggs [43, 44]. Miyajima and Okumura then collected adult mites from the soil and reared them in the laboratory. During rearing, they observed that eggs laid in the cages developed into chiggers. The chiggers collected from the rodents were morphologically identical to the chiggers hatched from eggs in the laboratory. They also attached the lab-hatched larvae to a Japanese monkey, which developed typical symptoms of tsutsugamushi disease, including eschar, regional lymphadenopathy, and fever. Only two of the 28 chiggers induced eschars, suggesting that only a small proportion of chiggers in an endemic area were infectious [42]. In this way, they observed the entire life cycle of *L. akamushi* and confirmed for the first time that only chiggers transmit the disease to warm-blooded animals. Furthermore, this finding suggests that transovarial transmission is the most plausible mechanism for the persistence of *O. tsutsugamushi* in nature. This mode of transmission was later demonstrated informally by Makie in 1946 [45] and formally by Yu and Wu in 1959 [46]. He also inoculated monkeys and guinea pigs with mite emulsion, then induced fever, and observed corpuscles of the same morphology ("Akamushi corpuscles") [47]. However, this experiment may not be able to distinguish rickettsiae from rickettsia-like bacteria present in the mite intestine, including *Bartonella* spp. Since 1902,, Miyajima had claimed that tsutsugamushi disease might be a rickettsial infection because tsutsugamushi disease and Rocky Mountain spotted fever were similar in topography and clinical features. In this regard, he invited Ashburn and Craig to Japan [48].

Masanori Ogata, Ishiwara, and Masanori's son, Ogata N, from the Department of Hygiene at Tokyo Imperial University, conducted a study on tsutsugamushi disease from 1905 to 1918. Ogata M was a pioneer in hygiene

and bacteriology in Japan. Every summer, they traveled from Tokyo to Niigata-ken and stayed there for three weeks. During their research, he observed variable sized amoeboid bodies within large cells in specimens from patients and autopsies. They believed these bodies were amoeboid protozoa [49, 50]. In 1910, Ogata M proposed that the protozoan "*Gregarina tsutsugamushi*" was the cause of the disease [51]. Later, Nagayo mentioned that the rickettsia-like inclusion bodies he observed were similar to those reported by Ogata M. Therefore, Ogata M may have observed rickettsiae but misclassified his finding as a protozoan, as this new organism was not well known at that time. From 1920, Kikutaro Ishiwara used intratesticular inoculation in various animals, including monkeys and guinea pigs, to isolate the causative agent of tsutsugamushi disease. In 1927, he began using this method in rabbits, and by 1930, he had succeeded in maintaining the organism in the laboratory for up to 111 passages [52]. The infected rabbit testes showed significant inflammation as the bacteria became more abundant and virulent with each passage. Additionally, the swelling of the infected testis could be grossly observed and the contralateral uninfected testis could be used as a control, making it easier to identify the infection status compared to the alternating passage between guinea pigs and monkeys used by Kitashima and Kawamura. The most practical advantage of this method is its low cost compared to the use of monkeys. Intradermal inoculation of the infected testicular tissue into other rabbits resulted in eschars, systemic symptoms, and occasional deaths. Typical symptoms of the disease were reproduced in monkeys using it, and Gram-negative pleomorphic bacteria were consistently observed in the cytoplasm of histiocytes in tissues obtained from the infected animals. In 1928, he postulated that the organism was *Rickettsia* [53]. In 1929, an infected rabbit was donated to Hayashi, who used it to reproduce tsutsugamushi disease in a monkey. During 1929–30, the term "tsutsugamushi rickettsia" was openly used at conferences in Japan, although no one formally reported the agent as rickettsia. Ishiwara's method, the intratesticular inoculation method, was a new technique in the study of rickettsial infections. He experienced a patient with a febrile disease following a rat bite in Niigata-ken, and later reproduced the disease in laboratory animals and observed spirochete-like bacteria in their blood. As a result, he was the first to identify the causative organism of rat-bite fever (sodoku), *Spirillum minus*. At that time, syphilis, a well-known spirochetal infection, was being studied through intratesticular inoculation and serial passage. It is therefore speculated that he was inspired to use this method while he was investigating the etiologic agent of rat bite fever. After these studies, he ceased his work on tsutsugamushi disease and focused his interest on the field of hygiene. Ogata N and Unno continued to use this method at Chiba University [54], and Ogata N donated the infected testicular homogenates to Nagayo and Kawamura in 1930.

Naoske Hayashi, from the Department of Pathology at Aichi University, studied the disease in Nagaoka every summer from 1905 and reported the results annually. He observed patients with multiple episodes of tsutsugamushi disease, with two episodes in thirteen patients and three episodes in three patients. Of the 16 patients, all but two had mild symptoms [55]. He observed intracellular bodies in various tissues from patients, including three autopsied cases. He summarized these findings and reported the protozoan "*Theileria tsutsugamushi*" as the cause of tsutsugamushi disease in 1920. He illustrated various developmental forms of this protozoan in plasma, red blood cells, and leukocytes [56]. In 1922, he first used the name *Rickettsia tsutsugamushi*, although it was not based on the characterization of the bacteria he cultured. In 1924, he presented a review of the characteristics of rickettsiae at a conference held by the Japanese Society of Pathology [57]. Later, Nagayo, Ogata N, and Kawamura used these characteristics as evidence that the bacteria they had cultured were *Rickettsia*.

The National Niigata Medical College was established in 1910, with Rinya Kawamura being appointed as a professor in the Department of Pathology and a member of the Committee for the Prevention of Endemic Diseases of Niigata-ken in 1911. Since then, he had conducted intensive studies on various aspects of tsutsugamushi disease, such as its pathology, clinical features, laboratory findings (including leukopenia), mites, tsutsugamushi disease in Taiwan, field trials of a live vaccine using the Pescadores strain, and fever therapy for general paresis using the same strain. In 1922, Kawamura provided a guinea pig infected with *O. tsutsugamushi* to Sellards. Sellards conducted further research on the bacterium in the United States, maintaining it through alternating passage between guinea pigs and monkeys, the same method used by Kawamura. In 1923, Sellards proposed the bacterium be named "*Rickettsia nipponica*." However, since the bacterium was grown in cell-free media, contamination likely occurred during the passage. Sellards emphasized the occurrence of hemorrhages in the tissue of guinea pigs as a characteristic of *O. tsutsugamushi* infection,

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while Kawamura did not observe this phenomenon [58]. Kawamura described the adverse outcomes of five pregnant women with tsutsugamushi disease. Although the prognosis of the disease in the pregnant women was good, all five miscarried. Blood from the umbilical vein of one of these women was injected into a monkey, which showed no symptoms. Autopsies were performed on three of the miscarriages. The findings were similar to those in adults, but milder: increased phagocytic cells in the spleen, lymph nodes, and bone marrow, and focal accumulation of leukocytes in the liver [59]. Twenty-two out of 90 patients experienced multiple episodes of tsutsugamushi disease: two episodes in 10 patients and three episodes in 12 patients. The reinfected patients displayed indistinct eschars and had a low mortality rate. Additionally, there were 33 cases of reinfection in an endemic area: two episodes in 30 patients and three episodes in the remaining patients. Seven of these patients developed the disease the following year, while 16 patients developed it within ten years of the first episode [59]. In 1931, the etiologic agent of tsutsugamushi disease was reported as "*Rickettsia akamushi*." The bacteria were clearly observed in the salivary glands of chiggers captured from wild rodents. He demonstrated the organisms in various animals infected through different routes, including intratesticular, intraocular, or intraperitoneal inoculation [60]. In 1947, the persistence of *O. tsutsugamushi* in a person who had previously received the Pescadores strain for treating general paresis was first documented by him and his colleagues [61]. He collaborated with Miyaji and Nishibe. Miyaji, from the Department of Hygiene and Bacteriology, joined the study of tsutsugamushi disease in 1914 and criticized previous reports that the microscopic bodies observed in tsutsugamushi disease were protozoa [5]. Nishibe actively studied the disease using tissue culture and laboratory animals. Unfortunately, he contracted laboratory-acquired tsutsugamushi disease during these experiments and passed away at the age of 41 [62]. Around the same time, there were reports of cases of laboratory-acquired rickettsial infections, possibly because researchers began culturing rickettsiae and handling specimens containing large amounts of rickettsiae [62-64].

From 1915 to 1930, Mataro Nagayo, Miyagawa, Mitamura, and later Tamiya at the Government Institute for Infectious Diseases studied the disease. They traveled to Yachimachi, Yamagata-ken, and spent a month there each summer, examining patients hospitalized for tsutsugamushi disease in a nearby isolation hospital. In 1915, they successfully reproduced tsutsugamushi disease by inoculating monkeys with unfiltered blood from patients, but not with filtered blood. Monkeys that had recovered from tsutsugamushi disease did not contract it again when given blood from another patient, demonstrating specific immunity. Using various staining methods, including the Giemsa method, they observed cytoplasmic bodies in large mononuclear cells from the lymph nodes and spleens of both patients and monkeys. Mice that received blood from patients did not show symptoms, but splenomegaly was observed in the infected mice [65]. In 1916, they collected chiggers, nymphs, and adult mites from the noxious area in Yamagata-ken. They reared the mites in the laboratory, described the detailed morphology of *T. akamushi*, and identified the unique morphological and biological characteristics of *T. akamushi*. Consequently, they proposed renaming *T. akamushi* to *L. akamushi*. The photogram included in this article clearly showed that as early as 1915, they were already wearing protective gear [66]. They discovered four new species of *Trombicula* mites and named them *T. pallida*, *T. palpalis*, *T. scutellaris*, and *T. intermedia*, which had previously been named differently by Tanaka (kedani and yasodani), Miyajima (coarse-haired and fine-haired types), and Kawamura (A, B, C, and D types) [17]. A study revealed the presence of *O. tsutsugamushi* in adult mites. In this experiment, tsutsugamushi disease was reproduced in three out of eight monkeys after inoculation with pools of adult mites. However, similar experiments conducted by Kawamura and Miyajima did not yield the same results [67]. In 1924, eschars were reproduced by intradermally inoculating monkeys with the blood of patients. Numerous rickettsia-like bodies were demonstrated in the cytoplasm of large histiocytes in smear preparations and sections of the eschars. No organisms were identified in the endothelial cells of the blood vessels adjacent to the eschars. The classification of this bacterium as a species of *Rickettsia* was postponed until it could be compared it with other known *Rickettsia* species [68]. However, the planned experiments were not performed until 1930, possibly due to difficulties in obtaining a sufficient amount of the bacteria [69]. In 1930, they inoculated clinical isolates into the anterior chambers of rabbit eyes. Intracellular organisms were observed in the endothelial cells of Descemet's membrane. Based on these findings, this bacterium was reported as *Rickettsia orientalis* [70, 71].

Ogata N reported this bacterium as *R. tsutsugamushi* in 1931, based on its characteristics of being non-filterable,

https://doi.org/10.3947/ic.2024.0095

non-motile, pleomorphic, difficult to cultivate, Gram-negative, and with intracellular localization, as well as arthropod vector transmission [72]. The formal name of this rickettsia remained controversial in Japan for a long time because Nagayo first named this bacterium *R. orientalis*, but was indebted to Ogata N for obtaining enough of the bacteria to make his experiment possible, while Ogata N was late in naming *R. tsutsugamushi*. In 1934, Kawamura proposed "*Rickettsia tsutsugamushi-orientalis*" to settle this dispute. Additionally, because Hayashi had priority for the name *R. tsutsugamushi*, Ogata N probably wanted to rename the bacterium and later mentioned the names "*R. tsutsugamushi* S. *kedani*" (1935) and "*R. tsutsuganomushi*" (1954). Meanwhile, Phillip listed *R. tsutsugamushi* as the formal binomial name of this bacterium in Bergey's Manual of Determinative Bacteriology (5th edition) in 1939. In the 6th edition of the book (1948), he described the rationale behind his judgment that three reports by Nagayo prior to 1930 were preliminary, with his final report being published in March 1931. Ogata N used the name *R. tsutsugamushi* in the report presented to the Eighth Congress of the Far Eastern Association of Tropical Medicine in December 1930, and published the formal report in October 1931 [72]. Philip concluded that Ogata N had named the bacterium before Nagayo, despite potential disagreements with his judgment. Ultimately, *R. tsutsugamushi* became the accepted name of the bacterium. In 1995, Akira Tamura et al. classified the bacterium as a new genus *Orientia* and renamed it *O. tsutsugamushi* [73]. Yoshida (1935) successfully cultured *O. tsutsugamushi* in tissue culture, using testicular, lung, and spleen tissues from infected rabbits [74].

After the etiologic agent of tsutsugamushi disease was identified, research interest in Japan declined significantly. Additionally, starting in 1929, the Great Depression and then World War II had a negative impact on the medical societies of Japan. At the same time, the number of clinical cases of tsutsugamushi disease steadily decreased until 1948, when sporadic cases of mild tsutsugamushi disease began to occur in various areas of Honshu and Shikoku, including a large outbreak in the Shichito Islands, called Shichito fever, which occurred between 1951 and 1952. Several explanations have been proposed for this trend [5]. First, knowledge about the disease was well established and widely disseminated. A Japanese medical textbook published in 1907, *Inoue Naika Shinsho*, described "Tsutsugamushi disease (Kedani disease)" (in Japanese) in a separate chapter, with an 8-page volume and including photograms of an eschar and rash, a fever chart, and chiggers. As early as 1910, the Yamagata Prefectural Government conducted mass education to prevent mite infestation. The installation of shower facilities near workplaces was emphasized. Spraying of insecticides and bulldozing of undergrowth were used for environmental control. Second, the river banks were renovated using modern technology, significantly reducing the floodplain areas. For example, the length of the Bandai Bridge, which crosses over the Shinano River, was reduced from 770 meters in 1886 to 270 meters in 1929. Third, and most importantly, the need to enter these riverside areas diminished as farmers became industrial workers or soldiers.

4) Old Japanese literature

Although there were several old publications on tsutsugamushi disease before 1878, most Japanese scientists were unaware of them. Tanaka was the first to search for old relevant Japanese and Chinese literature and published it twice [1]. However, Tanaka's publications received little attention from other researchers. Ogata N (1953) reviewed the history of research on tsutsugamushi disease in Japan [51, 75] and consulted Hiroshi Kanbara, an orthopedic surgeon and medical historian in Niigata-ken, to search for old literature on the disease [19, 76-78].

The oldest book describing Shashidu (tsutsugamushi disease) and Shegongdu (tick-borne rickettsiosis) in Japan is *Wakan Sansai Zue*, written by Ryoan Terajima in 1712. This encyclopedia illustrated the diseases based on *Bencao Gangmu* but did not indicate their actual occurrence in Japan. It also described Kidan as having clinical features similar to Zhongxidu (tsutsugamushi disease) [1]. The name Kidan is a synonym for Kedani.

Seishinhu is a book published in 1792 that describes the family history of the vassals of Shibata-han and their various achievements. The book recounts an episode that occurred during a mission to reclaim a swampy area as follows: Shibata-han sent a construction officer to a village in Nakanoshima, Nagaoka, in 1754 to reclaim a marshland. The officer told the village chief, "There are tsutsuganomushi in this area, so do not enter..." [5]. Masumi Sugae, a classical scholar and traveler, mentioned tsutsugamushi in a travelogue about Niigata-ken (1784) and described kedani in several chronicles of Akita-ken after he settled there in 1802: Okachi-gun (Okachi County) (1814), Hiraka-gun (1824), and Senboku-gun (1826). The 1801 Yearbook of Shibata-han, an official

https://doi.org/10.3947/ic.2024.0095

record, described several outbreaks of tsutsugamushi disease in Nakanoshima during those years, resulting in many deaths. The prefectural government discussed the management and prevention of the disease.

Hakuju Hashimoto (1810) published the monograph *Dandoku Ron* (Treatise on Eliminating Poisons) and insisted that smallpox, measles, syphilis, and scabies could be prevented through his new concept of infectious diseases, i.e., transmissibility, isolation, sterilization or disinfection, fomite, and immunity. The monograph described tsutsugamushi disease as an example of infectious disease, stating: "In Japan, Shegong, also called tsutsuga by the indigenous people, occurs along the banks of the Chikuma River." The Chikuma River is a tributary of the Shinano River and is located in Nagano-ken. Although Shegong is an engorged tick, Shegong and Shashi became indistinguishable at that time, as described in our previous report [1]. He described the disease in Kanji characters as *totoga*, but Mototaka Taki used *tsutsuga* in Japanese characters when quoting this sentence in his monograph *Jikan dokugasho* (1837). Genkei Ohtomo (1819) submitted the treatise *Treatment Experience of Kedani Disease* (in Japanese) to the Akita Prefectural Government. Every summer in Akita-ken, he experienced several patients with a febrile disease, diagnosed it as kedani disease after distinguishing it from epidemic Shanghan (typhoid fever), and treated it with a decoction that has an antipyretic effect. He removed the chiggers from the bite sites as a prophylactic measure. In 1836, Yonesawa-han (now merged into Yamagata-ken) published *Kedani-go* (Thoughts on Kedani), which contained replies from four experienced doctors in Akita-ken, including Ohtomo and his son, on the treatment of kedani disease. Until 1878, there were several other writings describing tsutsugamushi disease, but most of them were based on Chinese literature and did not add any new knowledge [19,76-78].

CONCLUSION

The topographical features of Niigata-ken, Akita-ken, and Yamagata-ken have created a unique ecological condition for mites. Annual flooding resulted in large undisturbed grassy areas, many of which have been gradually reclaimed over three centuries. The hot summers and high humidity have favored the growth of *L. akamushi*, resulting in the prevalence of the Kato serotype of *O. tsutsugamushi*. The influx of farmers from other prefectures to the reclaimed land, who would not have been immune to the Kato serotype, has contributed to the clinical recognition of the disease. Japanese researchers distinguished tsutsugamushi disease from typhoid fever and other infections. Their knowledge of entomology and microbiology led to further research on tsutsugamushi disease, including its transmission by chiggers and the discovery of the causative agent, *O. tsutsugamushi*. Collaboration between basic scientists and physicians was another key factor in this success. It was also important for the prefectural governments to provide research funds and space for decades, as well as to establish the surveillance system for tsutsugamushi disease.

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Funding
None.

Conflict of Interest
No conflict of interest.

Author Contributions
Conceptualization: MHC, JSK, JSL. Writing - original draft: MHC. Writing - review & editing: MHC, JSK, JSL.

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