Original Article

Seasonality and Geographical Distribution of Tsutsugamushi Diseases in Japan: Analysis of the Trends since 1955 till 2014

Hiroshi Yoshikura*

National Institute of Infectious Diseases, Tokyo 162-8640, Japan

SUMMARY: Data concerning the seasonality and geographical location of tsutsugamushi disease (TD) in Japan from 1955 to 2014 were compiled. After disappearance of “classical” TD in the mid-1960s, there was a long near-vacuum. Then, in 1975, a TD epidemic with October–December seasonality emerged in southern prefectures, and in 1979, that with May–June seasonality in northern prefectures. The current TD epidemic could be classified according to seasonality and geography into three types: a large October–December epidemic with a negligibly small May–June epidemic in the southern part of Japan, a large May–June epidemic with a mid-sized October–December epidemic on the Pacific coast of northern Japan, and a large epidemic in May–June with a negligibly small October–December epidemic on the Japan Sea coast of northern Japan. In addition, there were a few patients that were scattered throughout the year in various places in Japan. How these different epidemic patterns emerged is discussed.

INTRODUCTION

Tsutsugamushi disease (TD) is caused by *Orientia tsutsugamushi* (*Ot*), a cytoplasmic symbiont of mite *Leptotrombidium*. As only the unfed female larvae transmit *Ot* to humans, the timing of TD epidemics must be linked to the timing of larval maturation as female (1–2). Belonging to the order *Rickettsiales* together with *Wolbachia*, *Ot* causes feminization and parthenogenesis of their hosts (3–6).

TD was endemic for a long time in the northern part of Japan. In Tamiya’s review (2), we find a description of the “Kedani-Daimyojin” (hairy-mite-god) Shrine in Yamagata Prefecture dating back to 1860. The epidemic season was summer, when temperatures were above 25°C (1–2). This disease, known as “classical TD”, was transmitted by *L. akamushi*. It continued until the mid-1960s. The “new type” TD epidemics emerged in the early 1970s and are still prevalent today. TD has 2 epidemic peaks per year: from late spring to early summer, and from autumn to early winter (1–2). This seasonality was demarcated geographically: the former, May–June type, in the northern part of Japan and the latter, October–December type, in the southern part (7). In order to understand how the present seasonality emerged, we traced the TD epidemiology back to the mid-1950s, when the infectious disease reporting system was similar to the current one.

MATERIALS AND METHODS

The data used were obtained from the Infectious Diseases Statistics under the Infectious Disease Prevention Act (<http://www.mhlw.go.jp/toukei/list/densenbyou.html>) for 1955–1998, and the National Epidemiological Surveillance of Infectious Diseases (NESID) (<http://www.nih.go.jp/niid/ja/all-surveillance/2270-idwr/nenpou/3363-foward2011.html>) for those later than 1999. Under the Infectious Disease Prevention Act of 1954, which was effective until 1999, TD was reportable, but not notifiable. In 1999, when the infectious disease surveillance was placed under the NESID, TD was classified as a notifiable disease (compulsory reporting of all diagnosed cases).

Until 1999, the monthly incidence in Japan and the annual incidence in individual prefectures were disconnected. The weekly incidence in each prefecture became available only after the surveillance was placed under NESID. The temperature data were obtained from the Japan Meteorological Agency: Past Climate Data (<http://www.data.jma.go.jp/gmd/risk/obsdl/>). We used Microsoft Excel 2010 for graphics and statistical calculations.

RESULTS

Geographical distribution: Fig.1 shows the annual incidence of TD in 47 prefectures from 1955 to 2014. Initially, the TD that was reported almost exclusively from Akita and Niigata was “classical” TD. The “new type” TD appeared first in the foothills of Mt. Fuji in 1946 and 1948 (1), which was reported in large numbers from Shizuoka in 1960–1963. In the mid-1960s, TD became rare throughout Japan. Then, in the mid-1970s, prefectures in the northern part of Japan including Akita, Niigata, and Fukushima, and those in the central to the southern part of Japan including Fukushima, Saitama, Tokyo, Kanagawa, Shizuoka, Miyazaki, and Kagoshima

Received July 7, 2017. Accepted August 3, 2017.

J-STAGE Advance Publication October 31, 2017.

DOI: 10.7883/yoken.JJID.2017.073

*Corresponding author: Mailing address: Policy Planning and Communication Division, Department of Environmental Health and Food Safety, Ministry of Health, Labour and Welfare, 1-2-2 Kasumigaseki, Chiyoda-ku, Tokyo 100-8916, Japan. Tel: +81-3-5253-1111, Fax: +81-3-3503-7965, E-mail: yoshikura-hiroshi@mhlw.go.jp

started reporting TD patients every year.

Seasonality: Fig.2A shows the monthly incidence of TD in Japan from 1955 to 1998. Until 1963, the patients were reported mainly in July–September, which was the classical TD season in Akita and Niigata (compare lanes July–September in Fig. 2A and lanes 5 and 7 in Fig. 1A). There was a temporal increase in September–October in 1960–1962, which was attributable to the “new type” TD that emerged in Shizuoka (compare lanes September–November in Fig. 2A and lane 24 from 1960–1963 in Fig. 1A). After a long period of disappearance from the mid-1960s until the mid-1970s (see middle part of Figs. 1A and 2A), the current October–December/January and May–June epidemics emerged. As many prefectures started reporting many TD cases thereafter, it became impossible to collate the seasonal data (Fig. 2A) with the geographical data (Fig. 1A). Therefore, we tried to determine possible statistical correlations; i.e., if the number of reports from prefecture A was predominant in month B every year, the total number of patients per year in prefecture A would fluctuate synchronously with the total number of patients in month B. Pairs between Akita or Niigata vs. May or June and pairs between Miyazaki or Kagoshima vs. October, November, or December from 1981 to 1998 were chosen for this analysis. The annual number of TD

cases in a prefecture was plotted on the x-axis and the annual number of TD cases in a month on the y-axis. Correlations were significant between Akita and May (Fig. 2B-1), Niigata and June (Fig. 2B-1), and Miyazaki and November (Fig. 2B-2), respectively (correlation coefficient (cc): 0.94, 0.74, and 0.79, respectively). Thus, the May–June epidemic possibly occurred in Akita and Niigata, and the October–December epidemic in Miyazaki. This estimate agreed well with the data of weekly incidence in individual prefectures that became available later than 1999 (see below). Thus, it was concluded that the current geographical demarcation of the seasonality dated back to the late 1970s.

Data after 1999: Fig.3 shows the weekly incidence of TD in individual prefectures from 1999 until 2014. As the total number of TD cases per year was small, the data were pooled for 1999–2006 and for 2007–2014. The data for prefectures with epidemics mainly in October–December are shown in Fig. 3A and those with epidemics mainly in May–June in Fig. 3B.

The October–December seasonality was found in prefectures from the central to the southern part of Japan: that is, Chiba, Tokyo, Kanagawa, Gifu, Shizuoka, Aichi–Mie, Wakayama, Sanyo-Shikoku, Miyazaki, Kagoshima, and other prefectures in Kyushu (Fig. 3A). In the southern Miyazaki and Kagoshima prefectures,

Fig. 1. Annual incidence of TD in prefectures in 1955–2014 (Fig. 1A; Color online), PN, prefecture numbers, (Fig. 1B) and geographical location of prefectures (Fig. 1C). The prefectures are shaded similarly for Fig. 1B and for Fig. 1C.

the epidemic started approximately 2 weeks later than in the other prefectures (week 43 in contrast to week 41 in the others) and continued until January of the next year, which may suggest intrinsic temperature sensitivity of *Ot*-mite symbiotic pairs. In addition, the prefectures in the central part of Japan, e.g., Toyama, Gunma, Ibaraki, and Tochigi, had small peaks in May–June.

Fig. 3B shows prefectures with a large peak in May–June and smaller peaks in October–December. They are generally in the northern part of Japan and all characterized by cold, snowy winters. In Akita, Yamagata, Niigata, and Nagano on the Japan Sea coast, the October–December peaks were small, while in Aomori, Iwate, Miyagi, and Fukushima on the Pacific coast and in Shimane and Tottori on the Japan Sea coast in the southern part of Honshu mainland, the October–December peak was much larger.

In both figures, in addition to the main May–June and October–December peaks, there were small peaks that were scattered throughout the year, suggesting the presence of a small-scale epidemic that was ongoing

throughout the year.

DISCUSSION

The first question was whether the May–June and October–December TD epidemics were caused by the same *Ot*-mite symbiotic pairs twice a year or different *Ot*-mite symbiotic pairs once a year with different seasonality. The data appeared to support the latter. The reasons were (i) in Akita, for example (Fig. 3B), the May–June peak was dominantly larger than the October–December peak; if the May–June peak was produced by the overwintering *Ot*-mite symbiotic pairs, the May–June peak should have been much smaller than the October–December peak because “in autumn, after reaching a peak, the population (of infesting larvae) is gradually decreased during the winter, possibly due to the inactivity of the larvae” (1); (ii) among northern prefectures exhibiting May–June seasonality, the October–December seasonality was negligibly small in prefectures on the Japan Sea coast (left-side graphs in

Fig. 2. Monthly incidence of TD in Japan from 1955 to 1998 (Fig. 2A) and correlation between number of patients in a month (in the vertical axis) and number of patients in a prefecture (in the horizontal axis) (Fig. 2B-1: solid line: PN7 (Niigata) vs. June; dotted line: PN5 (Akita) vs. May; Fig. 2B-2: solid line: PN45 (Miyazaki) vs. November.

Fig. 3. Weekly incidence of TD in prefectures in Japan from 1999 to 2014. The solid lines indicate weekly number of cases totaled for the period of 1999-2006 and dotted lines the data for the period of 2007-2014. The horizontal axis indicates week of the year and the vertical axis incidence. In Fig. 3A prefectures mainly with the October-December seasonality are grouped and in Fig. 3B prefectures mainly with the May-June seasonality.

Fig. 3B), but was nearly the same as the May–June peak on the Pacific coast and in Shimane-Tottori (right-side graphs in Fig. 3B); this difference was best explained by postulating that the October–December epidemic invaded the northern part of Japan where the May–June type epidemic had prevailed [note: prefectures on the Pacific Ocean Coast were more connected to the prefectures where the October–December seasonality prevailed]; (iii) the May–June seasonality emerged several years later than the October–December seasonality (1979–1980 vs. 1974–1978, respectively) (Fig. 2A); if the same *Ot*-mite symbiotic pair caused the 2 seasonal epidemic types, they should have emerged simultaneously; and (iv) the seasonality was geographically demarcated even within a prefecture like Fukushima, where both types of the seasonality were found (8).

The next question was how the 2 types of TD seasonality emerged. The question was important in the tsutsugamushi epidemiology, but has not been given priority previously. As TD is caused by the engorgement of humans by unfed female mite larvae, the seasonality of TD is equal to the seasonality of occurrence of the unfed female larvae emerging immediately after hatching. In the mite eggs, *Ot* was initially undetectable with electron microscopy, but became abundant only before hatching

(10); i.e., the replication of *Ot* was suppressed during the early embryogenesis of the mite and became active only when the embryogenesis was complete. A question arises as to what suppressed the replication of *Ot* during the early mite embryogenesis in eggs.

The egg stage of the mite symbiotic to *Ot* was estimated to be from 9 weeks to 3 weeks before the TD epidemic because the time for embryogenesis before hatching was 6 weeks (6 weeks for *L. pallidum* and 10 weeks for *L. scutellare* at 25°C) [Table 13 in (1)], the start of engorgement was 2 weeks after hatching (1), and the patient's incubation time was 5–14 days (9). The egg phase derived from the plot in Fig. 4A was from mid-March (week 12) to late April (week 18) for the May–June epidemic in Akita, and from late September (week 40) to early November (week 46) for the October–December epidemic in Kagoshima (Fig. 4B). The estimate, at least for the October–December epidemic, agreed well with the rearing data obtained in natural conditions, which was from August/June to November [Fig. 4 in (1)] (for the May–June epidemic, no rearing data was available).

Using this information, we then examined temperatures experienced by the mite embryos in the egg shells. In the May–June epidemic in Akita, the temperature at the start of embryogenesis was 7°C, and the temperature at the end was 16°C; the initial temperature was 9°

Fig. 4. Estimated environmental temperature during the mite embryogenesis of *Ot*-mite symbiotic pairs. Fig. 4A: May–June epidemic in Akita; Fig. 4B: October–December epidemic in Kagoshima in 2007–2014. The upper panel: average environmental temperature in 2007–2014. The lower panel: total TD cases in 2007–2014 for each week of the year (●); cumulative number of TD cases in 2007–2014 starting from week 17 for Fig. 4A or from week 40 for Fig. 4B (○); and plots running 9 weeks ahead of the plots of the cumulative number of TD cases (dotted lines). The vertical axis: number of incidence; the horizontal axis: week of the year. Inserts like “7 → 15°C” mean the embryogenesis starts at 7°C and ends at 15°C. The thick part of the inserted horizontal bars indicates estimated period of the mite embryogenesis before hatching, and the thin part period after hatching till engorgement. The horizontal axis in the upper panel indicates month and the horizontal axis in the bottom panel indicates weeks of the year. Fig. 4C: Environmental temperature (mean of 1999 and 2000) of Okinawa, Kagoshima, Hokkaido and Akita. The vertical axis shows the temperature (°C) and the horizontal axis month starting from January. □: maximum; ○: mean; △: minimum temperature.

lower than the temperature at the end (Fig. 4A). In the October–December epidemic in Kagoshima, the initial temperature was 24°C, and the temperature at the end was 16°C; the initial temperature was 8°C higher than at the end (Fig. 4B).

As parthenogenesis can be driven by symbiotic *Ot* (3,5), and *Ot* variants with different optimum replication temperatures were known to exist (11), we examined the temperature slope during embryogenesis. The environmental temperature was 7°C at the start of embryogenesis and 16°C at the end for the May–June epidemic, and 24°C at the start and 16°C at the end for the October–December epidemic. The temperature at the start was not permissive for replication of *Ot* since it was lower or higher by 8–9°C than at the end when *Ot* initiated active replication. Although the temperature at hatching was 16°C for both seasonality types, their temperature sensitivities were entirely different: after hatching, *Ot* causing the May–June epidemic had to replicate under higher temperatures (up to 25°C), while *Ot* causing October–December seasonality replicated under lower temperatures (down to 10°C). This hypothesis explains why there were so few TD cases in Okinawa despite environmental temperatures similar to those of Kagoshima, where the temperature slope was far milder than in Kagoshima (Fig. 4C). Monitoring *Ot* genome copies from the start to the end of the egg stage under different temperatures and temperature slopes for *Ot*–mite pairs causing the 2 different seasonalities will clarify whether the temperature slope actually controlled *Ot* replication.

In Hokkaido, although the temperature slope was sufficiently steep (Fig. 4C), TD was nearly absent; however, rodents carrying rickettsia-positive mites were detected at low frequencies almost all year-round [see Fig. 31 in (2)]. In this connection, it was important to note that there were small peaks of TD that scattered throughout the year in Japan (Fig. 3). These observations suggested the presence of *Ot*–mite symbiotic pairs that were reproduced differently from those causing the seasonal TD epidemics.

As the *Ot*-positive rate of larval mites in the field in Japan was less than 1% (1), the seasonal TD outbreaks could be due to a large number of unfed female larvae produced through parthenogenesis driven by symbiotic *Ot* (5–6). As arthropods including mites can reproduce both sexually and parthenogenetically, the year-round small peaks could be produced by few female larvae that survived killing by symbiotic *Ot* or produced through sexual reproduction. Comparing the numbers of unfed female larvae between the high season and off-season, and that between sexual reproduction and parthenogenesis, will shed light on these issues.

The last question was about the origin of the TD epidemic of May–June seasonality. We first estimated the environmental temperature during the embryogenesis of *Ot* responsible for classical TD (Fig. 5A). It was 17°C (mid-June) at the start and 23°C (late July) at the end. The estimates fit well to the observed seasonality for the second generation of *L. akamushi* collected in Akita and reared in Saitama under natural environments, which were the egg stage in May–July and unfed larvae

Fig. 5. Environmental temperature during the mite embryogenesis estimated for the “classical” TD in Akita (Fig. 5A). Fig. 5A: See explanations Figs. 4A and 4B. Fig. 5B: Maximum, mean and minimum temperature in May, June and July in Akita from 1955 to 1970 (mean).

in June–August [Fig. 2 in (1)]. It was on the ascending slope similar to the TD epidemic of May–June seasonality. The temperature difference between the start and the end of classical TD, which was 5°C, was however almost within the temperature fluctuation range (Fig. 5B). Under such situations, *Ot* may have the chance to replicate from the start of embryogenesis while killing the host mite. Repeated such events will select for *Ot*–mite symbiotic pairs, which initiate embryogenesis in the steeper ascending limb of the environmental temperature. Such *Ot*–mite symbiotic pairs can be produced through the transmission of *Ot* from infected mammals to other mites (1) whose embryogenesis starts earlier under colder environment. The near vacuum of TD cases from the extinction of classical TD until the appearance of TD with May–June seasonality (Figs. 2A and 2B) could be attributed to the incubation time for emergence of such an *Ot*–mite symbiotic pair through micro-evolution. Comparative genome analysis of *Ot* that caused classical TD and *Ot* that are causing the current May–June seasonality will shed light on this matter.

Finally, in order to know possible genomic diversity of *Ot* in Japan and to obtain a clearer picture of tsutsugamushi epidemics in Japan, it is imperative to have information on the genome sequence of *Ot* variants present in Japan.

Conflict of interest None to declare.

REFERENCES

1. Kawamura A Jr, Tanaka H, Tamura A, editors. Tsutsugamushi Disease in Japan. Tokyo: University of Tokyo Press; 1995.
2. Tamiya T, editor. Recent Advances in Studies of Tsutsugamushi Disease in Japan. Tokyo: Medical Culture Inc. 1962.
3. Takahashi M, Urakami H, Yoshida Y, et al. Occurrence of high ratio of males after introduction of minocycline in a colony of *Leptotrombidium fletcheri* infected with orientia tsutsugamushi. Eur J Epidemiol 1997;13:79-86.
4. Perlman SJ, Hunter MS, Zchori-Fein E. The emerging diversity of *Rickettsia*. Proc Biol Sci. 2006;273:2097-106.
5. O'Neill SL, Hoffmann AA, Werren JH. editors. Influential Passengers: Inherited Microorganisms and Arthropod Reproduction. Oxford: Oxford University Press; 1997.
6. Beukeboom LW, Perrin N, editors. The Evolution of Sex Determination. Oxford University Press; 2014.
7. Yoshikura H. Geographical distribution of Japanese spotted fever and tsutsugamushi disease in Japan – possible effect of environmental temperature. Jpn J Infect Dis. 2017;70:349-51.
8. Yaginuma S, Yuki T, Abe T, et al. [Epidemics of Tsutsugamushi in Fukushima Prefecture. Annual Report.] Fukushima Prefectural Institute of Public Health. 2010;28: 39-42. Japanese.
9. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare. Tsutsugamushi disease and Japanese spotted fever in Japan, as of December 2005. Infect Agents Surveillance Rep. 2006;27:27-9. Japanese.
10. Urakami H, Takahashi M, Tamura A, et al. Electron microscopic observations of the embryo *Leptotrombidium* (*Leptotrombidium*) *pallidum* naturally infected with *Rickettsia tsutsugamushi*. Microbiol Immunol. 1988;32:967-72.
11. Tamura A, Makisaka Y, Enatsu T, et al. Isolation of *Orientia tsutsugamushi* from patients in Shikoku and finding of a strain which grows preferentially at low temperatures. Microbiol Immunol. 1999;43:979-81.