Human dermatitis caused by the natural infestation of larval trombiculid mites *Leptotrombidium akamushi* (Brumpt, 1910) (Acar: Trombiculidae) at the hot spot of Tsutsugamushi disease in Akita Prefecture, Japan

Mamoru Takahashi*, 1, 4), Teruki Kadosaka2), Yumi Takahashi3), Hitoko Misumi1, 4), Hiroko Sato5), Chihiro Shibata5), Shihoko Saito5), Hiromi Fujita6), Nobuhiro Takada7) and Nobuyuki Matsumoto1)

*Corresponding author: Department of Anesthesiology, Saitama Medical University, Moroyama-machi, Iruma-gun, Saitama 350–0495 Japan (E-mail: m.takahashi@wish.ocn.ne.jp)

1) Department of Anesthesiology, Saitama Medical University, Moroyama-machi, Iruma-gun, Saitama 350–0495, Japan

2) Department of Infection and Immunology, Aichi Medical University School of Medicine, Nagakute-shi, Aichi 480–1195, Japan

3) Faculty of Medicine, Saitama Medical University, Moroyama-machi, Iruma-gun, Saitama 350–0495, Japan

4) Matsuyama High School, 1–6–10 Matsuyama-cho, Higashimatsuyama-shi, Saitama 355–0018, Japan

5) Akita Prefectural Research Center for Public Health and Environment, 6–6 Sensyukubota-machi, Akita 010–0874, Japan

6) Mahara Institute of Medical Acarology, 56–3 Korekuni, Aratana, Anan, Tokushima 779–1510, Japan

7) Faculty of Medical Sciences, University of Fukui, 23–3 Shimoaizuki, Matsuoka, Eiheiji-cho, Yoshida-gun, Fukui 910–1193, Japan

(Received: 7 November 2012; Accepted: 8 February 2013)

**Abstract.** We observed the prognosis of clinical symptoms and histopathological alternations of human dermatitis in a 61-year-old man, who had been bitten by the unfed larval trombiculid mites *Leptotrombidium akamushi* (Brumpt, 1910), during a field survey of trombiculid mites at the hot spot of Tsutsugamushi disease on the river side of the Omonogawa River, Omagari, Akita prefecture. The victim first noticed bites by the *L. akamushi* larvae on 12 sites comprised thin or tender areas in the neck, elbow folds, armpits, and around the nipples approximately 12 hours’ post-infestation. The average mite-feeding period was 59.3 hours. The pain persisted for 24–96 hours after each larva detached. The small erythematous macules lasted for several days to 1 week and then subsided gradually, leaving residual pigmentation for approximately 1–2 weeks. Histopathological examinations of the victim’s skin at 30, 40, and 54 hours’ post-infestation revealed that the larvae had formed mesenchymal stylostomes that penetrated through the epidermis into the dermis. Inflammatory cells such as histiocytes, lymphocytes, and neutrophils were observed around the stylostomes, but few eosinophilic leukocytes were seen. This report differs from those of animal experiments because it clarifies the natural infestation time of *L. akamushi* larvae on the human body.

**Key words:** *Leptotrombidium akamushi*, trombiculid mite, stylostome, dermatitis, infestation, histopathology

**INTRODUCTION**

In August 2008, a 17-year-old woman was diagnosed with classical Tsutsugamushi disease that was suspected to have been caused by the bite of a *Leptotrombidium akamushi* mite harboring Kato-type rickettsiae on the banks of the Omonogawa River (Sato et al., 2010). We conducted an investigation to confirm whether *Orientia tsutsugamushi*-infected unfed larvae of *L. akamushi* inhabited the site (hot spot) where the patient was thought to have been bitten by the infected larvae. During this investigation, one of the authors (the victim) was bitten on many sites of his body by unfed *L. akamushi* larvae. Only a few cases of stylostome formation by *L. akamushi* have been reported in the literature (Obata and Aoki, 1958).

Herein, we describe the pain changes, stylostome formation, and histopathological changes of each bite site on the human body in detail. This study is clearly different from an animal experiment since it clarifies the natural time lapse of human infestation with *L. akamushi* larvae.

**MATERIALS AND METHODS**

**Observation of the Skin Reactions to *L. akamushi* Infestation**

We collected unfed *L. akamushi* larvae in the hot spot at the bank of the Omonogawa River, Omagari, Daisen-shi, Akita Prefecture, Japan, between 17 : 40 and 18 : 30 on August 4, 2011 using a black cloth. The victim (61-year-old man) first noticed many bites from the unfed *L. akamushi* larvae at approximately 07 : 00 on August
5, 2011. The victim’s skin reactions to the bites and larval trombiculid mites were observed using a magnifier (HANSA DX LUPE $4 \times$) daily at 08:00 and 20:00 by one of the authors until August 9, 2011.

**Histopathological Observations of the Skin Tissue**

To examine the skin lesions and stylostome formation on the human skin resulting from the different feeding periods, each small piece of skin tissue bitten by the *L. akamushi* larvae was surgically removed at approximately 30, 40, and 54 hours post-infestation. Each skin specimen was treated using the same method as described in our earlier report (Misumi et al., 2003). Namely, the skin samples were fixed in Carnoy’s solution for 30 min and then placed in 70% ethyl alcohol. The tissues were embedded in Technobit 7100 (Kulzer Histo-Technik), serially sectioned into 5-μm slices, and stained with hematoxylin-eosin for microscopic observation.

**Results**

**Bite Sites on the Victim’s Body**

After the survey, one of the authors (the victim) noticed 12 bite sites on his body caused by *L. akamushi* larvae at approximately 12 hours post-infestation because of the characteristic skin reactions to the bites. He did not exhibit any subjective symptoms until that time. Each of the 12 sites was thin or tender, and all were located in the neck, elbow folds, armpits, or around the nipples (Fig. 1).

**Feeding Period of Larvae on the Victim**

At 38 hours post-infestation, the larvae were still present on 11 of the body sites. By 50 hours post-infestation, 4 larvae (at site Nos. 1, 5, 8, and 9) had detached from the skin. By 62 hours post-infestation, 4 more larvae (at site Nos. 4, 6, 7, and 11) had detached from the skin. The average feeding period on human skin was 59.3 hours, while the longest period exceeded 74 hours (at site No. 3). This larva was manually detached between 74 and 86 hours’ post-infestation (Table 1). The fully engorged larvae were approximately 360 μm long and 230 μm wide.

**Symptoms Caused by the Larval Bite**

Using a magnifier, a biting larva could be observed as a minute red point in the center of each pale anemic macule (Fig. 2B). In each bite site, only a small erythematous macule (approximately 3–5 mm in diameter) (Fig. 2A) was observed surrounded by an erythematous halo. All macules were similar in size. The victim felt severe pain at each of the bite sites, as though a tiny thorn had penetrated and remained within the skin, and each site was especially painful when it was rubbed with clothing or by hand, but the sites were not itchy. The pain persisted after the larvae detached from the skin. In fact, the pain at 2 sites (Nos. 4 and 5) persisted for 24 and 36 hours, respectively, after detachment. Conservative estimates indicated that the longest period of pain was 96 hours after the larva detached from site No. 9. The small erythematous macule lasted for several days to 1 week and then subsided gradually, leaving residual pigmentation for approximately 1 to 2 weeks.

**Stylostome Formation on the Victim by the *L. akamushi* Larvae**

Each stylostome formed by an *L. akamushi* larva was a cone-shaped structure. The size at 30 hours post-infestation was as follows: length, 100.5 μm; proximal width, 47.3 μm; middle width, 37.8 μm; and distal width, 35.5 μm (Fig. 3B). The size at 54 hours post-infestation was slightly extended as follows: length, 105.8 μm; proximal width, 40.2 μm; middle width, 38.5 μm; and distal width, 35.8 μm (Fig. 3D). The stylostome clearly penetrated through the epidermis into the dermis. Therefore, the stylostome was identified as mesenchymal stylostome according to the classification of Hase et al. (1978). Each stylostome consisted of a food cavity, central canal, central canal wall, inner layer, central layer, outer layer, and epidermal coat (Figs. 3C and 3D) (Misumi et al., 2003). The substance that constituted the inner and outer layers stained well with eosin. In particular, the wall of the central canal was very thin and appeared to stain well with hematoxylin (Fig. 3C). The wall was thought to comprise of trombiculid mite secretions. However, the central layer was a mass of uniform thickness that was chromophobic to hematoxylin and eosin, and appeared to be a homogeneous non-staining material. Since it differed from the eosinophilic hyaline substance seen within the mammalian tissue, it may have also been secreted by the trombiculid mite. The slightly wider tip of the stylostome (food cavity) opened into the upper part of the dermis of the host tissue (Figs. 3B and 3D). Microscopic observations indicated that the stylostome resulted from an alternating secretion and sucking process.

![Fig. 1. Natural human infestation by *Leptotrombidium akamushi*. Nos. 1–12 show the body sites bitten by unfed *L. akamushi* larvae.](image-url)
Table 1. The progression of symptoms at the body sites caused by the natural human infestation of *Leptotrombidium akamushi*.

<table>
<thead>
<tr>
<th>Site no.</th>
<th>Day noticed</th>
<th>Symptom</th>
<th>Skin bite date</th>
<th>Bitten larvae observed or not (symptom)(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aug. 5 (20 : 00)</td>
<td>Aug. 6 (08 : 00)</td>
</tr>
<tr>
<td>Feeding period</td>
<td>13</td>
<td>26</td>
<td>38</td>
<td>50</td>
</tr>
<tr>
<td>1</td>
<td>Aug. 5 (07 : 00)</td>
<td>pain</td>
<td>Yes (pain)</td>
<td>Yes (pain)</td>
</tr>
<tr>
<td>2</td>
<td>Aug. 5 (07 : 00)</td>
<td>pain</td>
<td>Yes (pain)</td>
<td>Yes (pain)</td>
</tr>
<tr>
<td>3</td>
<td>Aug. 5 (07 : 00)</td>
<td>pain</td>
<td>Yes (pain)</td>
<td>Yes (pain)</td>
</tr>
<tr>
<td>4</td>
<td>Aug. 5 (07 : 00)</td>
<td>pain</td>
<td>Yes (pain)</td>
<td>Yes (pain)</td>
</tr>
<tr>
<td>5</td>
<td>Aug. 5 (07 : 00)</td>
<td>pain</td>
<td>Yes (pain)</td>
<td>Yes (pain)</td>
</tr>
<tr>
<td>6</td>
<td>Aug. 5 (07 : 00)</td>
<td>pain</td>
<td>Yes (pain)</td>
<td>Yes (pain)</td>
</tr>
<tr>
<td>7</td>
<td>Aug. 5 (07 : 00)</td>
<td>pain</td>
<td>Yes (pain)</td>
<td>Yes (pain)</td>
</tr>
<tr>
<td>8</td>
<td>Aug. 5 (07 : 00)</td>
<td>pain</td>
<td>Yes (pain)</td>
<td>Yes (pain)</td>
</tr>
<tr>
<td>9</td>
<td>Aug. 5 (07 : 00)</td>
<td>pain</td>
<td>Yes (pain)</td>
<td>Yes (pain)</td>
</tr>
<tr>
<td>10</td>
<td>Aug. 5 (07 : 00)</td>
<td>pain</td>
<td>Yes (pain)</td>
<td>Yes (pain)</td>
</tr>
<tr>
<td>11</td>
<td>Aug. 5 (07 : 00)</td>
<td>pain</td>
<td>Yes (pain)</td>
<td>Yes (pain)</td>
</tr>
<tr>
<td>12</td>
<td>Aug. 5 (07 : 00)</td>
<td>pain</td>
<td>Yes (pain)</td>
<td>Yes (pain)</td>
</tr>
</tbody>
</table>

\(^1\) Yes, biting larva observed; No, biting larva detached
Fig. 3. Stylomate formation by natural *Leptotrombidium akamushi* infestation in human skin seen after hematoxylin and eosin staining. 

A, *Leptotrombidium akamushi* larva 54 hours after natural infestation of human skin. Note the distal portion of the stylomate within the dermis. Many inflammatory cells aggregated near the stylomate. B, Stylomate formation 30 hours after natural infestation of human skin showing complete penetration of the epidermis and half the length of the stylomate invading the papillary layer of the dermis. Disintegrated epidermal cells surround the stylomate (100.5 µm long). C, Stylomate formation 40 hours after the natural infestation of human skin. The distal portion of the stylomate is seen in the dermis where many blood vessels and nerve endings are distributed. Collagenous bundles surrounding the top of the stylomate are believed to have been digested by the larva’s salivary enzymes. D, Stylomate formation 54 hours after the natural infestation of human skin. Degenerated epidermal cells surrounded the stylomate. Inflammatory cells, mainly histiocytes with twisted nuclei, lymphocytes, and neutrophils are aggregated to the stylomate (105.8 µm long).

Abbreviations: ca, central canal; ce, central layer; ec, epidermal coat; de, dermis; di, disintegrated cell; ep, epidermis; fc, food cavity; i, inner layer; La, *Leptotrombidium akamushi* larva; o, outer layer; s, stylomate; w, wall of central canal.
Histopathological Findings in the Skin Caused by the Larval Bite

The 30-hour sections of site No. 12 (Fig. 1) to which an *L. akamushi* larva had attached showed a stylostome that clearly penetrated the epidermis (Fig. 3B). Although the stratum corneum of normal skin is a rough structure, a narrow area became dense at the *L. akamushi* larval bite site. A homogeneous eosinophilic cell- and nuclear-free zone (central canal) extended from the mouthparts of the larva to the inner side of the host epidermis. The stylostome is this tubular structure. One half of the stylostome was located within the papillary layer of the dermis with dense aggregation of adjacent inflammatory cells such as histiocytes with twisted nuclei, lymphocytes, and neutrophils after destruction of the epidermal cells by enzymes injected by the larva. In addition, the collagenous fibers around the stylostome had degenerated and become heavily eosinophilic due to the salivary secretions of the trombiculid mites.

The 40-hour sections of site No. 2 (Fig. 1) showed that the stylostome had bored through the epidermis of the host tissue (Fig. 3C). In addition, heavy infiltration of inflammatory cells, including many degenerated or destroyed cells of the stratum granulosum and stratum spinosum were observed adjacent to the stylostome.

The 54-hour sections of site No. 10 (Fig. 1) showed further extension of the stylostome deep within the dermis (Figs. 3A, D). Approximately two-thirds of the stylostome tip penetrated the epidermis into the dermis. The stylostome was covered with a thick layer of degenerated epidermal cells and formed a hyperplastic epidermis. In particular, a dense aggregation of inflammatory cells, mainly histiocytes, lymphocytes, and neutrophils (eosinophilic leukocytes were rare), was identified in the papillary layer of the dermis and penetrated the upper part of the reticular layer of the dermis. Overall, the histopathological changes in human skin caused by the *L. akamushi* larvae bites were restricted to a narrow area adjacent to each stylostome.

**Discussion**

Unfed larval trombiculid mites usually feed on the surface of host epidermis. They do not suck blood; rather, they insert their feeding structures (chelicerae) into the host skin and secrete skin-digesting saliva that contains enzymes that destroy the host tissue. The mite larvae then feed upon the destroyed tissue or the liquefied epidermal cells as their primary diet. Hardening of the surrounding skin results in the formation of a feeding tube (stylostome) over the course of a few days in the host’s tissue (Jones, 1950; Hase et al., 1978; Kaneko and Kadosaka, 1994; Shatrov, 2000). Misumi et al. (2003) showed a morphological illustration of the stylostome based on many histological sections of stylostomes formed by *Leptotrombidium* larvae.

The moment of actual invasion is usually unnoticed, and bite sites appear to consist of moist areas and thinner skin where clothing is restricted, such as the ears, genitalia, axillae, mammmae, groin, and cubital and popliteal fossae (Gordon and Lavoipierre, 1962; Uchikawa and Otaki, 1999). When the author collected unfed larvae from the soil surface, the unfed larvae may have first become parasitic on his arms and then spread to his upper body. His lower body was protected by clothing and a belt.

The average feeding period of trombiculid mite larvae on human skin differs among trombiculid mite species and individual victims, 20–55 hours in *Leptotrombidium scutellare* (Takahashi et al., 2000; Misumi et al., 2003), 69 hours in *L. intermedium* (Misumi et al., 2000), 49–75 hours in *L. akamushi* (Obata and Aoki, 1958; Takahashi et al., 1991), and 53–76 hours in *L. pallidum* (Misumi et al., 2003). In the present case, *L. akamushi* fed for approximately 59.3 hours and became fully engorged. This finding is similar to that of an earlier report and almost the same as that in an experiment using mice (Takahashi et al., 1991).

The natural human infestation of *O. tsutsugamushi*-uninfected *L. akamushi* larvae in the present study did not cause any symptoms until approximately 12 hours’ post-infestation. The victim felt pain but no itching. Inhabitants of the endemic areas within Niigata, Akita, and Yamagata Prefectures in Japan call this sensation “ira” in the local dialect, which has been confirmed in experimental human infestations, although a few victims also felt itching (Obata and Aoki, 1958; Ito and Obata, 1961; Takahashi et al., 1991).

The characteristic symptoms such as a severe painful reaction caused by *L. akamushi* bites is considered to be because of specific irritants secreted from the saliva that stimulate nerve fibers in the dermis and epidermis where many different kinds of nerve endings are broadly distributed.

The clinical pictures generally differ among trombiculid species, including severe itching by *Eutrombicula wichmanni* (Takahashi et al., 2009), pain by *L. palpale* (Kadosaka, personal communication), mild or hardly felt by *L. pallidum*, *Shoengastia hanmyaensis*, *L. scutellare*, and *L. intermedium* (Suzuki, 1976; Misumi et al., 2003; Arai, 1955; Sasa, 1956; Toriyama et al., 1988). These differences may depend on the irritation quality of the saliva of the different chigger species.

The victim in the present study had the anamnesis of being bitten by various trombiculid mites, such as *L. palpidum*, *L. scutellare*, *L. intermedium*, and *L. akamushi*. As such, his clinical symptoms were carefully observed whether there would be an immunological cross-reaction to the various salivary substances secreted by different species of larvae. The small erythematous macule with halo at each bitten site is considered to be the result of immunological cross reaction. However, it needs further immunological examination in detail.

Regardless of the trombiculid mite species, extremely severe clinical pictures such as blister formation, severe itching, and severe pain caused by the bites of *L. scutel-
lare, L. intermedium, and L. pallidum were observed (Misumi et al., 2000, 2003). These severe reactions are considered to be the result of hypersensitivity to the antigenic substances secreted by larval trombiculid mites.

It is generally agreed that the initial penetration of the epidermis by unfed larval trombiculid mites is usually by the same process in the host tissues regardless of mite species (Schmacher and Hoeppli, 1963; Voigt, 1970; Hase et al., 1978; Kaneko and Kadosaka, 1994). Once the mites insert their chelicerae into the epidermis, they inject a complex salivary secretion to penetrate the epidermis and form the stylostome (Schmacher and Hoeppli, 1963; Hase et al., 1978). Thus, the stylostome is formed by the interaction between the larval secretions and the host tissue.

The histopathological changes in the human skin sections taken at 30 hours post-infestation of L. akamushi larvae were almost the same as those at 40 and 54 hours post-infestation. The most peripheral layers of the stratum corneum in normal human skin, called stratum disjunctum, this stratum disjunctum was not observed at the sites larvae were almost the same as those at 40 and 54 hours post-infestation taken at 30 hours post-infestation.

In emerging and reemerging infectious diseases (H21-32 Med. Entomol. Zool.), the histopathological changes in the human skin sections taken at 30 hours post-infestation of L. scutellare and L. pallidum (Misumi et al., 2003). Inflammatory cells such as histiocytes, lymphocytes, and neutrophils were identified near the stylostome, whereas macrophage types such as resident macrophages, moving macrophages, and Langerhans cells were not identified. However, it is thought that these cells began to function as scavenger cells to phagocyte the necrotic epidermal cells.

Additionally, with regard to the recovery process of the host skin damaged by the stylostome formation, Kadosaka (1996) reported on the expulsion of the stylostome formed in the mouse ear by L. pallidum larvae. After an engorged larva dropped off from the mouse skin, the remaining stylostome was surrounded by neutrophils and macrophages, and then pushed up by new epidermal cells. The recovery process of human skin damaged by stylostome formation may be the same as that of mouse skin. Further research using immunostaining techniques is required to examine the recovery process in detail.

Acknowledgements

We wish to express our sincere thanks to Prof. E. Arai, Division of Pathology, Saitama Medical University, Saitama Medical Center, Kawagoe, Japan, for kindly providing valuable comments.

This study was supported by a grant for Research on Emerging and Reemerging Infectious Diseases (H21-Shinkou-Ippan-06) from the Ministry of Health, Labour and Welfare, Japan.

References


