[SHORT COMMUNICATION]

Human Infestation by *Amblyomma testudinarium* (Acari: Ixodidae) in Malay Peninsula, Malaysia

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(Received 9 July 2012; Accepted 30 July 2012)

ABSTRACT

A Japanese male repeatedly infested with *Amblyomma testudinarium* in Malaysia was reported. He visited to Ulu Gombak, Malay Peninsula, Malaysia on April and May 2007, and he recalled three times of tick bite during traveling. The first tick bite was by one nymph infested on the inner side of the brachium of the patient. After a few days, erythema with a diameter of 2 cm was found at the site of tick attachment. Pain of the site remained for 20 days. The second tick bite was by larvae infested on the skin surface of the abdomen, basal portion of the thigh, and scrotum of the patient. He felt a pain at the moment of tick infestation. The pain remained for 15 days. The third tick bite was by a larva, and the tick was found in the phyma of his back immediately after his return Japan.

Key words: hard tick, *Amblyomma testudinarium*, tick bite, molecular identification, imported case, Malaysia

Ticks transmit a greater variety of pathogenic microorganisms than any other arthropod vector groups, and are one of the most important vectors carrying diseases to humans, livestock, and companion animals (Parola and Raoult, 2001; Jongejan and Uilenberg, 2004). Although about 104 species of ticks are found in south-eastern Asia, little is known about the epidemiology of tick-borne disease (Petney *et al.*, 2007). Very little is known about human infestation with hard ticks in the area. The comprehensive survey on the tick fauna and tick-causing problems is required in this area.

We report herein a case of human infestation by ticks in the University of Malaya Ulu Gombak Studies Centre (3°19’N 101°45’E, 250 m a.s.l.), Selangor, Malay Peninsula, Malaysia.
CASE REPORT

The patient was a 32-year-old Japanese male, resident of Tokyo, Japan. He visited Ulu Gombak to survey insects, especially ants, in the field from April 20 to May 7, 2007.

On April 21, the patient noticed a tick attached to the inner side of his brachium (Fig. 1) just after walking about the grassland. He twisted the tick slowly and removed in himself. The detached tick was preserved in 70% ethanol. After a few days, erythema with a diameter of 2 cm was found at the site of tick infestation. Although pain of the site remained for 20 days, he did not go to a hospital and no medical treatment for the tick infestation was performed.

On May 5, he noticed ticks attached to the abdomen (four individuals), basal portion of the thigh (four), and scrotum (three) (Fig. 2). At the time, he was sitting on the ground of the field to observe ants. He felt a severe pain at the moment of tick infestation. He removed ticks in himself, and preserved them in 70% ethanol. Rubors with a maximum diameter of 1.5 cm and faint tumidities were found at the sites of tick infestations. He felt a pain such as the burn at the sites when he touched. The pain remained for 15 days.

On May 6, the patient found a phyma with a diameter of 5 mm in his back. On the following morning, he took off Kuala Lumpur International Airport, Malaysia, and arrived at Narita Airport, Tokyo in the evening. Just after arrival, he went to a Japanese style pub in Tokyo. When he touched the phyma in intervals drinking, an engorged tick was removed from the phyma.

Fig. 1. Nymphal Amblyomma testudinarium attached to the inner side of brachium.
Although blood flowed from the phyma, he did not feel a pain. The phyma disappeared a few days later.

**TICK IDENTIFICATION**

*Morphological observation*

The ticks were examined under binocular and light microscopes. On the basis of Voltzit and Keirans (2002) and Fujita and Takada (2007), the tick collected on April 21 and others were identified as a nymph and larvae of *Amblyomma testudinarium* Koch by their morphological characteristics, respectively.

*Molecular phylogenetic analysis*

Sequence data were analyzed using MEGA4 software (http://www.megasoftware.net) (Tamura et al., 2007). After alignment using the CLUSTAL-W (ver. 1.6) software package, the neighbor-joining (NJ) phylogenetic tree construction and bootstrap tests were carried out according to the Kimura 2-parameter distances method (Kimura, 1980; Saitou and Nei, 1987). Pairwise alignments were performed with an open-gap penalty of 15, and a gap extension penalty of 6.66. Multiple alignments were also performed using the same values. All positions containing alignment gaps and missing data were eliminated in pairwise sequence comparisons (pairwise deletion). By phylogenetic analysis, these ticks were related with *A. testudinarium* in Japan (Fig. 3).

![Larval Amblyomma testudinarium attached to the scrotum.](image-url)
Fig. 3. Neighbor-joining tree based on the mitochondrial 16S ribosomal RNA gene sequence for *Amblyomma* ticks. The evolutionary distances were computed using the Maximum Composite Likelihood method. Bootstrap values are indicated for nodes gaining more than 70% support (1000 replications). All positions containing alignment gaps and missing data were eliminated in pairwise sequence comparisons (Pairwise deletion option). There were a total of 477 positions in the final dataset.
DISCUSSION

Amblyomma ticks mainly inhabit tropical or subtropical areas. In Asia there are 14 species, and only three species in the Palearctic (Voltzit and Keirans, 2002). Very little information is available on the diseases relationships of members of Amblyomma in south-eastern Asia although in other areas of the world some species of the genus are important vectors of disease to man (Petney and Keirans, 1995; Estrada-Peña and Jongejan, 1999).

*A. testudinarium*, which sometimes carries *Rickettsia tamurae* (Imaoka *et al.*, 2011), is the most common *Amblyomma* in south-eastern Asia (Petney and Keirans, 1995; Voltzit and Keirans, 2002). The species, originally described from Java (Koch, 1844), ranges throughout the tropical wooded regions of Asia from Sri Lanka and India, through Myanmar, Thailand, Laos, Vietnam, Malaysia, Indonesia, Borneo, Sarawak, the Philippines, Taiwan, mainland China and Japan (Voltzit and Keirans, 2002). In the present study, all ticks were identified as larvae or a nymph of *A. testudinarium* based on their morphological characteristics. Moreover, the phylogenetic analysis of 16S ribosomal RNA gene sequence indicated a close relationship between the ticks and Japanese *A. testudinarium* reported by Yamauchi *et al.* (2010). On the other hand, it seems that the significant genetic differentiation is present in the widely distributed species because of the distance value between the ticks collected in Ulu Gombak and Japan.

Patients may experience the itch, mild pain, or tenderness at the sites of tick infestations (Uchikawa and Ohtaki, 1999). Domrow and Nadchatram (1963) stated that the larval *A. testudinarium* readily attached to man and the bite caused severe irritation for weeks in Gunong Jerai, Malay Peninsula, Malaysia. This resembles the present case. On the other hand, the severe pain at the moment of infestation have never been reported in immature *A. testudinarium* in Japan. The patient of the present study was often infested by unidentified hard ticks in Japan, but had not felt such a pain so far. *A. testudinarium* is a common species in Ulu Gombak and well known to original inhabitants as “painful tick”. The severe pain may be a characteristic of *A. testudinarium* population in Malay Peninsula.

ACKNOWLEDGMENTS

We wish to express our gratitude to Dr. Toshio Kishimoto (Japan Wildlife Research Center) for providing a bottle containing 70% ethanol when one of us (M. M.) collected the larval tick in Tokyo, Japan. This study was supported in part by Grant-in-Aids for Scientific Research on Emerging and Re-emerging Infectious Diseases from the Japanese Ministry of Health, Labour and Welfare (H21-shinkou-ippan-005, -006).

REFERENCES


