



## A FIRST REPORT OF RICKETTSIA JAPONICA DETECTED FROM AMBLYOMMA TESTUDINARIUM

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<p><b>Article Info</b> Received 15/08/2015 Revised 27/08/2015 Accepted 30/09/2015</p> <p><b>Key words:</b> Japanese spotted fever; <i>Rickettsia japonica</i>; <i>Amblyomma testudinarium</i>.</p>	<p><b>ABSTRACT</b> A 61 years old man presented as many as 31 red spots on his lower abdomen, back, and buttock. Seven ticks were found on the red papules. The ticks were the nymphs of <i>Amblyomma testudinarium</i>. Both of <i>Amblyomma testudinarium</i> and human DNA fragment were identified in the 7 ticks attached to the patient without presence of the clinical sign for rickettsia infection.</p>
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### INTRODUCTION

Japanese spotted fever is a systemic inflammatory disease by *Rickettsia japonica* infection transmitted by tick bite in the endemic areas. Here, we report the first case of *Rickettsia japonica* detected from *Amblyomma testudinarium*, suggesting *Amblyomma testudinarium* might be the potent carrier of *Rickettsia japonica*. We also discuss the efficiency of transmission of *Rickettsia japonica* by a carrier-tick bite.

### CASE

A 61 years old man consulted the Mie Prefectural Shima Hospital complaining itching small nodules on his groin. He worked as a forest manager during the afternoon of the day before consultation in a forest in Shima-city area, where is an endemic area of Japanese spotted fever (JSF). At the first visit, he presented as many as 31 red spots on his lower abdomen, back, and buttock (Figure 1). 7 ticks were found on the red papules. The ticks were uniformly-sized with 6 legs, and were suspected as the larvae of *Amblyomma testudinarium* (*A.testudinarium*) from a morphological perspective. He was afebrile and

showed no systemic skin lesions including diffuse erythema except for lower abdomen. All the ticks were removed using tweezers. Clinical laboratory investigation revealed minimum elevation of the liver enzymes: aspartate aminotransferase (AST) 49 IU/l and alanine aminotransferase (ALT) 39IU/l without elevation of inflammatory markers including C-reactive protein (CRP). He was treated with topical corticosteroid for the erythematous lesions. No clinical symptoms for JSF developed and the laboratory examination data was normalized during seven days following-up.

Using DNA extracted from the collected seven ticks by using QIAamp DNA mini kit (QIAGEN, Germantown, MD, USA) according to manufacture's instructions, the tick specific DNA sequence was amplified by a conventional polymerase chain reaction (PCR) with the tick species-specific primer sets [1]. The direct sequences of the amplified bands from the 7 tick samples were identical to that of *A. testudinarium*. Simultaneously, conventional PCR was performed using primers targeting the 17-kilo Dalton (17K) genus-common antigen gene of



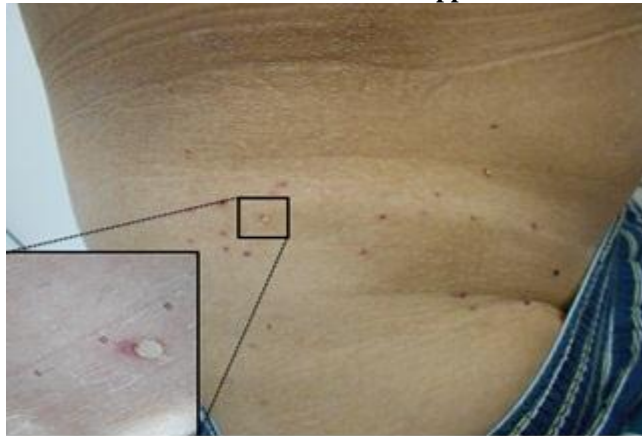
*R. japonica*, which has been commonly used for the screening of *R. japonica* infection in Japan [2]. The Rick PCR system with sensitive diagnosis of *Rickettsia* infection was also performed using a new primer pairs we originally designed for the inner targeting amplified sequence of the nested PCR primers [3]. And outer membrane of protein A (OmpA) gene of *R.japonica* was amplified with the specific primer sets we have designed recently. The sequence data of the rickettsia PCR confirmed one sample as that of *R. japonica*, and other samples were suspected as

*R. japonica* (Table 1). We also searched the human mitochondrial DNA fragment in the extracted DNA from the ticks by real time PCR using Tag man method. Human mitochondrial DNA was identified in all of the samples, which confirmed sucking of the patient's blood by all seven ticks. From the current result, all ticks were identified as the larva of *A. testudinarium* having *R. japonica*. In this study, serological studies and PCR methods were not performed using DNA extracted from the patient's blood or skin lesions for the diagnosis of JSF.

**Table 1. Observations**

Tick number	1	2	3	4	5	6	7
Rj5.10 primer set	-	-	-	-	-	-	+
Ricko1.2 primer set	+	+	+	+	+	+	+
RioA1.2 primer set	-	+	-	-	-	-	-

**Figure 1. Multiple inflammatory erythematous spot attaching ticks were observed. General symptom or annular erythema in case of spotted fever due to rickettsia infection was not appeared on the body.**



Rj5 (5'-CGCCATTCTACGTTACTACC-3')

Rj10 (5'-ATTCTAAAAACCATATACTG-3')

This primer sets are used for screening of Rickettsia species, targeting 17K genus-common antigen gene of *R. japonica*. Amplified product is 357bp.

Ricko1 (5'-AGTAATGCACCTACACCTACTC-3')

Ricko2 (5'-CGGGCGGTATGAATAAACAAG-3')

We originally designed for the inner targeting amplified sequence of PCR primers (Rj5, Rj10). This primer pairs were designed for detecting the common sequence of the various infectious rickettsia reported in Japan including the *R. japonica*, *R. tamurae* and *R. heilongjiangensis*. Amplified product is 121bp.

RioA1 (5'-GGAATCAGATAACGGCTAGAGG-3')

RioA2 (5'-CTACGGGATTAGTATTCGCAAC-3')

These are our original primer sets targeted for OmpA. OmpA is the adherence factor and the sequence of OmpA is rich in diversity. Amplified product is 156bp. The primer sets were designed for the identity of *R. japonica* specific gene.

All samples shared complete homology with *R. japonica* by using Ricko primer set.

No7 tick shared complete homology with *R. japonica* by using Rj primer set.

No2 tick was 94% gene homology with *R. japonica* by using RioA primer set.

These ticks might be thought as children of one mother because all of them were *A. testudinarium* in the same stage of larva in a small region. Therefore, they would receive a *R. japonica* from same mother carrying it.



## DISUSSION AND CONCLUSION

This is the first case report of *R. japonica* detected from *A. testudinarium*, suggesting *A. testudinarium* is one of the carriers of *R. japonica*. *A. testudinarium* might be the possible carrier of *R. japonica*. We followed up the patient carefully without prophylactic antibiotics therapy because of no report of the presence of *R. japonica* in *A. testudinarium*. In the current case, in spite of the presence of many red papules by tick bite on the skin lesions, only 7 ticks attached to the skin for sucking. The patient might have a history of tick biting because of loss of many ticks once attached to the patient's skin within about 20 hours. Basophil works advantageously for the acquired immunity against ticks [4]. The patient's basophil count was 12/ $\mu$ l at the first visit, 18/ $\mu$ l at the 3<sup>rd</sup> day, and returned to 5/ $\mu$ l at the seven<sup>th</sup> day. This basophil number profile implicates the presence of basophil-mediated action to ticks.

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In conclusion, this is the first case report of *R. japonica* detected from *A. testudinarium*.

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## CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

## STATEMENT OF HUMAN AND ANIMAL RIGHTS

All procedures performed in human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

