

DETECTION AND QUANTIFICATION OF *ORIENTIA TSUTSUGAMUSHI* IN THE PRE- AND POST-FEEDING *LEPTOTROMBIDIUM* CHIGGERS, USING REAL-TIME POLYMERASE CHAIN REACTION ASSAY

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Real-time PCR assays were conducted in an attempt to detect and quantify the amount of *Orientia tsutsugamushi* (Ot), a gram-negative intracellular bacterium, a causative of the scrub typhus. This zoonotic disease is transmitted from animal hosts (usually rodents) to human by the bites of larval mites (chiggers) of genus *Leptotrombidium*. Four groups of *Leptotrombidium* chigger (both non- and infected with Karp-strain *O. tsutsugamushi*) specimens of the pre- and post feeding on the 4-week-old ICR mice were used in this study: (a) unfed-non-infected chiggers (control group), (b) fed-non infected chiggers (control group), (c) unfed-infected chiggers, and (d) fed-infected chiggers. Each individual chigger was grinded and stored in sterilized/distilled water. Later, it was boiled in 30 μ l sterilized/distilled water. DNA sample was subjected to amplify in Thermal cycle (ABI 7700 SDS, Applied Biosystems). About 5 μ l of the DNA sample solution of each chigger was mixed with 0.25 unit of Taq Gold.

The rtq PCR assays were performed in triplicate reactions for each individual sample in order to determine the Ct value (Threshold cycle). Primers and probes did not cross-react with mock chigger DNA. Standard curves were constructed from tenfold of r47 plasmid serial dilutions within the quantification range to determine the amount of 47kDa. A recombinant r47 plasmid which contained probe sequence of the *O. tsutsugamushi*, was used as standard for copies number determination. Results obtained from 36 non- and scrub typhus-infected chiggers exhibited the number of Ot 47 kDa detected in the unfed, infected-scrub typhus chiggers (with an average of 30,173.37 copies) were 3 times higher than those in the fed, infected-scrub typhus chiggers (with an average of 12,924.13 copies). The highest number Ot 47 kDa detected in an individual unfed, scrub typhus-infected chigger was recorded at 59,471.5 copies with the lowest number at 11,203.4 copies. While, the highest number Ot 47 kDa detected in an individual fed, scrub typhus-infected chigger (chigger fed on ICR mouse) was recorded at 23,965.00 copies with the lowest number was as low as 603.80 copies. In summary, after the feeding period of the *O. tsutsugamushi*-infected chiggers on the 4-week-old ICR mice, the number of Ot 47 kDa were significantly decreased when compared to the unfed Lc-1 scrub typhus-infected chiggers. Our ongoing experiments are now focused on the older ICR mice (i.e., 10-week-old) in order to compare the amount of *O. tsutsugamushi* in the pre- and post-feeding *Leptotrombidium* chiggers as well as their immune response of the mice.

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