

Comparison of Nested Polymerase Chain Reaction and Real-Time Polymerase Chain Reaction Targeting 47kDa Gene for the Diagnosis of Scrub Typhus

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Abstract

Introduction: Scrub typhus is a zoonotic infection caused by *Orientia tsutsugamushi* which is transmitted by *Leptotrombidium* mites. The disease manifests as a mild-to-severe illness with non-specific clinical symptoms. Rapid diagnosis and prompt treatment are essential for patient management. Both serological and molecular methods are used for the diagnosis of scrub typhus. The present study assessed the usefulness of detection of the gene encoding the 47kDa outer-membrane protein (OMP) for the laboratory diagnosis of scrub typhus. **Materials and Methods:** Nested polymerase chain reaction (nPCR) and real-time PCR targeting 47 kDa OMP antigen gene of *O. tsutsugamushi* were performed on ethylenediaminetetraacetic acid blood samples. **Results:** Six of the 103 (5.8%) patients showed the presence of 47kDa gene by nPCR. Seventy of 103 (67.9%) cases showed the presence of 47kDa gene by qPCR. Among the 70 positive cases, the majority of them were females (40/70, 57.1%). The highest number of positive cases was observed during October–February. **Conclusion:** Real-time PCR targeting *O. tsutsugamushi*-specific 47-kDa gene is more sensitive than nPCR and may be the assay of choice for the detection of the organism in patients with suspected scrub typhus.

Keywords: 47kDa, nested polymerase chain reaction, real-time polymerase chain reaction, scrub typhus

INTRODUCTION

Scrub typhus is caused by *Orientia tsutsugamushi* and is the most prevalent human rickettsial infection. It affects one billion people in endemic areas and it is the cause of one million infections annually.^[1] The disease ranges from mild-to-severe, and mortality rates range between <1% and 50% depending on the time taken to start antibiotic treatment, the immune status of the infected host and characteristics of the strain of *Orientia*.^[2]

This zoonotic infection spreads by the bite of the larval stage of infected *Leptotrombidium* mites of the family *Trombiculidae*. The mites feed on human tissue fluids and non-specific symptoms such as fever, breathlessness, myalgia, vomiting and nausea occur 2–10 days after the bite.^[3,4] The disease may progress to a severe form with pneumonia, myocarditis, meningoencephalitis, acute renal failure, gastrointestinal bleeding, splenomegaly, hepatomegaly and seizures.^[5,6] Rapid diagnosis and prompt treatment are

essential for patient management. Serologic tests such as indirect immunofluorescence assay, immunoperoxidase test, enzyme-linked immunosorbent assay, passive hemagglutination test and immunochromatographic assay (rapid card test) are currently available and widely used. However, these serologic tests do not diagnose the disease in the early stages when antibody levels may be low. Polymerase chain reaction (PCR) detection of specific *O. tsutsugamushi* genes such as 56kDa, 47kDa and 16sRNA has been used for the rapid diagnosis of scrub typhus. The 47-kD gene, which is a specific outer-membrane protein (OMP) antigen gene is relatively conserved, with only 3.3% nucleotide sequence divergence.^[7]

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