Molecular detection of *Rickettsia* in peripheral blood of patients suspected of spotted fever rickettsiosis who tested sero-negative in indirect immunofluorescence assay

S Shiffana[1], RPVJ Rajapakse[2], SAM Kularathne[3], DS Thilakarathne[2]

Introduction and Objective: Spotted fever rickettsioses (SFR) are emerging tick-borne infections in Sri Lanka, particularly prevalent in the central hills of the country. Confirmatory diagnosis of this infection is rarely performed in referral laboratories where imported indirect immunofluorescence antibody test (IFAT) kits are used. However, the reliability of diagnosis using imported kits is questionable, as patients with typical clinical signs often test sero-negative. Therefore, this study aimed to use molecular methods to investigate the presence of SFR-causing bacteria in peripheral blood samples from patients suspected of SFR who tested negative in the IFAT.

Methods: The study encompassed peripheral blood samples submitted to the SFR diagnostic unit of the Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya from January to December 2020 (Ethical approval No: 2019EC63). All blood samples underwent screening using Vircell® *Rickettsia conorii* immunoglobulin G (IgG) IFAT. DNA was extracted from the pelleted blood of all IFAT-negative samples using the DNeasy Blood & Tissue Kit. The extracted DNA was then subjected to nested polymerase chain reaction (PCR) targeting the 17kDa gene of the spotted fever group of rickettsia.

Results: During the specified period, 144 blood samples suspected of SFR were submitted for IFAT. Of them, 104 (72.2%) tested sero-positive for rickettsia IgG, while 40 (27.8%) were identified as sero-negative. Nested PCR successfully detected rickettsial DNA in 60% (24/40) of the sero-negative samples. The percentage of rickettsial DNA positives in the IFAT-negative group was significantly higher ($p<0.001$) compared to that reported in a recent study conducted in Sri Lanka on IFAT-positive samples.

Conclusions: Although the detection of rickettsial IgG or IgM by IFAT is considered the gold standard reference, these tests have limitations, especially when using imported kits. Detection of rickettsial IgM is known to be less specific, and seroconversion to rickettsial IgG in patients can take up to two weeks. Detecting rickettsial DNA in peripheral blood of patients is less likely and expensive. However, the current study demonstrates that combining IFAT and nested PCR can efficiently confirm the diagnosis of suspected SFR patients who are IFAT negative.

Keywords: *Spotted-fever, rickettsioses, sero-negative, blood, PCR*

[1]*School of MLT (Peradeniya), Ministry of Health, Sri Lanka*
[2]*Department of Veterinary Pathobiology, Faculty of Veterinary Medicine & Animal Science, University of Peradeniya, Sri Lanka*
[2]*Department of Medicine, Faculty of Medicine, University of Peradeniya, Sri Lanka*

Address for correspondence: Dr. Dulari S. Thilakarathne, Department of Veterinary Pathobiology, Faculty of Veterinary Medicine & Animal Science, University of Peradeniya, Sri Lanka ; Telephone: 0778881842
Email: dsamanthikat@gmail.com https://orcid.org/0000-0002-8790-033X