Molecular detection of spotted fever group Rickettsia species from seropositive human blood samples

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Introduction and Objectives: Rickettsial infection is an emerging vector-borne disease worldwide. In Sri Lanka,the incidence of rickettsioses is on the rise and diagnosis relies on clinical manifestations and serology. Isolation or identification of the pathogen is not routinely performed. Therefore, the taxonomy of *Rickettsia* species occurring in Sri Lanka is unknown. Such information is crucial to develop specific diagnostics and for the implementation of efficient control measures. Success rates of molecular detection or isolation of *Rickettsia*are high when biopsies of cutaneous lesions are used.However, such clinical specimens are not as feasible as a blood sample. Attempts were made to identifywith molecular methods, the spotted fever group (SFG)*Rickettsia* in blood samples of patients who are sero-positive for immunoglobulin G (IgG) against *Rickettsia*.

Methods: Blood taken from 217 patients with suspected rickettsiosis from January 2020 to June 2021 was tested using the rickettsia indirect immunofluorescence antibody test (IFAT). Of the 217, 137 (63.1%) were seropositive for *Rickettsia conorii* IgG. Whole blood samples of 113 of the 137 were pelleted by centrifuging at 1500rpm for 10 minutes. .Whole blood samples of 113 of the 137 were pelleted by centrifuging at 1500rpm for 10 minutes. DNA was extracted using 100µL of pelleted blood and eluted in 50µL of elution buffer. A nested PCR was developed by designing primers targeting the 17kDa gene of SFG Rickettsia. The second PCR utilized the 1:10 dilution of the first-round product as the template. The product sizes of the first and second rounds were 407 and 246 base pairs respectively.

Results: Fifteen of the 113 samples (13.3%) were positive for *Rickettsia* DNA. As IFAT was conducted on single randomly timed sera, high sero-positivity observed in the tested population could also reflect a previous exposure to the pathogen rather than an acute infection. The observed percentage of PCR positives is reasonable as infected endothelial cells may not circulate in large numbers in the blood unless the infection is severe.

Conclusions: We will sequence the PCR products for phylogenetic analysis of locally circulating *Rickettsia* strains. We will culture *Rickettsia* PCR positive blood samples on Vero cellsfor isolation of the pathogen to develop a more specific in-house IFAT.

Keywords: Rickettsia, Spotted Fever Group, Immunofluorescence assay-antibody, PCR

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