Abstract

Rickettsioses are emerging infections in Sri Lanka. Diagnosis of these infections poses problems due to unavailability of validated tests. As development of serological tests are dependent upon antigen production which in turn is limited by the requirement of class III containment level laboratories, molecular tests are considered as the way forward in rickettsial diagnosis. Prior to the application of a molecular diagnostic test for routine clinical use, validation is necessary. Till such times that validated tests are available for routine laboratory diagnosis, clinical and epidemiological profile of patients established by means of laboratory confirmed cases in a given country provide aid in the clinical diagnosis of patients with suspected rickettsioses.

Thus the aims of this study were to map rickettsial infections in selected areas of Sri Lanka using serological tests and to validate two PCR based tests for the laboratory diagnosis of spotted fever and scrub typhus.

Serum samples were collected from 23 hospitals representing 8 provinces of Sri Lanka, from January 2009 to January 2011. These samples were categorized into three cohorts depending on the tests used for confirmation which in turn depended on the availability of kits. Cohort 1 (n=141, single sera) included samples tested using scrub typhus IgM and IgG ELISA. Cohort 2 (n=262, single sera) included samples tested using scrub typhus and spotted fever IgM and

IgG ELISA. Cohort 3 (n=212 of single sera and 44 paired sera) included samples tested using IFA.

In all three cohorts, the presence of rash and absence of eschar was significantly higher in patients with serologically confirmed spotted fever when compared to patients with serologically confirmed scrub typhus. Arthralgia and myalgia were also commoner in the spotted fever sero positive group while localized lymphadenopathy was commoner in the scrub typhus sero positive patients.

Spotted fever group antigens tested in cohort 3 were *Rickettsial conori, Rickettsia honei, Rickettsia rickettsii, Rickettsia australis, Rickettsia siberica* and *Rickettsia akarii*. Out of these, *Rickettsia honei* was implicated as the causative agent in 30 patients. The Gilliam strain was found to be predominant among patients seropositive to *Orientia tsutsugamushi*. Combined results of all three cohorts showed a preponderance of spotted fever in samples received from Base Hospitals Gampola, Nawalapitiya, and Matale, Teaching Hospital, Kandy and General Hospital, Badulla. Scrub typhus was seen as the predominant rickettsioses in samples received from GH Kurunegala, Base Hospitals Dambadeniya, Kuliyapitiya, , and Embilipitya and General Hospitals Anuradhapura and Matara. Timescale analysis in selected locations showed location specific patterns.

Both spotted fever and scrub typhus PCR tests were optimized and validated to the level possible with existing facilities. Detection limit for the spotted fever PCR was 7.52 ng while

the detection limit for scrub typhus PCR was 6.4 ng. Preferable sample for both assays was ETDA whole blood when compared with serum or plasma. Diagnostic sensitivity of both depended on the sample quality. Delay in sample collection, improper sample volumes and prolonged storage at room temperature or at – 20 ^{0}C degrees were the main factors found to impact the PCR test. Fulfillment of all criteria for validation was not possible due to practical constrains.

Both assays could be applied for patient diagnosis provided that the samples were collected in the early part of illness, to the volume indicated in container and transported in ice to the test venue within a few hours of collection and stored at – 70 ^{0}C.

This study helped to widen the knowledge about the epidemiology of rickettsioses in Sri Lanka and to identify the issues associated with offering molecular diagnostic tests in a resource limited setting.