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Isolation and characterization of *Leptospira interrogans* from clinically suspected leptospirosis patients from selected hospitals in Western Province, Sri Lanka

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Background: Culture isolation of human pathogenic *Leptospira* is a challenge. Isolation of *Leptospira* is confirmatory of the disease but is difficult and laborious to perform. However, culture has an epidemiological value mainly to identify responsible reservoir animals. The last report of isolation of *Leptospira* from an infected patient in Sri Lanka was published in 1973.

Objectives: To isolate and identify circulating *Leptospira* species from clinically suspected leptospirosis patients in Western Province, Sri Lanka.

Methods: Whole blood from 100 leptospirosis suspected patients were subjected to DNA extraction followed by *secY* PCR. Acute leptospirosis was presumptively diagnosed by detecting IgM using an immunochromatographic assay. Few drops of whole blood were inoculated into semi-solid Ellinghausen-McCullough-Johnson-Harris medium for culture and incubated at 30°C. Positive cultures were selected by presence of motile spiral bacteria under dark field microscopy and subjected to nested PCR targeting the *flaB* gene of pathogenic *Leptospira* and sequencing for phylogenetic analysis. Culture positive patients sera were subjected to microscopic agglutination test (MAT) to identify the infective *Leptospira* serogroups using a panel of 10 representative leptospiral serogroups.

Results: Among 100 patients, *secY* PCR found to be positive in 27(27%), while 26 patients gave positive IgM results. Only 2 patients were found to be culture positive (2%) using dark field microscopy. PCR of both cultures were positive for *flaB* gene from pathogenic *Leptospira*. The phylogenetic distance of *flaB* sequences suggested *Leptospira interrogans* while MAT identified infective serogroups as Sejroe and Canicola.

Conclusion: For early detection of pathogenic *Leptospira*, molecular based assays are more suitable than culture isolation. Serogroup Sejroe and Canicola of *L. interrogans* have more affinity to animal origin such as cattle and dogs respectively. Thus, in the current study we stress the importance of cross contamination of infected animals in human habitats.

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