

Environmental contamination of pathogenic and intermediate pathogenic *Leptospira* spp in two divisional secretariats of Kandy district

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Introduction

Leptospirosis is a global zoonotic disease which has a considerable public health impact upon the Sri Lankan population. Previous studies have isolated and identified pathogenic *Leptospira* spp. from infected animals. However, isolation and molecular classification of pathogenic strains from contaminated irrigation water sources used for paddy cultivations has not been conducted methodically. The aim of the current study was to isolate *Leptospira* spp. from irrigation water sources and characterize them using molecular methods.

Methods

Water samples were collected from surface water sources that are used for agricultural activities in two Divisional Secretariats, which included 20 samples from Udunuwara and 13 samples from Yatinuwara. The isolation of the bacterium was carried out by filtering the water samples through 0.45µm filters and culturing of the filter membrane in Ellinghausen-McCullough-Johnson-Harris medium. Spirochaetes in cultures were observed using dark field microscopy. Positive samples were subjected to DNA extraction and a partial region of *lipL32* gene and *flaB* gene were amplified by PCR. Phylogenetic analysis was conducted after sequencing the PCR amplicons. GPS mapping of spirochaete positive samples was done to identify clusters.

Results: Upon dark field microscopy observation, 18 (54.5%) of the 33 samples were spirochete positive. Nine (50%) of the 18 samples were positive for both *lipL32* and *flaB* and 2 (11.1%) positive only for *flaB*. The phylogenetic analysis of sequences of the *flaB* gene confirms the presence of *L. interrogans*, *L. santarosai*, *L. kmetyi* and *L. wolffii*, and *L. licerasiae* in the water samples. GPS location distribution analysis of isolates confirms the presence of a cluster of pathogenic *Leptospira* contaminated irrigation water sources in the Yatinuwara division.

Conclusions: These results show the presence of pathogenic leptospires in environmental surface water sources used for agricultural activities. The virulence of these environmental leptospires should be studied to confirm the risk of infectivity towards humans and animals.

Keywords: Leptospirosis, irrigation water, *flaB*, *lipL32*

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