

EVALUATION OF PRIMERS DESIGNED FOR NESTED PCR TO DETECT P2 CLADE OF LEPTOSPIRES CAUSING LEPTOSPIROSIS IN HUMANS AND ANIMALS

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The pathogenic *Leptospira* (P) bacterium can transmit to humans through direct or indirect pathways and cause leptospirosis. This zoonosis is considered endemic in developing countries like Sri Lanka. Each year, more than one million leptospirosis cases, including 60,000 deaths, are recorded globally. Leptospirosis diagnosis is predominantly based on pathogen detection in clinical samples by *flaB* nested PCR. Intermediate *Leptospira* species (P2) can also cause leptospirosis but are undetectable through current molecular tests. Therefore, this study aimed to evaluate the primers designed for nested PCR to detect the P2 clade using extracted DNA samples collected from the Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka. The primers used for leptospirosis-related research in the past 10 years were analyzed in the literature survey. Intermediate primers (*I-flaB*) were initially evaluated using the in silico-based method through NCBI (National Center for Biotechnology Information) Primer-BLAST. Both known and unknown samples were used for the wet lab primer evaluation. DNA from 12 *Leptospira* species used in the Microscopic Agglutination Test (MAT), P2 human and animal clinical samples, and intermediate pathogen (P011) were used as the known samples. Seventy-two stored clinical blood samples that were *flaB*-PCR negative were used as the unknown samples. *16s rRNA*-PCR was conducted for the unknown samples to detect the existence of bacterial DNA. The five P2 species, *L. inadai*, *L. broomii*, *L. wolffii*, *L. licerasiae*, and *L. fainei* were explicitly detected by *I-flaB* primers in the 'in silico' evaluation, whereas the detection was non-specific in the wet lab evaluation using known samples where two P2 samples gave negative results. Of the unknown samples, six samples were positive for *16s rRNA*-PCR, implying the presence of bacterial DNA. However, *I-flaB* nested PCR was negative for all these six samples. Therefore, the detection of available *I-flaB* primers is non-specific. The 'in silico' method should be used cautiously. Primer evaluation before the application of newly designed primers is vital.

Keywords: *16s rRNA* gene, *flaB* gene, In silico analysis, Intermediate *Leptospira* species, Zoonotic disease