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Development of a rapid DNA based detection method for *Leptospira interrogans* in environmental water samples

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Leptospirosis is a wide spread zoonotic disease in the world, with 1.03 million incidences and 58,900 deathsannually. More than 230 serovars classified into 31 serogroups of leptospires have been described up to date. *Leptospira interrogans* is a commonly found species, which is responsible for many disease outbreaks. Although the transmission of leptospires mostly occurs through environmental water contaminated with an infected host's urine, detection of pathogenic leptospires from environmental water is still challenging. This study aimed to develop a Polymerase Chain Reaction (PCR) based method for the rapid detection of *L.interrogans* from environmental water samples. The sample preparation procedure for DNA extraction was optimized. *L.interrogans* serovar Icterohemorrhagiae and *L.biflexa* serovar Patoc were used as pathogenic and saprophytic strains respectively. Hundred milliliters of each pathogenic and saprophytic *Leptospira* spp. spiked water samples (10^7 cells/mL) were used for the DNA extraction. Prepared samples were centrifuged at 5000 rpm for 30 minutes to pellet the cells. One milliliter of each concentrated cell suspension was used to extract DNA using QIAamp DNA minikit. Extracted DNA was amplified using PCR targeting the*LipL*41 gene, which is found only in pathogenic leptopires. The amplicon of *LipL*41 gene (249 bp) on 2% electrophoresis gel was observed only for *L.interrogens* while *L.biflexa* was negative. The limit of detection (LOD) of the developed method was tested using 10^2 - 10^6 *L.interrogans* cells/mL water samples, and it was found that LOD was 10^3 cells/mL. This method is rapid and inexpensive compared to the other detection method such as Culture and Microscopic Agglutination tests. The developed method can be used even with basic laboratory facilities. This method can be applied to the study of the distribution of *L.interrogans* in environmental water samples.

Key words: Leptospira, serovars, serogroups, *LipL* 41 gene

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