

Identification of Pathogenic Leptospiral Serovars by Detection of the *ompL37* Gene Using PCR

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ABSTRACT

Background and Objectives: Leptospirosis is a widespread zoonotic disease that is transmitted directly or indirectly from animals to humans. Humans mainly acquire pathogenic leptospires through mucosal or percutaneous exposure to environment contaminated with urine from an infected animal. We aimed to identify pathogenic leptospiral serovars by detection of the *ompL37* gene using polymerase chain reaction (PCR).

Methods: Sixteen pathogenic leptospiral serovars and a saprophytic serovar, *L. biflexa* were cultured in modified semisolid Ellinghausen-McCullough-Johnson-Harris medium containing 5% rabbit serum. Genomic DNA extraction was done using the phenol-chloroform method. The *ompL37* gene was amplified using specific primers. PCR products were analyzed by agarose gel electrophoresis.

Results: The *ompL37* gene was amplified only in the pathogenic leptospiral serovars. We detected no amplified fragment for the saprophytic serovar.

Conclusion: Leptospirosis may be confused with other infectious diseases, and therefore, its early and accurate diagnosis is crucial. We showed that molecular detection of pathogenic leptospires based on the *ompL37* gene could be used for laboratory diagnosis of leptospirosis.

Keywords: Leptospirosis, PCR, *ompL37* Gene, Pathogenic Leptospires.

INTRODUCTION

Leptospirosis is a widespread zoonotic disease caused by pathogenic leptospire (1). Over 500,000 new cases of the disease occur annually, with mortality rate of up to 70% (2). Humans mainly acquires these bacteria through mucosal surfaces and skin abrasions following contact with soil or water contaminated with urine of infected animals (3). Leptospirosis may be confused with other infectious diseases such as influenza, malaria, viral hepatitis, dengue fever and typhoid fever (4). Hence, early and accurate diagnosis of the disease is vital for proper and prompt treatment. Serological methods such as microscopic agglutination test and enzyme-linked immunosorbent assay are used for diagnosis of the disease. Bacteria isolation is time-consuming and needs complex culture media (5). For this reason, polymerase chain reaction (PCR) is utilized for detection of slow-growing or fastidious microorganisms and small numbers of leptospire from clinical samples (6, 7). PCR has been shown to be a rapid and sensitive diagnostic method, particularly in patients with acute leptospirosis (8).

OmpL37 is a surface-exposed outer membrane protein (OMP) of *Leptospira* expressed during infection. This antigen has potential roles in leptospirosis, including attachment to elastin-rich tissues, such as the lungs, dermis and vasculature. It has been demonstrated that the gene encoding for this protein is highly conserved in pathogenic serovars of *Leptospira*, and can be used for molecular detection of the disease (9, 10). This study aimed to detect the leptospiral *ompL37* gene using PCR.

MATERIALS AND METHODS

In this study, sixteen pathogenic leptospiral serovars including: *Leptospira interrogans* serovars *Sejroe* *Hardjo* (RTCC2810,2821), *L. interrogans Canicola* (RTCC2805,2824,2836), *L. interrogans Icterohaemorrhagiae* (RTCC2812,2823), *L. interrogans Pomona* (RTCC2815,2822), *L. Interrogans Grippotyphosa* (RTCC2808,2825), *L. Interrogans Autumnalis* (RTCC2802), *L. Interrogans Serjoe* (RTCC2817), *L. Interrogans Pyrogenes* (RTCC2835), *L. Interrogans Australis* (RTCC2840), *L. Interrogans Bataviae* (RTCC2842), and a saprophytic serovar

Leptospira biflexa (RTCC2819) were obtained from the *Leptospira* reference laboratory, Razi Vaccine and Serum Research Institute, Karaj, Iran. The bacteria were inoculated into the selective culture medium Ellinghausen-McCullough-Johnson-Harris medium (Difco, Sparks, USA) containing 10% rabbit serum and enrichment supplements. After 7-10 days of incubation under aerobic conditions at 28 °C, growth was evaluated under a dark-field microscope. The samples were centrifuged at 15000g for 20 min at 4 °C. Leptospiral genomic DNA was extracted by proteinase K treatment and using the phenol-chloroform extraction method (11). The extracted DNA was resuspended in 20 µl of TE buffer (pH 8.0) and stored at -20 °C. Quality and quantity of the extracted DNA was then evaluated by agarose gel electrophoresis.

The *ompL37* gene was amplified by PCR using the following primers: 5'-AAG GAT CCG ATC AGA TCA ACT TAG-3' (forward) and 5'-TGG GTA CCT TAA TTT TGT GTT TTT-3' (reverse) (9). For optimization of the PCR process, different concentrations of the primers (5, 10, 15 and 20 pmol) and DNA template (0.01 pg - 100 ng) were used in the reactions. The final volume (25 µL) of each reaction mixture contained 2.5 µL of 1X PCR buffer, 0.5 mM of 10 mM deoxyribonucleoside triphosphates (dATP, dCTP, dGTP and dTTP), 1 µM of each primer, 0.75 of 50 mM MgCl₂, 0.3 unit/µL of 0.5 Taq DNA polymerase and 1 µL of template DNA. The genomic DNA from *L. interrogans* serovars *Icterohaemorrhagiae*, *Pomona* and *L. biflexa* serovar *Semarang* were used as positive control. A blank containing no DNA was used as the negative control. A temperature gradient was used to determine the optimal annealing temperature for the PCR assay. The PCR program was initiated at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 53-66 °C for 1 min and 72 °C for 1 min, and final extension at 72 °C for 10 min. The PCR products were run on 1% agarose gel containing DNA safe stain and then visualized UV light.

To determine the primers specificity for the pathogenic leptospiral strains, the PCR assay was performed using specific primers for detection of the *ompL37* gene in the pathogenic serovars of *leptospira*. DNA extracted from *Salmonella enteritidis* (RTCC1621) was used

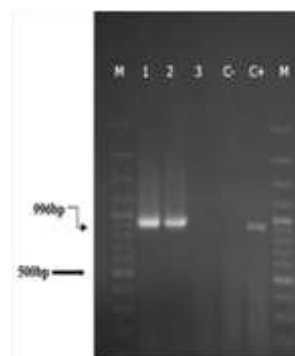
for further confirmation. In order to obtain the lowest amount of DNA for amplification and detection, concentration of an extracted DNA sample was determined using a spectrophotometer and diluted up to 0.001pg/ μ l.

PCR assay was eventually performed for each diluted sample.

Figure 1- Results of the primer sensitivity analysis (M: 100 bp DNA ladder, lane1: 100 ng/ μ l, lane 2: 10 ng/ μ l, lane 3: 1 ng/ μ l, lane 4: 100 pg/ μ l, lane 5:10 pg/ μ l, lane 6: 1 pg/ μ l, lane 7: 0/1 pg/ μ l, lane 8: 0/01 pg/ μ l, lane 9: negative control).



Figure 2- PCR amplification of the *ompL37* gene for detection of pathogenic serovars of *Leptospira* (M: 100 bp DNA ladder, lane 1: *L. pomona* (RTCC2815) , lane 3: *L. biflexa*, C-: negative control, C+: positive control):*canicola* (RTCC2805) , lane 2



The *ompL37* gene was amplified only in the pathogenic *Leptospira* serovars. The saprophytic serovar showed no amplified fragment (Figure 2).

DISCUSSION

Leptospirosis caused by different *Leptospira* species, is one of the most frequent zoonotic infections worldwide (12). It has a significant economic impact on the society and can spread from rural areas to cities (13). A major challenge in combating this disease is the application of basic research to improve diagnostics and vaccine development. Diagnosis is challenging because the available diagnostic tests are not always serovar-specific due to cross-reactivity with microorganisms in the same serogroup. Moreover, protein expression patterns in pathogenic leptospires differ when grown outside and inside the host. Therefore, the molecular mechanisms of leptospirosis pathogenesis remain unclear. Several virulence factors have been thought to contribute to the pathogenesis of leptospirosis. These include lipopolysaccharides, OMPs and

RESULTS

The results of PCR revealed a 996 bp fragment that represented amplification of the *ompL37* gene. Results of the primer sensitivity analysis also showed that the *ompL37*-specific primers could be amplified in DNA concentrations up to 1pg/ μ l and are suitable for the molecular detection of pathogenic *Leptospira* spp. with a high sensitivity (Figure 1).

other surface proteins, and adhesion molecules. Among these, OMPs may induce or enhance immune responses against the disease (12, 14).

Understanding the epidemiology of this disease is important for designing disease control programs. In untreated individuals, leptospirosis can progress rapidly and increase the risk of mortality, thus early diagnosis of the disease is crucial (15). PCR is more sensitive than culture for the detection of *Leptospira* and have been widely used for the direct detection of fastidious pathogenic microorganisms in clinical samples. Compared to other methods, PCR is more versatile for rapid diagnosis of early stages of leptospirosis (7). *OmpL37* can attach to elastin in host and play a key role in the pathogenesis of leptospirosis (10). PCR targeting of the

ompL37 gene can be useful for rapid diagnosis of the infection (16).

In the present study, we observed a 996 bp fragment corresponding to presence of the *ompL37* gene, which was absent in the saprophytic serovar. Several studies showed similar results when using other saprophytic serovars (17,18).

CONCLUSION

We detected the *ompL37* gene in pathogenic serovars of *Leptospira* using PCR. The detection of this highly conserved gene

could be useful for identification of pathogenic *Leptospira* and early diagnosis of leptospirosis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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