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Rapid Identification and Diagnosis of the Pathogenic Leptospiras through Comparison among Culture, PCR and Real Time PCR Techniques from Samples of Human and Mouse Feces

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Abstract : Leptospirosis is one of the most significant infectious and zoonotic diseases along with global spreading. This disease is causative agent of economoic losses and human fatalities in various countries, including Northern provinces of Iran. The aim of this research is to identify and compare the rapid diagnostic techniques of pathogenic leptospiras, considering the multifacetedness of the disease from a clinical manifestation and premature death of patients. In the spring and summer of 2020-2022, 25 fecal samples were collected from suspected leptospirosis patients and 25 Fecal samples from mice residing in the rice fields and factories in Tonekabon city. Samples were prepared by centrifugation and passing through membrane filters. Culture technique was used in liquid and solid EMJH media during one month of incubation at 30°C. Then, the media were examined microscopically. DNA extraction was conducted by extraction Kit. Diagnosis of leptospiras was enforced by PCR and Real time PCR (SYBR Green) techniques using lipL32 specific primer. Out of the patients, 11 samples (44%) and 8 samples (32%) were determined to be pathogenic Leptospira by Real time PCR and PCR technique, respectively. Out of the mice, 9 Samples (36%) and 3 samples (12%) were determined to be pathogenic Leptospira by the mentioned techniques, respectively. Although the culture technique is considered to be the gold standard technique, but due to the slow growth of pathogenic Leptospira and lack of colony formation of some species, it is not a fast technique. Real time PCR allowed rapid diagnosis with much higher accuracy compared to PCR because PCR could not completely identify samples with lower microbial load.

Keywords : culture, pathogenic leptospiras, PCR, real time PCR
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