## **Abstract**

Leptospirosis is a common zoonosis acquired by exposure to body fluids of infected animals, or to contaminated soil or water. Leptospirosis remains under-reported in Taiwan because of ignorance and the broad spectrum of clinical manifestations. Tests such as the microscopic agglutination test (MAT) or enzyme-linked immunosorbent assav (ELISA) for the detection immunoglobulin M are commonly used but have not been standardized. The 16s rDNA gene sequences have been shown to separate Leptospira sp. We can not readily detect Leptospiral pathogen by real-time polymerase chain reaction (rt-PCR). In the present study, we report the use of nested-PCR in the identification of leptospires directly from urine, CSF, serum or whole blood of leptospirosis-suspected patients. Out of 933 clinical samples, 167 leptospirosis cases were identified. DNA sequencing was performed of PCR products and then submitted for analysis using NCBI database. The prevalently endemic strains are L. borgpetersenii, L. interrogans, and L. myeri. The number of L. noguchii and L. santarosai, the most popular strain in Taiwan, are few in our clinical samples. We also found the sarprophytic "biflexa" group in clinical samples. These PCR-positive patients presented conjunctival suffusion, aseptic meningitis, infective endocarditis, adrenal insufficiency, and/or co-infections with other pathogens. We sequenced all PCR products and performed the phylogenic analysis, showing that there are three different genetic clusters. Our studies highlight under-recognition of the involvement of severe clinical symptoms with leptospirosis and underdiagnosis of leptospirosis in a region of high endemicity. Thus an appropriate surveillance and control measures need to be established.

**Key words: Leptospirosis, zoonosis, nested PCR**