Isolation and Molecular Characterization of *Leptospira interrogans* and *Leptospira borgpetersenii* Isolates from the Urban Rat Populations of Kuala Lumpur, Malaysia

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**Abstract.** Rats are considered the principle maintenance hosts of *Leptospira*. The objectives of this study were isolation and identification of *Leptospira* serovars circulating among urban rat populations in Kuala Lumpur. Three hundred urban rats (73% *Rattus rattus* and 27% *R. norvegicus*) from three different sites were trapped. Twenty cultures were positive for *Leptospira* using dark-field microscopy. *R. rattus* was the dominant carrier (70%). Polymerase chain reaction (PCR) confirmed that all isolates were pathogenic *Leptospira* species. Two *Leptospira* serogroups, Javanica and Bataviae, were identified using microscopic agglutination test (MAT). Pulsed-field gel electrophoresis (PFGE) identified two serovars in the urban rat populations: *L. borgpetersenii* serovar Javanica (85%) and *L. interrogans* serovar Bataviae (15%). We conclude that these two serovars are the major serovars circulating among the urban rat populations in Kuala Lumpur. Despite the low infection rate reported, the high pathogenicity of these serovars raises concern of public health risks caused by rodent transmission of leptospirosis.

**INTRODUCTION**

Leptospirosis, an infectious disease that affects both humans and animals, is recognized as one of the most widespread zoonoses worldwide. Annually, an estimated one-half million cases of severe leptospirosis are reported globally. However, this number is probably underestimated because of the lack of reported cases and the misdiagnosis of this disease in many countries.

Leptospirosis is caused by the pathogenic strains of the bacterium *Leptospira*. Currently, there are nearly 300 known serovars, and most of them have their primary reservoirs in wild and domestic animals, of which rodents and rats are the most common source worldwide. Infected rats shed *Leptospira* spp. in their urine over an extended period of time, and humans and animals get infected through direct or indirect contact with urine, water, or soil contaminated by *Leptospira* spp. Approximately one-half of the pathogenic serovars belong to *L. interrogans* or *L. borgpetersenii*.

Classically, the diagnosis of leptospirosis is based on serological tests, such as the microscopic agglutination test (MAT). In this test, reaction takes place between a leptospiral isolate and reference hyperimmune rabbit antisera. However, this method is laborious and time-consuming, and it requires extensive collection of reference strains and their corresponding rabbit antisera. Various molecular approaches have been developed, such as polymerase chain reaction (PCR)-based methods, to improve the diagnosis of leptospirosis. PCR has been successfully applied as a rapid, sensitive, and specific tool for the detection of several microorganisms, including *Leptospira*, in a variety of specimens from different hosts. The rapidity and reproducibility of pulsed-field gel electrophoresis (PFGE) makes it a very useful technique for typing *Leptospira* strains. PFGE is able to discriminate between closely related serovars of the *Leptospira* spp., which may aid in the identification of the different strains at serovar level.

**MATERIALS AND METHODS**

**Choice of the study sites.** Kuala Lumpur, the capital city of Malaysia, is characterized by a tropical climate of high temperature and high humidity year round, with temperatures ranging between 30°C and 36°C; rainfall is fairly even throughout most of the year but typically heavier between October and February during the monsoon season. The choice of the study sites was based on the suitability of the habitat for rodents to forage and breed and the possibility of spreading the diseases. The first site, Chow Kit market (*03°09′53.75″ N, 101°41′56.84″ E*), is the largest fresh food wet market, commonly found in Asian countries, in Kuala Lumpur. Here, water is used extensively to wash the floors, keep the fruits and vegetables fresh, and keep fish and shellfish alive. This market provides an extensive range of raw foods, including fruits, vegetables, seafood, and meat to the public. In turn, tons of rubbish are deposited into several steel containers. Excess garbage falls to the ground, forming temporary grounds for rats to forage. In contrast, Bangsar (*3°6′49″ N, 101°39′45″ E*) is an affluent residential suburb on the outskirts of Kuala Lumpur with mixed residential sites. The hawker centers, restaurants, and roadside stalls sell cooked food, and rodents found here thrive on leftovers.