Detection of pathogenic *Leptospira sp.* from body surface of cockroaches by real time polymerase chain reaction

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ABSTRACT

Introduction: Leptospirosis cases in Malaysia are increasing each year and are typically related to peridomestic rodents, specifically rats. They are the most important reservoirs of *Leptospira sp.* and have been significantly found sharing the same habitats with the cockroaches. Their interaction with humans is more or equal compared to the rats. Habitats that were contaminated with the urine of infected rats may contaminate the body of cockroaches with *Leptospira spp.* and probably their digestive tract too. This study has therefore attempted to demonstrate the potential role of cockroaches as carriers of pathogenic *Leptospira spp.* **Materials and Methods:** In this study, real time polymerase chain reaction (RT-PCR) method based on LipL32 gene was used to detect pathogenic *Leptospira spp* in selected locations in Selangor. One hundred thirty-seven cockroaches were captured using commercial cockroach traps from a wet market in Beranang, Selangor. Samples were taken from the body surface of each cockroach and cultured individually in EMJH media, then incubated in the dark condition for 2 month at 28°C, observed under x40 dark-field microscope to detect the presence of Leptospira spp. Genomic DNA was extracted from all 15 positive isolates which were subjected for RT- PCR assays using Agilent Brilliant III SYBR Master Mix which consists of a novel mutant Taq DNA polymerase. Primers LipL32-286R and LipL32-45F were used to amplify the targeted region of LipL32 gene, while probe Lip32-189P to detect the pathogenicity. Three pathogenic species (*Leptospira canicola, Leptospira javanica* and *Leptospira bataviae*) were used as positive controls and 2 negative control samples as references for this study. **Results:** Nine isolates from the body wash of the cockroaches produced Ct values ranging from 17.12 to 37.56, therefore were positive and pathogenic for *Leptospira spp.*, whereas 6 isolates (LS3, LS25, LS45, LS78, LS 87 and LS113 produced undetermined Ct values, therefore were free from any for pathogenic *Leptospira spp.* **Conclusion:** Cockroaches have the potential to be the mechanical carrier for Leptospira spp. Real Time PCR utilizing the mentioned set of primers and probes can be used to identify pathogenic *Leptospira spp.* from local cockroaches.