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A COMPARISON OF THREE MOLECULAR-BASED ASSAYS TO DETECT PATHOGENIC LEPTOSPIRES IN CATTLE URINE

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Leptospirosis is a globally widespread, emerging zoonotic disease that poses important public health threats in humid, tropical and subtropical areas. Leptospirosis is transmitted directly or indirectly from animals to humans through contact with contaminated soil, water, or body fluids of infected animals. Most feral and domestic mammals may serve as major reservoir hosts for *Leptospira* spp. However, there is little knowledge on the role of wild mammals, including the large number of rodents, as reservoir hosts of leptospires in Sri Lanka. The objective of this study was to compare three molecular-based assays to detect pathogenic leptospires in cattle urine samples. Mid-stream urine samples were collected from 50 cattle in a high risk area for leptospirosis (Mirigama) in the Gampaha district from May 2012 to February 2013. A Loop-Mediated Isothermal Amplification (LAMP) assay for the rapid detection of pathogenic *Leptospira* species was established using reference samples (*L. interrogans* strain RGA) through amplification of the lipL41 gene coding for the outer membrane protein LipL41. Qualitative Polymerase Chain Reaction (PCR) and quantitative real time PCR assays were established targeting a 202 bp fragment on the secY gene which is conserved among pathogenic serovars of *Leptospira.* Analytical sensitivity and specificity of each assay were tested using reference DNA samples. Each urine sample was tested by three molecular-based assays; namely, LAMP, qualitative PCR and quantitative real time PCR, with positive and negative controls. The repeatability of each assay was tested using two replicates of each sample. Concentrated urine samples were mixed with reaction buffer and directly applied for LAMP reaction. DNA was extracted from concentrated urine samples using a commercially available QIAGEN kit and the extracted DNA was used for both PCRs. Each molecular assay showed 100% analytical specificity. The analytical sensitivity (per reaction) of LAMP assay, qualitative PCR, and real time PCR were 5.8, 588, and 58.8 copies of bacteria, respectively. Of the 50 cattle urine samples, molecular-based assays confirmed *Leptospira* infection in 70% (35/50), 2% (1/50), and 10% (5/50) by LAMP assay, qualitative PCR, and real time PCR, respectively. In conclusion, we established three molecular-based assays to detect pathogenic *Leptospira* species in cattle urine samples. The highest number of positive reactors was detected with the new LAMP assay which utilises a simple DNA preparation step to detect pathogenic *Leptospira* species in urine. In contrast to PCR assays that use purified DNA samples from urine, the LAMP assay can amplify the target DNA without DNA purification and boiled urine samples are sufficient to prepare the DNA template. The results of these molecular-based assays showed that *Leptospira* spp. are circulating among the cattle tested in this study and pose a public health threat to farmers and farm workers in this area.

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