FINAL REPORT

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MOLECULAR AND SEROLOGICAL DETECTION OF LEPTOSPIRA AND RICKTETTSIA IN PATIENTS WITH ACUTE FEBRILE ILLNESS

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1.0 ABSTRACT

Leptospirosis is a zoonotic disease which is caused by the spirochete, *Leptospira interrogans* (*L. interrogans*). Rodents generally serve as the main carrier for Leptospirosis and transmit the disease to humans through cuts and abrasions in the skin or through mucous membranes of the eyes, nose or throat. High seroprevalence was recorded in Sabah, among people living within the national park is likely due to high exposure and contact with wild animals. Diagnosis of Leptospirosis is challenging due to a wide diversity of clinical symptoms which mimic regular symptoms of fever. LipL32 is a major outer membrane protein, which is present only in pathogenic strains of *Leptospira.* Studies show that ELISA is able to detect Leptospira-specific antibodies earlier than the gold standard method, the Microscopic Agglutination Test (MAT). In this study, we have used codon optimized synthetic gene encoding LipL32 and transfected into *E. coli* expression vector BL21 (DE3). The recombinant LipL32 protein was expressed after induction with IPTG which resulted in approximately a 40kDa protein. The purified recombinant LipL32 protein was used as an antigen for detecting Leptospira-specific antibody by ELISA. Preliminary results showed that the recombinant LipL32 antigen was able to detect Leptospira-specific IgM and IgG in human serum samples. Hence, this method could be used for diagnostic purposes and in epidemiological investigations of Leptospirosis.

ABSTRAK

Leptospirosis adalah penyakit zoonotik yang disebabkan oleh sejenis Spirochete yang dinamakan sebagai Leptospira interrogans (L. interrogans). Tikus merupakan pembawa utama bagi penyakit Leptospirosis dan menyebarkan ke dalam system badan manusia melalui luka pada kulit, selaput lender mata, hidung atau tekak. Sabah mencatatkan kadar kelaziman yang tinggi, bagi penduduk yang tinggal berdekatan dengan kawasan Taman Negara. Hal ini kerana, penduduk di kawasan tersebut lebih terdedah kepada hidupan liar. Diagnosis bagi penyakit Leptospirosis agak sukar memandangkan gejala yang dialami oleh pesakit Leptospirosis adalah sama dengan gejala demam biasa. LipL32 merupakan protein membran luar utama yang hanya terdapat pada strain patogenik bagi spesis Leptospira. Kajian menunjukkan bahawa ELISA mampu mengesan antibody khusus bagi Leptospira lebih awal daripada kaedah utama pengesanan Leptospira iaitu, ujian agglutinasi mikroskopik (Microscopic Agglutination Test). Dalam kajian ini, gen sintetik LipL32 digunakan dan telah ditransfekkan ke dalam bakteria E.coli BL21 (DE3). Protein rekombinan LipL32 telah di 'expressed' setelah ditambah IPTG, bersaiz 40kDa. Protein rekombinan LipL32 yang telah melalui proses penulenan, digunakan sebagai antigen dalam ELISA untuk mengesan antibody khusus bagi Leptospira. Keputusan awal menunjukan bahawa protein rekombinan LipL32 mampu mengesan antibody khusus Leptospira IgG dan IgM dalam sampel serum manusia. Oleh itu, kaedah ini boleh digunakan bagi tujuan diagnostic dan kajian epidemiologi bagi penyakit Leptospirosis.