UNIVERSITI PUTRA MALAYSIA

DETECTION OF INFECTION AND DETERMINATION OF BIOMARKERS FOR BRUCELLA MELITENSIS INFECTION IN GOATS

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DETECTION OF INFECTION AND DETERMINATION OF BIOMARKERS FOR *BRUCELLA MELITENSIS* INFECTION IN GOATS

By

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DETECTION OF INFECTION AND DETERMINATION OF BIOMARKERS FOR *BRUCELLA MELITENSIS* INFECTION IN GOATS

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Chairman: **Associate Professor Dr. Siti Khairani Bejo, PhD**

Faculty: **Veterinary Medicine**

Brucellosis is a chronic disease caused by *Brucella* spp. *Brucella melitensis* is one of the species of bacteria that infects animals and humans leading to undulant fever. The interaction between *B. melitensis* and immune system of the host could guide us towards the discovery of new biomarker to detect the infection as early as possible particularly understanding the unique pathway of *B. melitensis* and the intracellular features.

In this study, 288 samples of whole blood and serum were collected from a goat farm in Kedah, Malaysia, which was suspected to have been affected by brucellosis. Serological and molecular detections of brucellosis were performed, using Rose Bengal Plate Test (RBPT), Complement Fixation Test (CFT), conventional PCR and real-time PCR (RT-PCR). Each test was evaluated with respect to its sensitivity and specificity ensuring that the recommendations made are able to be use for the national brucellosis eradication program in Malaysia. Isolation and identification of *B. melitensis*

were also done in addition to conventional PCR and real-time PCR to detect *B. melitensis* from samples collected from vaginal swabs. CDs markers and its combination in different population of peripheral blood mononuclear cells (PMNC) were measured in detail, in different *B. melitensis* stages of the infection and in experimentally infected mice and goats by using specific monoclonal antibody. Histopathological picture was also been described in current study.

The sensitivity of RBPT was 89.04% whilst CFT was 97.02%. The specificity of each of RBPT and CFT were 99.06% and 96.38% respectively. Four (4) *B. melitensis* isolates were isolated from 300 vaginal samples and all isolate belonged to *B. melitensis* biotype 1. The real-time PCR was the easier and safer method for the confirmation of brucellosis in goat's population. The CDs biomarkers namely; CD14, CD4, CD25 markers were identified as good markers for the different stages of *B. melitensis* infection. A combination of a serological test namely RBPT and molecular technique, in particular real-time PCR based on the IS711 region of a hypothetical protein, showed promising results. This combination can be used to reduce the number of false positive results, which can cause severe economical loss during the implementation of proposed eradication programs.

Keywords*:* *Brucella melitensis*, biomarkers, Real-time PCR, CDs.

Abstrak thesis yang dikemukakan kepada Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Doktor Falsafah

DETEKSI INFEKSI DAN PENENTUAN BIOPENANDA JANGKITAN *BRUCELLA MELITENSIS* DI DALAM KAMBING

Oleh

MAGED AHMED MUTHANNA Al-GARADI

Mei 2011

Pengerusi: **Profesor Madya Dr. Siti Khairani Bejo, PhD**

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Brucellosis adalah penyakit kronik yang berpunca daripada *Brucella* spp. *Brucella melitensis* adalah sejenis spesies bacteria yang menjangkiti haiwan dan manusia lalu menyebabkan demam beralun. Interaksi antara *B. melitensis* dan sistem imun perumah boleh memberi panduan kepada kita untuk menemui biopenanda baru untuk mengesan jangkitan seawal yang mungkin. Dengan cara mamahami laluan unik *B. melitensis* dan ciri-ciri intraselularnya membantu untuk menemui penanda baru bagi jangkitan *B. melitensis*.

Asai reaksi rantai polimerase (PCR) telah terbukti sebagai pilihan yang baik untuk diagnosis bruselosis. Dalam kajian ini, 288 sampel darah penuh dan serum diambil dari ladang kambing di negeri Kedah, Malaysia, yang disyaki menghidap bruselosis. Pengesanan serologi dan molekul terhadap bruselosis telah dijalankan dengan menggunakan Ujian Plat Rose Bengal (RBPT), Ujian Pengikatan Pelengkap (CFT), PCR konvensional dan PCR masa nyata

(RT-PCR). Setiap ujian telah dinilai berhubung dengan kepekaan dan kekhususan untuk menentukan ujian mana yang patut digunakan dalam program pembasmian bruselosis di Malaysia. Pengasingan *B. melitensis* adalah tahap utama bagi pengenalpastian dan pengesahan bruselosis haiwan. Walau bagaimanapun, di Malaysia jangkitan *Brucella* spp. di dalam kambing semakin meningkat sejak kebelakangan ini dan tiada terdapat bukti bagi diagnosis biotip *Brucella* spp. yang menyebabkan penyakit dalam populasi kambing kecuali pengesanan kaedah serologi. Pengasingan dan pengenalpastian *B. melitensis* telah dijalankan melalui kaedah bakteriologi disamping PCR konvensional dan PCR masa nyata untuk pengesanan *Brucella melitensis* daripada sampel-sampel yang diambil daripada swab vagina di ladang yang disyaki. Penanda CD dan gabungannya dalam populasi yang berlainan bagi sel mononuklear darah periferi (PMNC) telah diukur dengan terperinci pada peringkat jangkitan *B. melitensis* yang berbeza didalam tikus dan kambing yang dijangkitkan secara kajian dan dengan menggunakan antiboldi monoklonal yang khusus. Gambar histopatologi turut dihuraikan dalam kajian ini.

Sebagai rumusan, gabungan ujian serologi dan teknik molekul, terutamanya PCR masa nyata berdasarkan kawasan IS711 daripada protein hipotesis adalah menggalakkan. Gabungan ini boleh digunakan untuk mengurangkan bilangan keputusan positif palsu, yang boleh menyebabkan kerugian ekonomi yang besar semasa perlaksanaan cadangan program pembasmian. Kepekaan RBPT adalah 89.04% sementara CFT adalah 97.02%. Kekhususan setiap RBPT dan CFT masing-masing adalah 99.06% dan 96.38%. Empat (4) isolat

Brucella melitensis telah diasingkan daripada 300 sampel vagina dan kesemua isolat tergolong dalam biotip 1 *B. melitensis*. PCR masa nyata adalah kaedah yang mudah dan selamat bagi pengesahan bruselosis di dalam populasi kambing. Biopenanda CD seperti; penanda CD14, CD4, CD25 telah dikenal pasti sebagai penanda yang baik untuk peringkat jangkitan *Brucella melitensis* yang berbeza. Gabungan kaedah serologi dan molekul adalah perlu untuk mencapai cadangan pembasmian menyeluruh bruselosis di Malaysia.

Kata kunci: *Brucella melitensis*, biopenanda, PCR masa nyata, CD.

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I certify that Examination Committee has met on 31st May 2011 to conduct the final examination of MAGED AHMED MUTHANNA Al-GARADI on his PhD thesis entitled "DETECTION OF INFECTION AND DETERMINATION OF BIOMARKERS FOR *BRUCELLA MELITENSIS* INFECTION IN GOATS" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the candidate be awarded the Doctor of Philosophy.
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This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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MAGED AL-GARADI
Date: 31 May 2011

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