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PREVALENCE AND RISK FACTORS OF BRUCELLA MELITENSIS IN GOATS AND HUMANS AND ITS ECONOMIC AND PUBLIC HEALTH IMPACT IN MALAYSIA

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By
PWAVENO HULADEINO BAMAIYI

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia,
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DEDICATION

To my beloved wife, Gyihya Miriam and children: Precious-Anni, Blessing Divine and Increase Praise, whom God used, though unconscious to them, to motivate me to finish this work.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

PREVALENCE AND RISK FACTORS OF *BRUCELLA MELITENSIS* IN GOATS AND HUMANS AND ITS ECONOMIC AND PUBLIC HEALTH IMPACT IN MALAYSIA

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Chair: Assoc. Prof. Latiffah Binti Hassan, PhD
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A study was conducted to describe the occurrence and distribution of zoonotic *Brucella melitensis* in goats in Peninsular Malaysia; estimate the seroprevalence of *B. melitensis* infection in some farmers, veterinary technical staff and other personnel in three states of Malaysia; identify some risk-factors associated with *B. melitensis* in humans in Peninsular Malaysia and do a case control study of brucellosis in goat farms in four states; estimate the economic impact of brucellosis in farms in Peninsular Malaysia and isolate *B. melitensis* and molecularly characterize the isolates.

Using serosurveillance data of the last decade (2000-2009) involving 119,799 goats and 3555 farms, the seroprevalence of brucellosis among goats was generally low (0.91%) and general farms seroprevalence was 7.09%. The odds of brucellosis increased significantly ($P<0.05$) in the later part of the decade, with increase in herd size and in the western region of Malaysia.

The seroprevalence rate of brucellosis among 446 farmers and non-farmers (veterinary technical staff and others) was 1.35%. Occupation, age and drinking unpasteurized milk though not statistically significant but were considered risk factors for brucellosis based on their OR values using multivariate logistic regression at 95% confidence level. The odds of having brucellosis increased by 7.19 times in farmers compared to non-farmers (95% CI=0.82, 63.45), it increased 7.16 times in individuals 40 years and below compared with those above 40years old (95% CI=0.82, 62.97) and 4.45 times among those who drink unpasteurized milk compared to those who do not (95% CI= 0.78, 25.33).

In the case control study of 42 goat farms: introduction of new animals (OR=5.25; 90% CI=1.46, 18.88); older category of farms (OR=5.53; 90% CI=1.09, 21.66) and having only single breeds of goats on the farm (OR=8.50; 90% CI=1.27, 41.97) were significant risk factors for brucellosis on the farm using multivariate logistic regression at 90% confidence level.

Comparing fifteen farms when they had no brucellosis infection and after they were infected with brucellosis using the culling of the goats and farm value as criteria the

fifteen farms had a financial loss of at least RM 156,212.50 (USD 50,391.13) which was found to be significant ($P<0.05$) at 95% confidence level.
Brucella melitensis was isolated from 7 (5.22%) out of 134 goats in 4 states in Malaysia with vaginal swabs giving the highest isolation rate (57.14%). The isolates were phylogenetically related to other isolates from India, Iran, Israel but most closely related to isolates reported from Singapore. This indicates a wide geographical distribution of the genotypes.

This study highlights the current status of brucellosis in Malaysia and the need for more proactive measures to control and eradicate the infection from the animal and human populations. It shows that goats and humans are infected with brucellosis and certain risk factors encourage the persistence of the infection. Great losses are suffered by farmers due to brucellosis infection on their farms. There is a need to study further other aspects of the epidemiology of brucellosis in Malaysia especially the role of wild life and importation of goats.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

PREVALENS DAN FAKTOR RISIKO *BRUCELLA MELITENSIS* PADA KAMBING DAN MANUSIA DAN IMPAK KE ATAS EKONOMI DAN KESIHATAN AWAM DI MALAYSIA

Oleh

PWAVENO HULADEINO BAMAIYI
Disember, 2013

Pengerusi: Profesor Madya Latiffah Binti Hassan, PhD
Fakulti: Perubatan Veterinar

Satu kajian telah dijalankan bagi menggambarkan kejadian dan taburan *Brucella melitensis* di kalangan kambing di Semenanjung Malaysia; menganggarkan seroprevalens *B. melitensis* di kalangan sesetengah petani, kakitangan teknikal veterinar dan kakitangan lain di tiga negeri di Malaysia; mengenal pasti beberapa faktor risiko yang berkaitan dengan *B. melitensis* di kalangan manusia di Semenanjung Malaysia dan kajian kes-kawalan brucelosis di ladang-ladang penternakan kambing di empat negeri; menganggarkan kesan ekonomi ke atas bruselosis di ladang di Semenanjung Malaysia; dan menciri *B. melitensis* secara molekul.

Dengan menggunakan data pengamatan serum sedekad yang lalu (2000-2009) yang melibatkan 119,799 ekor kambing dan 3555 buah ladang, seroprevalens bagi bruselosis di kalangan kambing secara umumnya adalah rendah (0.91%) dan pada tahap ladang adalah 7.09%. Kemungkinan jangkitan bruselosis telah meningkat dengan ketara ($P<0.05$) pada akhir dekad tersebut, dengan meningkatnya bilangan ternakan terutamanya di kawasan barat Malaysia.

Kadar seroprevalens bruselosis di kalangan petani dan bukan petani (kakitangan teknikal veterinar dan lainnya) seramai 446 orang adalah 1.35%. Jenis pekerjaan, umur dan pengambilan susu yang tidak dipasteur merupakan faktor risiko yang ketara bagi bruselosis, menggunakan regresi logistik pada aras keyakinan 95%. Kebarangkalian untuk mendapat brucelosis telah meningkat sebanyak 7.19 kali di kalangan petani berbanding bukan petani (95% CI=0.82, 63.45), ianya meningkat sebanyak 7.16 kali pada individu berumur 40 tahun ke bawah berbanding mereka yang berumur melebihi 40 tahun (95% CI=0.82, 62.97) dan 4.45 kali di kalangan mereka yang meminum susu yang tidak dipasteur berbanding mereka yang tidak berbuat demikian (95% CI= 0.78, 25.33).

Dalam kajian kes-kawalan yang melibatkan 42 ladang ternakan kambing: kemasukan haiwan baru (OR=5.25; 90% CI=1.46, 18.88); kategori umur ladang (OR=5.53; 90% CI=1.09, 21.66) dan bilangan baka kambing di ladang tersebut (OR=8.50; 90% CI=1.27, 41.97) adalah faktor risiko yang ketara ki bruselosis menggunakan regresi logistik multivariat pada aras keyakinan 90%.

Dalam membandingkan 15 ladang ternakan, iaitu sebelum ia dijangkiti bruselosis dan selepas ia dijangkiti bruselosis dengan menggunakan penakaian kambing dan nilai ladang ternakan sebagai kriterianya, kesemua 15 ladang ternakan mencatatkan kerugian sekurang-kurangnya RM 156,212.50 (50,391.13 USD) yang didapati beerti ($P<0.05$) pada aras keyakinan 95%.

Brucella melitensis yang diasingkan pada 7 (5.22%) daripada 134 kambing di 4 negeri dalam Malaysia menggunakan swab vagina menunjukkan kadar pengasingan tertinggi (57.14%). PCR mengesahkan bahawa kesemua 7 isolat tersebut secara filogenetiknya adalah berkait dengan isolat lain dari India, Iran, Israel tetapi paling berkait rapat dengan isolat dari Singapura. Ini menunjukkan taburan geografi yang luas bagi genotip tersebut.

Kajian ini menonjolkan keadaan semasa bruselosis di Malaysia dan perlunya kepada langkah-langkah yang lebih proaktif bagi mengawal dan membasmi jangkitan tersebut daripada populasi haiwan dan manusia. Ia menunjukkan bahawa kambing dan manusia dijangkiti bruselosis dan faktor risiko tertentu menggalakkan penerusan jangkitan tersebut. Kerugian besar telah dialami oleh petani disebabkan oleh jangkitan bruselosis di ladang mereka. Terdapat keperluan untuk mengkaji dengan lebih lanjut aspek epidemiologi bruselosis lain di Malaysia, terutamanya peranan hidupan liar dan pengimportan kambing.

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APPROVAL SHEETS

I certify that a Thesis Examination Committee has met on the 26th December, 2013 to conduct the final examination of Pwaveno Huladeino Bamaiyi on his Thesis entitled "Prevalence and risk factors of *Brucella melitensis* in goats and humans and its economic and Public Health Impact in Malaysia" in accordance with 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 student be awarded the Doctor of Philosophy.

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This Thesis was submitted to the Senate of the Universiti Putra Malaysia and 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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DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
CBA	Cost Benefit Analysis
PCR	Polymerase Chain Reaction
CFT	Complement Fixation Test
RBPT	Rose Bengal Plate test
SAT	Serum Agglutination Test
FAT	Fluorescent Antibody Test
ELISA	Enzyme Linked Immuno-Sorbent Assay
AGPT	Agar Gel Precipitation Test
OIE	Office of International Epizootics
OIEISS	Office of International Epizootics International Standard Serum
FAO	Food and Agriculture Organization
RM	Malaysian Ringgit
USD	United States Dollars
$	United States Dollars
R	Correlation coefficient
Backward-LR	Backward Logistic Regression
χ^2	Pearson's Chi-Square
RBC	Red Blood Cells
Ad Libitum	Unrestricted, at their pleasure
FV	Farm value
ZN	Ziehl-Neelsen
TSI	Triple Sugar Iron agar Test
Sulfide	Indole Motility Test
SNP	Single Nucleotide Polymorphism
dNTPs	Deoxyribonucleotide triphosphate
MOU	Memoranda of Understanding
DVS	Department of Veterinary Services
CI	Confidence Interval
mL	Millilitres
hrs	Hours
mins	Minutes

CHAPTER 1
INTRODUCTION

1.1 Study background

Brucella melitensis is a Gram negative coccobacilli bacteria responsible for the serious and economically important zoonotic disease Brucellosis, which affects goats and other domestic animals such as cattle, sheep, dogs, pigs and camels (Seleem *et al.*, 2010). It has also been reported in marine animals (El-Tras *et al.*, 2010), wild life (Godefroid *et al.*, 2010) and various other species of animals (Adone *et al.*, 2008). It is mainly an occupational disease in man but has also been known to affect other humans who may come in contact with infected animal materials such as aborted foetuses, vaginal discharges from infected animals or consumed infected animal products such as milk, cheese and other dairy products (Sofian *et al.*, 2008).

The disease is a worldwide zoonosis infecting about 500,000 individuals annually but is more endemic in developing than the developed nations (Pappas *et al.*, 2006; Seleem *et al.*, 2010). The clinical signs of the infection in goats and other animals include: abortion, still births, infertility, low milk production and retained placenta (Poester *et al.*, 2010). The symptoms in humans are more diverse including: intermittent fever, sweating, body aches, joint pain, fatigue, dizziness, weakness, headache, depression, irritability, loss of appetite, dyspnoea, chest pain, abdominal pain and enlarged liver or spleen (Mukhtar, 2010; Sathyanarayanan *et al.*, 2011). This infection causes losses in the form of abortion, low milk yield, infertility, culling and condemnation of infected animals and can be used as a potential bioterrorism weapon of the class B (Kaufmann *et al.*, 1997; Moran, 2002).

The Malaysian government has a policy aimed to ensure food security for the rapidly growing population by increasing and improving the goat population size in Malaysia to a sustainable size that can cater for the animal-protein and dairy need of the population. In order to achieve this, more breeder goats have been brought into the country to enrich the genetic pool of the goats and many of these goats are from countries endemic for brucellosis thereby increasing the risk for the disease among susceptible livestock (Bahaman *et al.*, 2007). Recent years have seen sporadic reports of marked increase of Brucella-positive animals (Khairani-Bejo *et al.*, 2006; Al-Garadi *et al.*, 2011a, 2011b). The marked increase in the incidence and prevalence has remained unabated in spite of over 30 years of the government- initiative test and slaughter program that aims at eradicating the infection. Every year millions are spent in an attempt to eradicate the infection since the 1980s (Bahaman *et al.*, 2007). Humans who are in constant contact with these animals are at risk of contacting the highly pathogenic *B. melitensis* with its attendant consequences and problems. Yet there is dearth of information on the epidemiology, economic and public health impact of the disease in Malaysia (Bahaman *et al.*, 2007).

1.2 Justification of the study

Brucellosis in goats in Malaysia causes significant losses due to abortion and still births thereby decreasing the gross number of animals available for the population to meet the needs of food security. Milk yield is affected from infected animals which can also infect man when raw milk is consumed. Enormous animal resources are lost due to culling and condemnation of infected animals. The government spends enormous financial resources on funding research on brucellosis and compensation of farmers whose animals are culled and also in monitoring and surveillance programs as part of its eradication policy. This pathogen poses a great threat to public health more so that it is a potential bioterrorism weapon (Moran, 2002). In addition to these, the effect of the disease in humans is debilitating and grossly compromises productivity resulting in low productivity of individuals (Sathyanarayanan *et al.*, 2011).

Consequently, a few pertinent research questions that will be addressed in this study include the following:

1. What is the extent of *Brucella* infection among goats in Peninsular Malaysia?
2. What are the factors that contributed to the occurrence of *B. melitensis* among goat populations in Peninsular Malaysia?
3. What is the economic impact of Brucella infection on the animal industry in Malaysia?
4. What is the level of impact the disease in livestock has on health of the public?

1.3 Hypotheses

Three hypotheses were formulated to answer the above questions:

1. Individuals exposed to infected goats are at a higher risk of contracting brucellosis caused by *B.melitensis*.
2. Several risk factors significantly contribute to the occurrence of brucellosis
3. Brucellosis causes a significant financial impact on the animal industry.

1.4 Objectives

The hypotheses will be addressed by the following specific objectives:

1. Describe the occurrence and distribution of *B. melitensis* in goats in Peninsular Malaysia
2. Determine the seroprevalence and discuss the health impact of *B. melitensis* on farmers, veterinary technical staff and other personnel in selected states
3. Identify the risk-factors associated with *B. melitensis* in goat farms
4. Estimate the production losses attributable to *Brucella* infection
5. Isolate and molecularly characterize *B. melitensis* using single nucleotide polymorphisms and phylogenetic analysis.

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