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

PWA VENO HULADEINO BAMA IYI

FPV 2013 21
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By

PWAVENO HULADEINO BAMAIIYI

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, In Fulfilment of the Requirements for the Degree of Doctor of Philosophy

December, 2013
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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

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PWAENO HULADEINO BAMAIYI

December, 2013

Chair: Assoc. Prof. Latiffah Binti Hassan, PhD
Faculty: Veterinary Medicine

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 40 years 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 MELITENSI S PADA KAMBING DAN MANUSIA DAN IMPAK KE ATAS EKONOMI DAN KESIHATAN AWAM DI MALAYSIA

Oleh

PWA VENO HULADEINO BAMA IYI
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 bererti (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.
ACKNOWLEDGEMENTS

First and foremost all thanks are to God for His mercy and sustenance in my studies and research work and for always helping me despite my weaknesses and shortcomings before Him.

I wish to thank Assoc. Prof Dr. Latiffah Hassan, chairman of my supervisory committee for her active role in shaping my PhD work, for her patience in reviewing my thesis chapters and for her insightful comments that have improved this thesis. To my supervisory committee member Assoc. Prof Dr Siti Khairani-Bejo and Prof Dr Mohamed Zainal Abidin for their technical expertise in bacteriology and economic analysis, without them this work cannot be accomplished. They have helped me transform and adjust quickly from working with parasites all my life to now working with bacteria at PhD level. I am very grateful.

I wish to express profound thanks and gratitude to the Federal Government of Nigeria’s Education Trust Fund Scholarship and the Adamawa State University, Mubi, Nigeria, whom God used to sponsor my coming to Malaysia for studies. I thank the Vice-Chancellor, Registrar, Dean of Faculty of Agriculture, Adamawa State University for their personal interest in my progress.

I wish to acknowledge the Department of Veterinary Services of Malaysia and the Veterinary Research Institute, Malaysia for their immense contribution to this work specially data collection and laboratory work.

I wish to thank Dr Kumar Vijay whose inspiring workshops in UPM helped to make my research journey a blessed one and also my Peer Support Group friends especially Azita Asadi for being supportive and providing a friendly atmosphere that cushions the effects of academic challenges.

I must thank staff and students of the Faculty of Veterinary Medicine Universiti Putra Malaysia especially the untiring staff of the Bacteriology Laboratory and Public Health Laboratory for their patience and perseverance during the course of this research and for responding to my calls for help even at night hours! I wish to acknowledge insightful advice given to me by our visiting Professor from Cornell University, Professor Husni Mohamed, especially at a difficult time during my data analysis.

I must acknowledge specially my family, especially my kids, for bearing to stay many lonely hours when daddy was in the laboratory or away to collect samples and data and busy in the library and other places writing. I thank my beloved wife Miriam for standing by me through “thick and thin” even when a lot was at stake. I will never forget my little daughter Blessing at age 2 telling me “daddy, go do your work”.

I must thank the hard working and very diligent staff of the various states and district departments of veterinary services who laboured with me in the rural parts of Malaysia to locate farms even where GSM networks have failed to reach. The Department of Veterinary Services drivers were just too good and their four wheel drives could go where my car could not go! Thanks a lot for a great job. These staff has proved again that there is dignity in labour and that one must be proud of his
vocation at all times and do it with the whole of his heart. In this direction also, I express profound thanks to Dr Anas Bin Saleh who went to almost every farm with me until towards the end when Dr Salim wrapped it up with the remaining farms. Their personal interest in my research and taking it like their own gave me great impetus to complete the project.

I am grateful to my mum who set my feet on this part many years ago as a seasoned educationist herself. She never grew tired of calling and inquiring how I was doing in Malaysia. Thanks to all my blood brothers and sister who share in my joys and challenges and who have always been there for me.

I wish to thank all my friends in the Veterinary faculty and all over UPM too numerous to mention by name who at one time or the other assisted in small and big ways. My brothers, friends and sisters in Hope Serdang and RCCG Malaysia especially RCCG TLC Serdang and RCCG Maranatha Praise, you provided another closely knitted family for me here in Malaysia.

I must thank the Mendeley research bibliography manager company UK, Singapore and Malaysia for appointing me an Advisor of Mendeley in Malaysia and for adding more colours to my academic activities in Malaysia.

Lastly I thank all and sundry whose names if I should write down will make a thesis of its own for being part of the story of my life and my PhD research journey in UPM.
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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| Name of Member of Supervisory Committee: ZAINAL ABIDIN MOHAMED |

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