UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF BOVINE HAEMOPARASITES AND RISK FACTORS ASSOCIATED WITH TRYPANOSOMA EVANSI INFECTION IN MALAYSIA

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By

NUR MAHIZA BINTI MD ISA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Veterinary Science

June 2010

DEDICATION

Dedicated with love to my parents, husband and my son,

Muhammad Umair Darwisy

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Veterinary Science

PREVALENCE OF BOVINE HAEMOPARASITES AND RISK FACTORS ASSOCIATED WITH *TRYPANOSOMA EVANSI* INFECTION IN MALAYSIA

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June 2010

Chairman: **Reuben Sharma, DVM, MVSc, PhD, CBiol, MiBiol**

Faculty: **Faculty of Veterinary Medicine**

A cross sectional study was carried out in Peninsular Malaysia to determine the prevalence and intensity of bovine haemoparasites, and to investigate the spatial distribution, seroprevalence and the risk factors associated with *Trypanosoma evansi* infection. A total of eleven farms were sampled throughout the country involving 1562 heads of cattle from seven breeds. Blood and serum were collected from each animal in order to determine the parasitological prevalence of haemoparasites and the seroprevalence of *T. evansi* respectively. Giemsa-stained thin blood films were prepared and examined for haemoparasites. *Theileria* sp. was the most prevalent haemoparasite, being present in 16.4% of the cattle, followed by

Mycoplasma sp. (11.2%), *T. evansi* (0.6%), *Anaplasma marginale* (0.1%) and *Babesia bigemina* (0.1%). The highest prevalence and intensity of haemoparasite infection were found in the imported Nelore and Mafriwal breeds. In contrast, the local Kedah Kelantan (KK) was found to have lower prevalence and intensities of the same organisms.

In order to determine the spatial distribution and seroprevalence of *T. evansi*, Peninsular Malaysia was stratified into six geographical zones. Haematocrit Centrifugation Technique (HCT) and the CATT/*T. evansi* kit were employed to detect active parasitaemia and circulating anti-RoTat1.2 antibodies specific for *T. evansi*, respectively. In order to qualify the use of the CATT/*T. evansi* kit, 41 field isolates of *T. evansi* were screened by PCR for the presence of the RoTat1.2 VSG gene within the genome. Molecular analysis revealed that all the local isolates examined possessed the said gene, rendering the CATT/*T. evansi* kit valid for serodiagnosis. The overall parasitological prevalence of *T. evansi* in the country was 2.1% while the seroprevalence was 52.0%, much higher than previously assumed. The highest parasitaemia and seroprevalence was found in the southeastern (4.5%) and southwestern (73.1%) zones, respectively.

The risk factors associated with local bovine *T. evansi* infection was also identified by collating data from direct observations and structured questionnaires. Using binary logistic regression analyses, the cattle breed was identified as a significant risk factor for parasitaemia, while the breed, age, sex and cattle production type were identified as significant risk factors

for the presence of circulating anti-*T. evansi* antibodies. Higher risk of active *T. evansi* infection was found in the Nelore and Droughtmaster, while the Jersey crosses, Mafriwal and Friesian crosses have a higher risk of seropositivity. In conclusion, bovine haemoparasites are widespread in Peninsular Malaysia with different prevalences between the various zones. All local *T. evansi* isolates examined possess the RoTat 1.2 VSG gene, qualifying the use of the CATT/*T. evansi* kit locally. Cattle in Peninsular Malaysia are exposed to a number of pathogenic and economically important haemoparasites, with the imported exotic breeds showing a higher susceptibility compared to the indigenous KK cattle. From the parasitological viewpoint, it is apparent that the KK and its crosses maybe more suitable to develop in order to meet the demands of the expanding local beef industry.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

PREVALENS PARASIT DARAH LEMBU DAN FAKTOR RISIKO YANG BERKAITAN DENGAN JANGKITAN *TRYPANOSOMA EVANSI* DI MALAYSIA

Oleh

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Jun 2010

Pengerusi: Reuben Sharma, DVM, MVSc, PhD, CBiol, MiBiol

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Satu kajian rentas telah dijalankan di Semenanjung Malaysia untuk menentukan prevalens dan intensiti parasit darah lembu dan menyiasat taburan spatial, seroprevalen dan faktor risiko yang berkaitan dengan jangkitan *Trypanosoma evansi*. Sebanyak sebelas ladang telah diambil sampel yang melibatkan 1562 ekor lembu daripada tujuh jenis baka. Darah dan serum diambil daripada setiap haiwan untuk menentukan prevalens parasit darah dan seroprevalens *T. evansi*. Calitan darah nipis-Giemsa disediakan dan diperiksa untuk parasit darah. *Theileria* sp. adalah parasit darah yang paling prevalens, dengan kehadiran 16.4% dari lembu-lembu, diikuti dengan *Mycoplasma* sp. (11.2%), *T. evansi* (0.6%), *Anaplasma*

marginale (0.1%) dan *Babesia bigemina* (0.1%). Prevalens dan intensiti tertinggi untuk jangkitan parasit darah dijumpai pada baka Nelore dan Mafriwal yang diimport. Berbeza dengan baka tempatan Kedah Kelantan (KK) dijumpai mempunyai prevalens dan intensiti yang rendah dengan organisma yang sama.

Di dalam menentukan taburan spatial dan seroprevalen *T. evansi*, Semenanjung Malaysia dibahagi kepada enam zon berdasarkan geografi. Teknik 'Haematocrit Centrifugation Technique' (HCT) dan CATT/*T. evansi* telah digunakan untuk mengesan parasitemia yang aktif dan peredaran antibodi anti-RoTat1.2 terhadap *T. evansi.* Untuk memastikan kelayakan dalam penggunaan alat CATT/*T. evansi* , 41 isolasi lapangan *T. evansi* disaring dengan PCR untuk kehadiran RoTat1.2 VSG gene didalam genom. Molekular analisis menunjukkan kesemua isolasi tempatan yang diperiksa mempunyai gen yang disebutkan, menjadikan alat CATT/*T. evansi* sah digunakan untuk serodiagnostik. Keseluruhan prevalens *T. evansi* di negara ini adalah 2.1% manakala seroprevalens adalah 52%, lebih daripada sangkaan sebelum ini. Zon mempunyai ketinggian parasitemia dan seroprevalens yang tertinggi masing-masing dijumpai di timurselatan (4.5%) dan baratselatan (73.1%).

Pengenalpastian faktor yang berisiko untuk penyakit *T. evansi* di dalam lembu juga dikenalpasti melalui pemerhatian langsung dan soal selidik. Dengan menggunakan binari logistik regresi, hanya baka dikenalpasti sebagai faktor risiko yang signifikan untuk parasitemia manakala baka,

jantina dan jenis produksi telah dikenalpasti sebagai faktor berisiko berdasarkan pengenalpastian peredaran antibodi terhadap *T.evansi*. Risiko aktif infeksi *T.evansi* yang lebih tinggi dijumpai pada baka Nelore dan Droughtmaster, manakala kacukan Jersey, Mafriwal dan kacukan Friesian mempunyai risiko lebih tinggi untuk seropositif . Kesimpulannya, parasit darah lembu hadir meluas di Semenanjung Malaysia dengan prevalens dan seroprevalensi yang berlainan di zon yang berlainan. Kesemua isolasi *T. evansi* tempatan menunjukkan kehadiran gen RoTat 1.2, melayakkan penggunaan alat CATT/*T. evansi* untuk kegunaan tempatan. Lembu-lembu di Semenanjung Malaysia terdedah dengan beberapa parasit darah yang patogenik dan berkepentingan ekonomi, dengan baka eksotik yang diimport menunjukkan lebih mudah terkena berbanding dengan lembu KK tempatan. Dari segi pandangan parasitologikal, kemungkinan KK dan kacukannya adalah lebih sesuai dibangunkan dalam memenuhi keperluan untuk perkembangan industri lembu pedaging tempatan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Veterinary Science. 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 citations 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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NUR MAHIZA BINTI MD ISA

Date: 17 June 2010

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
BCT	Buffy Coat Technique
ca.	approximately
CDC	Centre of Disease Control
CATT	Card Agglutination Test for Trypanosomiasis
CATT/*T. evansi*	Card Agglutination Test for *Trypanosoma evansi*
DVS	Department of Veterinary Services
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
FISH	Fluorescence *in situ* Hybridization
GIS	Geographical Information System
HCT	Haematocrit Centrifugation Technique
IFA	Immunoflorescent Antibody
Inc	Incorporated
kDNA	kinetoplast -specific DNA
KK	Kedah-Kelantan
LID	Local Indian Dairy
MAECT	Miniature Anion Exchange Centrifugation Technique
MEGA	Multiplex Endonuclease Genotyping
MGE	Mobile Genetic Elements
OIE	World Organization for Animal Health
PATT	Procyclic Agglutination Test for Trypanosomiasis
PCR	Polymerase Chain Reaction

PNA	Peptide Nucleic Acid
RAPD	Random Amplification of Polymorphic DNA
rbcs	red blood cells
RFLP	Restriction Fragment Length Polymorphism
RoTat 1.2	Rode Trypanozoon Antigen Type 1.2
sp.	Species
SSR	Simple Sequence Repeat
TBF	Thin Blood Film
VATs	variable antigen types
VSGs	Variant Surface Glycoproteins

CHAPTER 1

GENERAL INTRODUCTION

Parasitism is recognized as a major impediment to economic livestock farming in many parts of the world, especially in the humid tropics (McLeod, 1995). Domestic ruminants in general, succumb to a wide array of parasites representing the major classes of helminths, arthropods and protozoa. While certain parasite taxa exert minimal pathological effects on their ruminant hosts, the haemoparasites appear to be more detrimental, causing both acute and chronic disease states (Simon *et al*., 1996). A number of haemoprotozoa and rickettsial pathogens are commonly isolated from cattle worldwide, including members of the genus *Trypanosoma*, *Theileria*, *Anaplasma*, *Babesia* and *Mycoplasma* (*Eperythrozoon*). These organisms have been recognized as important disease agents causing serious economic losses in cattle due to mortality, reduced growth rates and production, lowered working efficiency and abortions (Levine, 1985; Luckins, 1988; Kamio *et al*., 1990). With the global economic crisis and escalating costs of treatment, these pathogens pose a serious challenge to the global cattle industry.

Ruminant livestock farming is an important component of the agriculture sector in Malaysia. The government, in its efforts to achieve self-sufficiency in meat, has embarked on various plans to promote economic livestock production. Over two decades ago, the Department of Veterinary Service

Malaysia (DVS, 1986) had stressed the importance of disease surveillance and identified haemoprotozoal infections as a major parasitic condition affecting cattle in the country. The spread of these organisms are often difficult to control as they are effectively transmitted by biting arthropods, that are abundant in the tropical climate of Malaysia which favours its survival and multiplication throughout the year (Saharee and Fatimah, 1993).

Trypanosomosis, theileriosis, babesiosis, anaplasmosis and mycoplasmosis have been identified as diseases affecting bovine livestock in Malaysia (Amin-Babjee, 1978; Salleh, 1984; Abas Mazni and Zainal Abidin, 1985; Sani *et al*., 1995; Sharifah, 2001, Chin, 2007). As such, the Malaysian government requires imported cattle to be vaccinated against babesiosis and anaplasmosis prior to importation as part of its preventive measure to control the spread of these diseases (DVS, 2009). However, the problem persists particularly among naïve imported breeds which succumb to the infection, resulting in outbreaks and considerable mortality and production losses (Rajamanickam, 1977 Amin-Babjee, 1978; ~~Rajamanickam, 1977 ;~~ Fadzil and Ragavan, 1986; Chin, 2007). Theileriosis is known to cause losses in milk production and body condition among cattle (Levine, 1985; OIE, 2009) but this disease has yet to be associated with mortality in the country, albeit the high prevalence of *Theileria* in farmed cattle (Kamio *et al*., 1990). Conversely, studies in other countries have shown that this rickettsia may be highly pathogenic especially in immuno-compromised individuals (Montes *et al*., 1994).

Bovine trypanosomosis (surra) is a disease that is characterized by a myriad of nonspecific clinical signs often causing a chronic disease state that leads to loss in production, abortions and death (Hoare, 1972). These conditions often hinder the development of the bovine livestock industry in many tropical countries. Presently, there are no prevention and control measures for trypanosomosis in Malaysia and imported cattle are not screened for trypanosomes. Ironically, in Southeast Asia, the movement of livestock was identified as a key factor for the spread of *T. evansi* (Luckins, 1988; Payne *et al*., 1990; Reid, 2002). The introduction of *T. evansi* into a new environment could result in severe outbreaks of the clinical disease (surra) if appropriate control measures are not taken. In Indonesia for example, surra has been known to cause high mortality among imported buffalo and cattle (Payne *et al*., 1991a), which may be naïve to local strains. With the ever increasing trans-boundary trade in livestock, it is imperative that proper screening protocols be implemented to curtail the spread of this infectious haemoparasitic diseases. As a result, it is necessary to determine the prevalence of *T. evansi* and the current status of bovine trypanosomosis in Malaysia, in order to facilitate the implementation of proper control measures.

Despite the economic importance and worldwide distribution of bovine trypanosomosis, there remains a dearth of published reports on the epidemiology of the disease in Southeast Asia. In addition, little is known about the potential risk factors associated with the infection. Studies in Africa have shown that trypanosomes persists in certain breeds of cattle,

and this factor has been implicated in the susceptibility and occurrence of bovine trypanosomosis (Agyemang *et al*., 1992; Murray *et al*., 2004). In addition, age, sex, rainfall, and the presence of tabanid fly densities were identified as factors related to the susceptibility and infection.

While numerous studies and surveys on the occurrence of bovine haemoparasites have been conducted elsewhere (Osiyemi and Agbonlahor, 1980; Payne *et al*., 1988; El-Metenawy, 2000; Bell-Sakyi *et al*., 2004), there remains a paucity of current data on the prevalence and intensity of these pathogens among local and imported breeds of cattle in Malaysia. Therefore, this study was designed to provide baseline epidemiological information which could facilitate the planning and implementation of effective control measure to curtail bovine haemoparasite infection in the country. In addition, several putative risk factors will be investigated for its association with *T. evansi* infection in locally farmed cattle.

Aim of Research

The aim of this study is to investigate the prevalence of bovine haemoparasites in Malaysia, and to determine the spa~~zoogeographica~~tial~~l~~ spread and risk factors associated with *T. evansi* infection in various breeds of cattle in local farms.

Research Hypothesis

The hypotheses of this study are:

1. Bovine *T. evansi*, theileriosis, mycoplasmosis, anaplasmosis and babesiosis are present in Peninsular Malaysia and the prevalence and intensities of these infections varies between breeds.
2. Spatial distribution and seroprevalence of bovine *T. evansi* are different with zones and locations in Peninsular Malaysia.
3. Several risk factors such as zone, location, breed, age, sex and herd size ~~and origin~~ of cattle are associated with the prevalence of bovine *T. evansi.*

Specific objectives

The specific objectives of the study are to:

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1. Determine the prevalence and intensity of haemoparasite infection among various breeds of cattle in Peninsular Malaysia.
2. Investigate the spatial distribution ~~zoogeographical spread~~ and seroprevalence of *T. evansi* in Peninsular Malaysia.
3. Identify the risk factors associated with local bovine *T. evansi* infection~~trypanosomosi~~.

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