UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF BOVINE ANAPLASMOSIS IN MALAYSIAN FARMS

NABIL MILAD ALTHABET MIRWAN

FPV 2003 14

DEDICATION

TO THE MEMORY OF MY MOTHER

TO MY FATHER, MY STEP- MOTHER ZUHRA, MY WIFE FATMA, MY CHILDREN MOAYED, WALLA AND MOHAMMED, AND MY BROTHERS AND SISTERS, FOR THEIR MORAL SUPPORT AND ENCOURAGEMENT.

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine, in partial fulfillment of the course VPD 5908-Project.

PREVALENCE OF BOVINE ANAPLASMOSIS IN MALAYSIAN FARMS

BY

NABIL MILAD ALTHABET MIRWAN

Nov 2003

Supervisor: Dr. Siti Zubaidah Ramanoon

The main objective of this cross sectional study was to determine the prevalence of bovine anaplasmosis in Malaysian farms involving Selangor and Pahang and to determine the potential risk factors related to the seroprevalence. A total of 180 animals from seven dairy farms and 12 beef farms were involved in this study.

A questionnaire was used to collect data on cattle health and management practices. Seven dairy and six beef farms participated. Sixty-eight blood samples in heparin from dairy cattle were tested for Anaplasma sp. using Giemsa thin smear and no parasitemia was observed. One hundred and eighty sera from dairy and beef cattle farms were tested for antibodies to *A. marginale* using competitive enzyme linked immunosorbent assay kit (cELISA kit).

The seropositivity of *A. marginale* was 87.7% (158/180) when a cutoff of ≥ 30% was considered as positive. The seroprevalence of bovine anaplasmosis in smaller farms (30-60 animals) were significantly ($p < 0.05$) higher at 96.7% compared to bigger farms (> 60 animals) (78.8%). Farm type, breed, age, and sex were not significant factors. There were also no correlation between antibody prevalence status and packed red cell volume, body condition score and rectal temperature in the dairy animals studied.

Keywords: Cattle, anaplasmosis, prevalence, questionnaire, cELISA

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi keperluan kursus VPD 5908 – Projek.

PREVALENS ANAPLASMOSIS LEMBU DI LADANG- LADANG MALAYSIA

OLEH

NABIL MILAD ALTHABET MIRWAN

Nov 2003

Penyelia: Dr. Siti Zubaidah Ramanoon

Objektif utama kajian melintang adalah untuk mengkaji prevalens anaplasmosis lembu di ladang- ladang Malaysia melibatkan Selangor dan Pahang dan mengkaji faktor- faktor penyebab yang berkaitan dengan seroprevalens. Sejumlah 180 ekor lembu dari tujuh ladang lembu tenusu dan 12 ladang lembu pedaging terlibat dalam kajian ini. Soalselidik telah digunakan untuk mengumpul data kesihatan lembu dan amalan pengurusan. Enam puluh lapan sampel darah dalam heparin dari lembu tenusu telah diuji untuk Anaplasma sp. menggunakan pewarna Giemsa dimana tiada parasitemia dikesan. Seratus lapan puluh sampel sera dari lembu tenusu dan pedaging telah diuji untuk antibodi terhadap *A. marginale* menggunakan *competitive enzyme linked immunosorbent assay kit* (cELISA kit).

Seropositiviti untuk *A. marginale* adalah 87.7% (158/180) apabila nilai "cutoff" sama atau lebih dari 30% dianggap positif. Seroprevalens anaplasmosis lembu di ladang kecil (30-60 lembu) adalah signifikan ($p<0.05$) pada 96.7% berbanding ladang besar (> 60 lembu) (78.8%). Faktor jenis ladang, baka, umur dan jantina adalah tidak signifikan. Tiada korelasi di antara prevalens antibodi, sel darah merah mampat, skor badan dan suhu rektum pada lembu tenusu yang di kaji.

Kata kunci: Lembu, anaplasmosis, prevalens, soalselidik, cELISA

ACKNOWLEDGMENT

All praise to Almighty Allah, the Merciful and the Benevolent. Had it not been due to his will and favour, the completion of my study would not have been possible.

I would like to express my sincere gratitude and appreciation to my supervisor, Dr. Siti Zubaidah Ramanoon; she has devoted a lot of her time for invaluable advice, guidance, support and patience and kind encouragement throughout my study.

I wish to express my sincere gratitude to my co-supervisor Associate Professor Dr. Abdul Rahman Omar for his kind encouragement, guidance and sincere constructive comments.

Special thank go to Dr. Goh Yong Meng and Dr. Nadzri Salim for their assistance in the data analysis, Dr. Siti Khairani Bejo and Maureen for their assistance in collecting samples from Pahang, Dr. Zainor Mohamad and his assistants (JPH, Pahang) for the questionnaire for Pahang farmers, and Dr. Chandrawathani of the Veterinary Research Institute, Ipoh who provided me useful information on bovine anaplasmosis status in Malaysia.

I am especially grateful to the Ministry of Education and Al-Fateh University, Libya for providing the scholarship during my study.

I would also like to thank all farmers in Selangor and Pahang who allowed me to carry out this project using their precious animals.

I also acknowledged the skillful assistance and untiring help rendered by the staff of the parasitology and biologic laboratories, Mr. Lee Chu Chong, Encik Rashid Abdul Rahman and Mrs. Rodiah Husin and other colleagues who had in one way or another

helped to make the project a success. I am very grateful to Mr. Mior Mohd Nizam and Mr. Ghazali and those who were involved either directly or indirectly during my study. Continuous moral support and encouragement from my father, step-mother Zuhra Serkez, my sisters and brothers during my study are deeply appreciated.

Last but not least, grateful thanks are also conveyed to my supportive wife Fatma Ali Algriany and beloved children Moayed, Walla, and Mohammed for their moral support, encouragement and sacrifice especially during the periods I have been away. To them I remained indebted.

CERTIFICATION

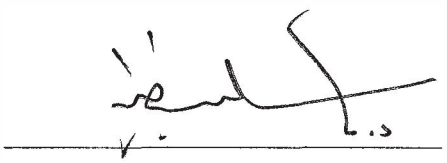
It is hereby certified that we have read this project paper entitled "Prevalence of Bovine Anaplasmosis in Malaysian farms" by Nabil Milad Althabet Mirwan and in our opinion; it is satisfactory in terms of scope, quality and presentations as fulfillment of the requirement for the degree of Master of Veterinary Medicine, VPD 5908 Project.

DR. SITI ZUBAIDAH RAMANOON
DVM (UPM), MSc. (Guelph)
Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

ASSOC. PROF. DR. ABDUL RAHMAN OMAR
DVM (UPM), PhD (Cornell)
Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-supervisor)

DECLARATION

I hereby declare that the project paper is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any degree at UPM or other institutions.



Nabil Milad Althabet Mirwan
Date: 14.11.03

TABLE OF CONTENTS

LITST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

(aa)	Amino acid
BCS	Body Condition Score
CAT	Card Agglutination Test
cELISA	competitive Enzyme Linked Immunosorbent Assay
CFT	Complement Fixation Test
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
HRP	Horseradish Peroxidase
IFAT	Immunofluorescent Agglutination Test
IgG	Immunoglobulin G
ILRAD	International Laboratory Research of Animal Diseases
kg	Kilogramme
LAT	Latex Agglutination Test
MAb	Monoclonal Antibody
MBP	Maltose Binding protein
mg	Miligramme
MHC	Major Histocompatibility Complex
ml	Milliliter
MSP	Membrane Surface Protein
nm	Nanometre
nPCR	nested Polymerase Chain Reaction

O.D.	Optical Density
PCR	Polymerase Chain Reaction
PCV	Packed Red Cell Volume
PI	Percentage Inhibition
RBC's	Red Blood Cells
RIA	Radioimmunoassay
r.p.m	round per minute
rs	Correlation Coefficient
SPSS	Statistical Package for the Social Science
°T	Rectal Temperature
µl	Microlitre
µm	Micrometre
VMRD	Veterinary Medical Research & Development

CHAPTER 1

INTRODUCTION

Bovine anaplasmosis is an infectious, non contagious, hemoparasitic disease caused by *Anaplasma marginale* (*A. marginale*) a member of Rickettsiales. It is transmitted usually by biological arthropods and mechanically.

A. marginale has the widest geographical distribution among the pathogenic anaplasma (Rodostits *et al.,* 2000) where their vectors are abundant. It can affect cattle, buffaloes, camel and wild ruminants like deer (Wanduragala and Ristic, 1993). Throughout the tropics, an estimated 600 million cattle are exposed to anaplasmosis and babesiosis (ILRAD, 1991). Anaplasmosis in livestock has received little attention in Malaysia with only occasional reports in cattle (Chandra & Lee, personal communication). Therefore information on the extent of infections throughout this country is not adequate.

In Peninsular Malaysia, the cattle population was estimated at 676,847 in 2001 (Malaysia, 2001). This figure comprised 650,663 heads of beef cattle and 26,184 heads of dairy cattle respectively. Cattle farms may be exposed to anaplasma infection in this country if a systemic control programme is not be established.

Diagnosis of hemoparasitic diseases in livestock in lesser developed countries is impeded by the lack of sensitive and specific assays (Guglielmone, 1995).

A number of epidemiological surveys on bovine anaplasmosis caused by *A. marginale* have been reported using a variety of serological test in the tropical and subtropical zones (Paull, 1980; Teclaw *et al.,* 1985; World Organization of Animal Health, 1996). Recently, competitive enzyme linked immunosorbent assay (cELISA) has become available for serological diagnosis and survey (Ndung'u *et al.,* 1995; Torioni de Echaide *et al.,* 1998, Renya-Bello *et. al.,* 1998; Bowles *et al.,* 2000).

It is indeed a need to study the prevalence of this disease in order to establish the design of effective control measures and to obtain data on bovine anaplasmosis among Malaysian cattle where no previous studies have been reported on this disease.

The objectives of this study were to determine the:

1. prevalence of *A. marginale* infection and antibody in selected small cattle farms and association with selected risk factors such as herd size, herd type, sex, age, and breed.
2. correlation between the antibody prevalence and body condition score (BCS), packed red cell volume (PCV) and rectal temperature (T^{o}) of the animal.

CHAPTER 2

LITERATURE REVIEW

2.1 Definition, Etiology and prevalence

Bovine anaplasmosis is a tick-borne disease of cattle and wild ruminants caused by *Anaplasma marginale* (*A. marginale*), which are obligating intraerythrocytic parasites and belonging to the order Rickettsiales.

The organism was first described as "marginal points" in bovine erythrocyte and the term Anaplasma, implies that the organism is devoid of cytoplasma and the species names marginale and centrale refer to the location of the organism within infected erythrocyte (Daniel, 2000). However, identification depends upon its appearance in the infected erythrocyte as a coccoid or ring like body 0.2-0.4μm in diameter, staining dark violet by Giemsa and mainly located near the periphery of the cell in the species *marginale* (Wanduragala and Ristic, 1993).

As presently classified, the primary pathogenic Anaplasma species is *A. marginale* infection in cattle and by *A. ovis* infection in sheep and goats. Historically, a third species, *A. centrale,* has been isolated by Theiler as causing mild disease in cattle (Daniel, 2000). Recently *A. centrale* has been used as a live vaccine to induce partial protection against *A. marginale* (Rodostits *et al.,* 2000).

The prevalence of bovine anaplasmosis varied with diagnostic techniques used throughout the world. This disease has remained a serious constraint to livestock production in tropical and subtropical regions (Rodostits *et al.,* 2000).

However, it is also a significant problem in temperate regions. Table 1. 2 shows the prevalence status in different countries throughout the world. The prevalence of infection in cattle in tropical and subtropical regions (endemic areas) is high with seropositivity rates varying from 98.60% to 57.7%, while in other regions which are not endemic, seropositivity rates also varied from 2.2% to 48.6%.

Table 2.1: Prevalence of bovine anaplasmosis worldwide

Country (Region)	No. of [a] cattle sampled	Diagnostic method [b]	No. and % cattle seropositivity	References
Nigeria*	573	CAT	34	Obi Tu., (1978)
Venezuela*	376	IFAT	57.7	James *et al.*, (1985)
		LAT	48.6	
Nigeria*	500	IFAT	79.4	Ajayi and Dipeolu, (1986)
		CAT	25	
Gambia**	184	CFT	0	Kuttler *et al.*, (1988)
		CAT	0	
St. Lucia, Guyana*	249	IFAT	70	Hugh-Jones *et al.*, (1988a)
Zambia*	1,784	CAT	38.6	Jongejan *et al.*, (1988)
Louisiana**	11,085	CAT	7.8	Morley and Hugh-Jones, (1989)
Paraguay*	1,228	CAT	92	Payne and Osorio, (1990)
Cameroon**	524	Blood smear	2.2	Ndi *et al.*, (1991).
Austria**	5076	CFT	2.1	Baumgartner *et al.*, (1992)
Mexico*	368	CFT	69.2	Cossio-Bayugar *et al.*, (1997)
		PCR	54.6	
Illinois, USA**	4994	CAT	6.4	Hungerford and Smith, (1997)
South Africa*	151	cELISA	98.60	Dreyer *et al.*, (1998)
Brazil*	410	cELISA	87.5	Vidotto *et al.*, (1998)
Morocco**:		cELISA		Sahibi *et al.*, (1998)
Gharb zone	258		12.7	
Haouz zone	217		4.1	
Bolivia**	380	ELISA	32.1	Carrique *et al.*, (2000)

[a]=beef & dairy cattle, [b]= cELISA (competitive enzyme liked immunosorbent assay),
CAT (card agglutination test),
IFAT (immunofluorescent antibody test),
PCR (polymerase chain reaction),
LAT (latex agglutination test),
CFT (complement fixation test)

* = endemic area, ** =non endemic area

2.2 Epidemiology

Anaplasmosis in cattle is common on all six continents, being present in South Africa, Australia, Asia, the former USSR, South America and the United States (Wanduragala and Ristic, 1993).

It is transmitted by a diverse group of biological and mechanical vectors. Infection in cattle is endemic in tropical and subtropical areas that support large populations of these vectors and infection occurs more sporadically in temperate climate (Rodostits *et al.,* 2000). Endemic regions reflect a high tick infection rate and are maintained by the prevalence of both competent arthropod vectors and persistently infected carrier cattle. These carrier cattle, which are typically asymptomatic, are efficient reservoirs for tick-borne transmission (Eriks *et al.,* 1993).

In the United States the disease has occurred beyond the boundaries of tick-infested areas and the area distribution in Europe has been advanced northward in recent years with sporadic cases in France, Switzerland, the Netherlands, Hungary and Austria (Rodostits *et al.,* 2000). Infection is endemic in Southeastern and much in the west coast of the United States (Kuttler, 1979). The principal biological vector in west coast is *Dermacentor occidentalis and* the southeastern region where tabanid flies are the mechanical vectors (Kuttler, 1979).

In Australia infection is closely related to the distribution of *Boophilus microplus* which is restricted to the northern areas (Bock *et al.,* 1999). Differences in enzootic and epizootic areas in South Africa are also related to tick distribution and climate (Potgieter, 1981).

In Malaysia, the existence of cattle infected with *A. marginale* has been noted in a local area using conventional methods (Giemsa stain) (Chandra, personal communication). Other than that no epidemiological studies of Anaplasmosis have been reported. In addition the determinants of tick- and fly-borne transmission are not well understood

2.3 Methods of transmission

The source of infection is always the blood of an infected animal. Once infected the animal remains a carrier for many years, probably for life, even though the parasite may not be always demonstrable in the blood (Kiesser *et al.,* 1990). Rickettsemia can be demonstrated, however by inoculation of splenectomized calves with carrier blood (Luther *et al.,* 1980) and by nucleic acid probe analysis (Eriks *et al.,* 1989a; Goff *et al.,* 1990).

A variety of species of wild ruminants in both North America and Africa can be infected and may have been significant reservoirs for the disease (Potgieter, 1979; Zaugg, 1985). Wild deer have been regarded as equally important reservoirs with cattle and Anaplasma may survive in nature through deer-to-deer (Wanduragala and Ristic, 1993).

Spread from animal to animal occurs chiefly by insect vectors. A variety of arthropods may act as vectors but significant natural vectors are ticks in the *Ixodidae* and flies in the family *Tabanidae*. Of the ticks, the one host *Boophilus* spp. are of major importance in tropical and subtropical regions and the three host *Dermacentor* spp. of major importance in the western US (Wanduragala and Ristic, 1993).

In Australia, the ticks *Boophilus microplus* are the most vectors of bovine anaplasmosis (Bock *et al.,* 1999) which, also probably the most vector of this disease in Malaysia (Rajamanickam, 1987) and in South Africa *B . microplus, B. decoloratus,* and *Rhipicephalus simus* (Daniel, 2000). In the United States *B. annulatus, D. andersoni*, *D. variables, Argas persicus* and biting flies of tabanid species and eye gnats (*Hippelates pusio*) (Wanduragala and Ristic, 1993) also act as vectors.

The organism undergoes a complex developmental cycle in the gut cells of ticks and the final infective stage is present in the salivary gland (Kocan *et al.,* 1992). Ticks transmit *A. marginale* biologically after extensive multiplication occurs in several tick tissues. Tick transmission is from stage to stage (transstadial) or within a stage (intrastadial), but transovarial transmission from one generation to the next does not occur.

Male ticks develop persistent generalized infection in which many tick cell type become infected with *A. marginale* (Kocan *et al.,* 1992). Intrastadial transmission by male ticks is believed to be an important mechanism of transmission because male ticks can become infected after a short feeding period on an infected bovine and then transmit infection during repeated feeding on multiple susceptible cattle (Kocan *et al.,* 2000).

Male ticks, therefore, serve as a reservoir of infection. They readily become infected after feeding on persistently infected cattle and the percentage of infected ticks is related to the parasitemia during feeding (Eriks *et al.,* 1993). However, once ticks become infected, the infection rate in individual ticks is similar because of extensive

multiplication in tick cells. Tick infection rates are unaffected by bovine antibodies ingested during tick feeding on *A. marginale* immune cattle (Kocan *et al.,* 2000).

Bovine anaplasmosis may also be spread mechanically via any blood-contaminated fomite, including contaminated needles, dehorning instruments, nose tongs, tattooing instruments, ear tagging devices, embryo transplants and castration instruments (Rodostits *et al.,* 2000).

2.4 Pathogenesis, clinical signs and post mortem findings

Anaplasmosis generally is categorized into four stages: incubation, developmental, convalescent, and carrier. The incubation period is the time from which the organism is introduced into the susceptible animal until one percent of red blood cells (RBC's) become infected (Wanduragala and Ristic, 1993). The length of this stage seems to vary directly with the number of organisms the animal is exposed to and no clinical signs will be seen at this time (Richery *et al*., 1977). During the incubation period, the packed cell volume (PCV) will remain constant, as RBC's will be produced at the same rate they are destroyed (Susan, 1998).

The developmental stage and clinical onset of anaplasmosis is determined by the incubation period, which can be from 15 to 45 days depending on the animal.

The convalescent stage extends from the appearance of reticulocytes to the return to normal of the various blood values. The length of this stage varies greatly and may extend from a few weeks to a few months. The differentiation between developmental and convalescent stages is evidence of increased erythropoiesis on

the stained blood smears. The signs of increased erythropoiesis in the peripheral blood, which identifies the convalescent stage, are reticulocytes, polychromatophils, basophilic stippled cells, normoblasts, increased hemoglobin and an increase in the total white cells.

The carrier stage is usually thought to as that time extending from the disappearance of discernible anaplasma bodies sometime during the convalescent stage to the end of the animal's life. Clinically recovered animals remain carriers with a non-detectable parasitemia and thus act as a reservoir of the disease. (Wanduragala and Ristic, 1993).

Clinical signs begin to appear as RBC production drops and more erythrocytes are parasitized and destroyed (Richery *et al.,* 1977). Studies indicate that some animals show signs at as little as ten percent loss of RBC's, (Wanduragala and Ristic, 1993) while others indicate a 65% loss before onset of clinical signs, with a mean some-where between 35% and 50% (Susan, 1998; Zaugg *et al.,* 1985). Erythrocyte count, PCV, and hemoglobin values will be severely reduced following the developmental period (Susan, 1998). As the anemia becomes more severe, the acutely infected animals lose condition rapidly. Icterus, weight loss, dehydration, constipation with hard dry feces shaded green, dark yellow urine, and progressive respiratory signs may become evident. Moreover, aggressive behavior, abortion of pregnant animals, and death due to hypoxia may occur.

An animal that survives about with anaplasmosis requires a convalescent period of up to three months, with hematologic parameters (blood values) returning to normal.