



UNIVERSITI PUTRA MALAYSIA

***COMPARATIVE DIAGNOSIS, PATHOGENICITY AND
IMMUNOSUPPRESSIVE EFFECT OF *Trypanosoma evansi* INFECTION
IN GOATS***

IBRAHIM ABUBAKAR ANKA 1

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IMMUNOSUPPRESSIVE EFFECT OF *Trypanosoma evansi*
INFECTION IN GOATS**

By

IBRAHIM ABUBAKAR ANKA 1

**This Thesis Submitted to the School of Graduate Studies, Universiti
Putra Malaysia, in Fulfillment of the Requirement for the Degree of
Doctor of Philosophy**

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DEDICATION

This study is dedicated to:

- our master and seal of the Prophets, Muhammad (P.B.U.H);
- my late father Alhaji Abubakar Anka 1 (Allah Rufa Asiri) and my mother Hajiya Fatima `Yar-Hawwa;
- my wife Rukayyah Usman Danladi and children: Ahmad (‘Karibullah) and Hamidah;
- my late brother Alhaji Abdullahi (Mai-Gwandu) and late sister Hajiya Maryam (Yalwa), may their souls rest in Allah’s Rahma, amin!
- Every healthy mind. A mind which puts truth to test and knows it from the wreck of the wrong. A mind which weighs all that has been said in the scale of justice and always comes out in favour of reason. A mind which compares words and sayings, and has the ability to distinguish between the logical and not so logical, and between strong and feeble.

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

COMPARATIVE DIAGNOSIS, PATHOGENICITY AND IMMUNOSUPPRESSIVE EFFECT OF *Trypanosoma evansi* INFECTION IN GOATS

By

IBRAHIM ABUBAKAR ANKA 1

January 2012

Chairman: Assoc. Prof. Rehana Abdullah Sani, PhD

Faculty: Faculty of Veterinary Medicine

Conflicting information exist on the pathogenicity of *T. evansi* infection (Surra) in goats. This has made the diagnosis and reservoir status of goats in the epidemiology of *T. evansi* infection uncertain. Therefore, Surra remains a significant challenge and threat in endemic and disease free areas. This study was therefore designed to answer the questions whether one, the pathogenicity of *T. evansi* infection in goats depends on the isolate? two, CATT/ *T. evansi*[®] kit can be used in seromonitoring Malaysian isolates of *T. evansi* infection in goats? and three, *T. evansi* infection can compromise caprine immune response to intranasal pneumonic Mannheimiosis vaccination (IPMV)?

Experimental infection of goats was carried out for the assessment of CATT/*T. evansi*[®] in the detection of predominant RoTat 1.2 antibodies in three Malaysian (two bovine and a deer) isolates of *T. evansi* to determine its usefulness in the diagnosis of caprine Surra in relation to parasitaemia. During the same experiment comparison of the pathogenicity of two out of the three *T. evansi* (bovine and deer) isolates was investigated; and a field survey was carried out in eight goat farms from six districts in the state of Selangor to determine the prevalence of *T. evansi* infection in 228 goats using CATT/*T. evansi* and Haematocrit Centrifugation Technique (HCT). In another experiment, the immunosuppressive effect of *T. evansi* infection in goats vaccinated with IPMV was investigated using cellular and antibody (lung IgA and serum IgG) responses.

The first experiment showed that all infected goats remained positive for *T. evansi* until termination of the experiment on day 62 post-infection following treatment with diminazine diacetate injection. Both isolates of *T. evansi* induced fever (except in one goat), anaemia (more severe in the group infected with the bovine *T. evansi* isolate), neutrophilia, eosinophilia, lymphocytosis and monocytosis, and hyperproteinemia, hypoalbuminemia, decreased albumin-to-globulin ratio, hyperbilirubinemia, decreased alanine transaminase and creatine kinase and mild weight loss. No mortality was recorded among the infected goats which recovered after treatment.

The CATT/*T. evansi* results for the three infected groups maintained similar high scores average of >3 until the animals were treated when the scores dropped drastically thereafter with a score average of <2. No trypanosomes were detected after treatment.

The prevalence study shows high seroprevalence of 61.4% using CATT/*T. evansi*. Only 6 (4.3%) among the seropositive goats had CATT/*T. evansi* score of >3. However, no parasite was detected by HCT.

Neutrophilia and eosinophilia in the peripheral blood suggests evidence of immunosuppression in the experiment. However, no significant difference ($P>0.05$) was found in the serum and lung lavage fluid antibody titers between the *T. evansi* infected, infected-treated and control groups.

It was concluded that firstly, the CATT/*T. evansi*[®] is useful in the seromonitoring of *T. evansi* infection in goats, secondly, the two isolates of *T. evansi* induced similar pathogenicity in goats, thirdly, there was high seroprevalence of *T. evansi* in the study area suggesting that the goats were exposed to the natural infection and fourthly, at a given infective dose of 10^4 *T. evansi* per animal, did not prevent goats from mounting protective immune response against lethal *Mannheimia haemolytica* challenge following IPMV.

Abstrak tesis ini dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Doktor Falsafah

**PERBANDINGAN DIAGNOSTIK, KEPATOGENAN DAN KESAN
IMMUNOSUPRESIF OLEH JANGKITAN *Trypanosoma evansi* DI
DALAM KAMBING**

Oleh

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Kekeliruan fakta berlaku mengenai kepatogenesis jangkitan *T. evansi* di dalam kambing. Ini menyebabkan diagnostik dan status takungan ('reservoir) di dalam epidemiologi *T. evansi* tidak menentu. Oleh yang demikian, Surra kekal sebagai cabaran yang signifikan dan ancaman di kawasan yang endemik mahupun di kawasan bebas penyakit. Kajian ini direka bagi menjawab kepada soalan-soalan seperti: satu, kepatogenesis jangkitan *T. evansi* dalam kambing bergantung kepada isolasi? dua, Kit CATT/*T. evansi*[®] boleh digunakan dalam pemantauan isolasi Malaysia dalam jangkitan *T. evansi* dalam kambing? tiga, dan infeksi *T. evansi* boleh mengkompromi respons

keimunan kambing untuk 'intranasal pneumonic Mannheimiosis vaccination (IPMV)'?

Ujikaji jangkitan dalam kambing dijalankan dalam mentaksirkan kit CATT/*T. evansi*[®] dalam pengesanan antibodi predominan RoTat 1.2 dalam tiga isolasi *T. evansi* Malaysia (dua lembu dan satu rusa) untuk menentukan kegunaannya dalam diagnosis Surra di dalam kambing dalam hubungannya dengan parasitemia. Semasa ujikaji yang sama, perbandingan patogenik dalam dua isolasi *T. evansi* (lembu dan rusa) disiasat; dan kajian lapangan dijalankan dalam lapan ladang kambing daripada enam daerah Selangor untuk menentukan jangkitan *T. evansi* prevalensi dalam 228 kambing dengan menggunakan CATT/*T. evansi* dan Haematocrit Centrifugation Techniques (HCT). Dalam ujikaji yang berlainan, kesan immunosupresif dalam infeksi *T. evansi* dalam kambing yang diberi pemvaksisan dengan IPMV disiasat menggunakan selular dan antibodi (IgA paru-paru dan serum IgG) jawapan.

Keputusan ujikaji yang pertama menunjukkan bahawa kesemua kambing yang dijangkiti kekal positif untuk *T. evansi* sehingga penghabisan ujikaji hari ke 62 iaitu diikuti dengan suntikan diminazine diacetate. Kedua-dua isolasi menyebabkan demam (kecuali dalam seekor kambing), anemia, neutrofilia, oesinofilia, limphositosis dan monositosis, dan hyperproteinemia, Hypoalbuminemia, pengurangan albumin-kepada-globulin ratio, hyperbilirubinemia, pengurangan alanine transaminase dan creatine kinase

dan kehilangan berat badan. Tiada kematian direkodkan dalam kambing yang dijangkiti dimana pulih selepas rawatan.

Keputusan CATT/*T. evansi* untuk dua kumpulan kambing yang dijangkiti kekal sama dengan skor tertinggi ialah >3 sehingga haiwan tersebut dirawat dimana skor jatuh secara drastic dengan purata <2 . Tiada trypanosome ditemui selepas rawatan kepada kambing.

Prevalens kajian menunjukkan seroprevalens yang tinggi 61.4% dengan menggunakan CATT/*T. evansi*. Hanya 6 (4.3%) dikalangan kambing yang seropositif mempunyai CATT/*T. evansi* >3 . Akan tetapi, tiada parasit ditemui dalam HCT.

Neutrofilia dan eosinofilia didalam darah periferal menunjukkan bukti berlakunya immunosupresi di dalam ujikaji immunosupresif. Akan tetapi, perbezaan yang signifikan dijumpai dalam antibodi serum dan 'lavage' cecair peparu diantara haiwan yang dijangkiti *T. evansi*, dijangkiti *T. evansi* dan dirawat dan kumpulan kawalan.

Dapat disimpulkan bahawa, pertamanya, CATT/*T. evansi* adalah berguna di dalam kawalan serologi dalam jangkitan *T. evansi* dalam kambing, kedua, dua isolasi *T. evansi* mendorong kepatogenan yang sama di dalam kambing, ketiga, terdapat seroprevalens yang tinggi dalam kawasan kajian yang mencadangkan kambing adalah kerap terdedah kepada infeksi semula jadi

dan, keempatnya, untuk dos 10^4 *T. evansi* (per haiwan), jangkitan tidak akan mencegah kambing untuk meninggikan respons keimunan setelah cabaran dengan *Mannheimia haemolytica* maut susulan daripada IPMV.



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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 institutions.

Ibrahim Abubakar Anka 1
Date: 11th January 2012

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LIST OF ABBREVIATIONS

-	Negative
±	Weakly positive
+	Positive
++	Strongly positive
+++	Very strongly positive
°C	Degree celcius
ALT	Alamine aminotransferase
AST	Aspartate aminotransferase
CATT	Card Agglutination for Trypanosomiasis
CATT/ <i>T. evansi</i>	Card Agglutination for Trypanosomiasis for
CATT/ <i>T. evansi</i>	<i>Trypanosoma evansi</i>
CK	Creatine kinase
DNA	Deoxyribonucleic acid
DVS	Department of Veterinary services
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
HCT	Haematocrit centrifugation technique
Hgb	Haemoglobin
ID	Identification number
IPVM	Intranasal pneumonic Mannheimiosis vaccination

MCHC	Mean haemoglobin concentration
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volum
PCR	Polymerase chain reaction
RBC	Red blood cell
RoTat 1.2	Rhode antat 1.2
Sp.	Species
T Lymphocyte	Thymus dependant lymphocyte
TP	Total protein
VSG	Variable surface glycoprotein

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CHAPTER ONE

INTRODUCTION

Trypanosoma evansi is a flagellated obligate blood protozoa, and is transmitted mechanically by biting flies mainly tabanids (Reid, 2002). It has the widest geographical distribution among the pathogenic trypanosome species, existing in Africa, South America, most of Asia and some parts of Europe. *T. evansi* infection affects a wide range of domestic and wild mammals including horses, donkeys, camels, buffaloes, cattle, sheep, goats and pigs. The disease is called "Surra" and causes serious economic losses due to vaccination failure, weight loss, abortion and mortality (Gutierrez *et al.*, 2006).

It was previously thought that goats are refractory host for *T. evansi* (Gill, 1977). However, existing literature show conflicting reports on the pathogenic effect of *T. evansi* in goats. Some studies have reported severe disease with mortalities (Manuel, 1998; Gutierrez *et al.*, 2005), while others documented mild and asymptomatic infections in both naturally and experimentally infected goats (Otieno and Gacanja, 1976; Boid *et al.*, 1981). This could be attributed to variation in pathogenicity and virulence of the *T. evansi* strains involved, different breeds of goats, size of inoculum, route of

infection, host and geographical origin of the parasite (Dia, 1995; Queiroz *et al.*, 2000). However, there are no comparative studies on the pathogenicity using different isolates of *T. evansi* in goats, which could enable proper assessment of the role of goats in the epidemiology of Surra.

The diagnosis of caprine Surra is difficult due to the lack of pathognomonic clinical signs, low levels of parasitaemia coupled with the few available reports on prevalence and incidence of caprine Surra, has made the reservoir status of goats uncertain (Gutierrez *et al.*, 2006). The Card Agglutination Test for Trypanosomiasis (CATT/*T. evansi*), is a widely used rapid serological test for detection of *T. evansi* (Bajyana Songa and Hamers, 1988). The test is only capable of detecting antibodies against the RoTat 1.2 predominant antigen of *T. evansi*, which is found to be absent in certain strains in Kenya (Ngaira *et al.*, 2004) and Sudan (Salim *et al.*, 2011). Therefore, it has become necessary to evaluate the effectiveness of CATT/*T. evansi* for local strains of *T. evansi* before it can be used for screening of samples in any geographical entity.

T. evansi infection is considered endemic in Peninsular Malaysia (Zainal-Abidin, 1992) where natural infection with occasional outbreaks have been reported in cattle, buffaloes (Abas-Mazni *et al.*, 1987; Sani *et al.*, 1989, 2009; Cheah *et al.*, 1999; Md-Isa 2010), horses (Ng and Vanselow, 1978; Ikede *et al.*, 1983; Cheah and Azman, 2000), deer (Adrian *et al.*, 2010), pigs (Arunasalam *et al.*, 1995), dogs (Rajamanickam *et al.*, 1985) and rhinoceros

(Vellayan et al, 2004). Information is lacking, however, on the prevalence of *T. evansi* in goats in Malaysia (Zainal-Abidin, 1992). Goats are important in the Malaysian livestock industry with a growing population reaching about 500,000 heads due to high importation rate (DVS, 2008) and considered as potential reservoir of *T. evansi* infection (Gutierrez *et al.*, 2006). Therefore, it is necessary to investigate its possible role in the epidemiology of *T. evansi* infection in Malaysia.

Immunosuppression is a common feature associated with trypanosomosis in chronically infected animals, which can lead to immune compromise in vaccinated animals and secondary infection (Sileghem *et al.*, 1994). Previous studies (Singla *et al.*, 2010) had investigated the immune responses of *T. evansi*-infected animals which had been parenterally administered with vaccines against bacterial infections and correspondingly, there is no literature that documents the immunosuppressive effect of *T. evansi* in animals vaccinated with intranasal vaccines against pneumonic manheimiosis, in which vaccination failure has been frequently observed in Malaysia (Zamri-Saad and Sabri, 2008).

Therefore, Surra remains a significant challenge and threat in endemic and disease free areas, respectively. This study was therefore designed to address issues concerning the prevalence, diagnosis, comparative pathogenicity and immunosuppressive effect of *T. evansi* infection in goats.

The research hypotheses of the study are:

1. CATT/*T. evansi* can detect infection in goats;
2. *T. evansi* infection is endemic in goats in the state of Selangor, Malaysia;
3. pathogenicity depends on virulence of *T. evansi* isolate and;
4. *T. evansi* infection can compromise immune response of intranasal Pneumonic Mannheimiosis vaccine.

The objectives of the study are to:

1. determine the prevalence of *T. evansi* in goats in Selangor;
2. compare and assess the Card Agglutination Test for Trypanosomiasis (CATT) for the detection of antibodies in experimental infection of goats with three Malaysian field isolates of *T. evansi*;
3. compare the clinical and haematological changes in goats experimentally infected with two isolates of *T. evansi*;
4. compare the biochemical changes in goats experimentally infected with two isolates of *T. evansi* and;
5. determine the immunosuppressive effect of *T. evansi* infection in goats given intranasal Pneumonic Mannheimiosis vaccine.

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