
**UNIVERSITI PUTRA MALAYSIA**

# *STRESS PROFILING OF THE HEMATOLOGICAL, BIOCHEMICAL AND ACUTE PHASE PROTEIN RESPONSES TO CAPRINE TRYPANOSOMA EVANSI INFECTION*

**WONG MENG TACK**

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# STRESS PROFILING OF THE HEMATOLOGICAL, BIOCHEMICAL AND ACUTE PHASE PROTEIN RESPONSES TO CAPRINE *TRYPANOSOMA EVANSI* INFECTION

**WONG MENG TACK**



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

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**STRESS PROFILING OF THE HEMATOLOGICAL, BIOCHEMICAL AND ACUTE PHASE PROTEIN RESPONSES TO CAPRINE *TRYPANOSOMA EVANSI* INFECTION**

By

**WONG MENG TACK**

**October 2011**

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

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*Trypanosoma evansi* is a cosmopolitan haemoflagellate and is the most widely distributed of the pathogenic animal trypanosomes affecting domesticated livestock and wild animals. Goats appear to be resistant to the disease but various reports have indicated that they may serve as potential reservoirs of the infection. The dynamics of the disease and the interaction between the caprine host and parasite remains unclear. The present study was undertaken to ascertain the response of the Hypothalymic-pituitary-adrenal (HPA) axis to *T. evansi* infection in goats with reference to cortisol levels; to determine the alterations in putative haematological and serum biochemical stress markers, as well as to investigate the acute phase response of haptoglobin and serum amyloid-A. The dynamics

of *T. evansi* infection was studied in 10 female Boer goats 8-10 months of age. The goats were divided into two equal groups (A and B), kept in adjacent pens in a fly-proof house and maintained in a similar manner throughout the experiment. Animals of group A were infected with 1 x $10^4$ *T. evansi* trypomastigotes intravenously while animals in group B were injected with Phosphate Buffered Saline with 10% Glucose (PBSG) at the start of the experiment. The animals were bled every alternate day from day 0 to day 62 post infection (pi). Infected animals were treated with diminazene diaceturate on day 62 pi. Blood was collected every six days for a month after the treatment. Whole blood was examined for parasitaemia as well as levels of circulating leukocytes, neutrophils and lymphocytes. Plasma was analysed for cortisol, albumin, globulin, creatinine, lactate, haptoglobin (Hp) and serum amyloid-A (SAA). Three peaks of parasitaemia were apparent in the infected animals; the first and highest peak occurred on day 6 pi followed by the second peak on day 14 pi and a third minor peak on day 28 pi. The infected group showed three main peaks of cortisol increment on days 4, 20 and 28 pi, correlating with the peaks of parasitaemia. Plasma creatinine did not differ significantly in both experimental groups with the exception of one spike in the infected goats on day 12 pi which corresponded to the first major parasitaemia peak. Elevations in plasma lactate were apparent from day 16-24 pi, but dropped to basal values thereafter. The infected animals also showed a significant and progressive decrease in plasma albumin and a corresponding increase in plasma globulin throughout the course of the infection. The haemogram in the infected goats revealed leukopaenia and lymphopaenia early in the infection with marked chronic neutropaenia. The mean plasma levels of Hp and SAA were significantly elevated in the infected group, corresponding to the high levels of

circulating parasites. Collectively, the changes observed in plasma cortisol, serum biochemistry, haematology and acute phase proteins indicate that *T. evansi* has the potential to elicit a measurable stress response in goats, with each metabolite having a unique pattern of expression. These ruminants are also potential chronic carriers of the parasite, making them efficient reservoirs of the pathogen and an important contributor to the epidemiology of livestock trypanosomiasis in the country.

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

# PROFIL STRES HEMATOLOGI, BIOKIMA DAN TINDAKBALAS PROTIN FASA AKUT DALAM JANGKITAN *TRYPANOSOMA EVANSI* PADA KAMBING

Oleh

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*Trypanosoma evansi* ialah sejenis haemoflagellate kosmopolitan di mana ia disebarkan secara meluas diantara trypanosome patogenik haiwan dan mempengaruhi ternakan tempatan dan haiwan liar. Kambing didapati mempunyai ketahanan terhadap penyakit ini tetapi pelbagai laporan menunjukkan bahawa haiwan ini berpotensi sebagai hos simpanan terhadap jangkitan tersebut. Dinamik penyakit dan interaksi antara kambing dan parasit masih tidak jelas. Kajian ini dijalankan untuk menyiasat tindak balas paksi Hypothalymic-pituitary-adrenal (HPA) dalam jangkitan *T. evansi* pada kambing berdasarkan tahap kortisol; untuk menentukan perubahan tindak balas dalam hematologi dan biokimia plasma yang dianggap sebagai penanda tekanan oleh jangkitan *T. evansi*,

serta untuk menentukan tindak balas fasa akut dari tahap haptoglobin dan serum amyloid-A. Dinamik jangkitan *T. evansi* dijalankan dengan menggunakan 10 ekor kambing 'Boer' betina berumur 8-10 bulan. Kambing itu dibahagikan kepada dua kumpulan dengan jumlah yang sama (A dan B). Kedua kumpulan kambing itu disimpan di dalam kandang secara bersebelahan di dalam rumah kalis lalat dan juga diurus dengan cara yang sama. Haiwan kumpulan A dijangkitkan dengan 1 x $10^4$ *T. evansi* manakala haiwan dalam kumpulan B disuntikkan dengan 'phosphate-buffered saline 10% glucose' (PBSG) secara intravena pada hari permulaan eksperimen. Semua haiwan diambil darah setiap dua hari sepanjang jangkitan tersebut dari hari ke-0 sehingga hari ke-62. Kambing dalam kumpulan A dirawati dengan diminazene diaceturate pada hari ke-62. Darah diambil setiap 6 hari selama sebulan selepas rawatan. Kesemua darah yang diambil dijalankan pemeriksaan parasitaemia dan tahap edaran leukocyte, neutrofil serta limfosit. Plasma dianalisis untuk tahap kortisol, albumin, globulin, kreatinin, lactate, haptoglobin (Hp) dan serum amyloid-A (SAA). Tiga puncak parasitaemia yang jelas kelihatan dalam kambing yang dijangkiti; puncak yang pertama dan tertinggi berlaku pada hari ke-6 diikuti oleh puncak kedua pada hari ke-14 dan puncak kecil ketiga pada hari ke-28. Kumpulan kambing yang dijangkiti menunjukkan tiga puncak yang utama bagi peningkatan kortisol pada hari ke-4, ke-20 dan ke-28 selepas jangkitan yang berhubungkait rapat dengan puncak parasitaemia. Plasma kreatinin tidak menunjukkan perbezaan ketara antara kedua kumpulan kecuali satu puncak yang tinggi pada kambing yang dijangkiti pada hari ke-12 di mana berhubung kait dengan puncak parasitaemia utama yang pertama. Peningkatan plasma laktat adalah jelas daripada hari ke-16 sehingga hari ke-24 selepas jangkitan, tetapi menurun ke nilai asal selepas itu. Haiwan berjangkit itu menunjukkan pengurangan

secara ketara dan progresif pada plasma albumin dan peningkatan yang sesuai pada plasma globulin sepanjang kursus jangkitan itu. Hemogram di dalam kambing jangkitan menunjuk bahawa leukopenia dan limfopenia berlaku pada awal jangkitan bersama tandaan neutropenia kronik. Tahap min plasma Hp dan SAA adalah meningkat secara ketara di dalam kumpulan berjangkit, berkait rapat dengan edaran parasit yang bertahap tinggi. Secara kolektif, perubahan yang dijumpai pada plasma kortisol, biokimia plasma, hematologi dan protin fasa akut berpotensi untuk mengukur tindak balas tekanan di dalam kambing yang dijangkiti *T.evansi*, di mana setiap metabolit mempunyai ungkapan corak yang unik. Ruminan ini juga dianggap sebagai pembawa kronik bagi parasit tersebut, menjadikanya hos simpanan yang cekap bagi *T.evansi* dan penyumbang yang penting kepada epidemiologi ternakan trypanosomiasis di dalam negara.

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Finally, I personally dedicate this thesis to my family members, my dad, my mum, sisters and brothers, thanks for the love and support. Last but not least, I would like to thank all my postgraduate friends and my best friends for their caring and sharing.

I certify that a Thesis Examination Committee has met on 7 Octo 2011 to conduct the final examination of Wong Meng Tack on his thesis entitled "Stress Profiling of the Hematological, Biochemical and Acute Phase Protein Responses to Caprine *Trypanosoma evansi* Infection" in accordance with the Universities and Universiti 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 Master of Science.

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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 currently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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**WONG MENG TACK**

Date: 7 October 2011

# TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| ACTH | adrenocorticotropic hormone |
| APP | acute phase protein |
| AVP | arginine vasopressin |
| $C_6H_{12}O_6.H_2O$ | glucose (monohydrate) |
| ca. | approximately |
| CPM | counts per minute |
| CRH | corticotrophin releasing hormone |
| G | gauge |
| Hp | haptoglobin |
| HPA | hypothalamic-pituitary-adrenal |
| IL-1-β | interleukin-1-beta |
| IL-6 | interleukin-6 |
| K2 EDTA | dipotassium ethylenediaminetetracetic acid |
| KCl | potassium chloride |
| kDNA | kinetoplastic deoxyribonucleic acid |
| $MgSO_4$ | magnesium sulphate |
| $Na_2HPO_4$ | disodium hydrogen phosphate |
| NaCl | sodium chloride |
| $NaH_2PO_4$ | sodium dihydrogen phosphate |
| PCV | packed-cell-volume |
| SAA | serum amyloid-A |
| TNF-α | tumor necrosis factor-alpha |
| VSG | variant surface glycoprotein |

# CHAPTER 1

## GENERAL INTRODUCTION

Trypanosomiasis caused by *Trypanosoma evansi* is the most widely distributed among the pathogenic animal trypanosomiasis (Hoare, 1972; Stephen, 1986). Various domestic and wild animals such as equids, camelids and ruminants are known to be infected by this parasite in Asia, Africa, Central and South America (Hoare, 1972; Stephen, 1986; Luckins, 1988; Brun *et al*., 1998). The pathogenesis and outcome of the disease called `surra' often depends on the virulence of the trypanosome strain and the species of host it infects. *T. evansi* is mechanicallly transmitted through haematophagous insects such as tabanids, *Stomoxys* and vampire bats (Hoare, 1972). Unlike the other members of the Trypanozoon group, this haemoflagellate does not undergo cyclic development in the insect vector, a phenomenon attributed to the lack of maxicircle kinetoplast DNA which encodes for proteins involved in insect-stage developmental regulation (Borst *et al*., 1987). Due to this simple and effective means of transmission, the spread of this cosmopolitan parasite and the debilitating disease it causes, is of concern to livestock farmers in the humid tropics (Luckins, 1988; Reid, 2002).

The infected animal with acute form of surra may show anaemia, high fever, marked depression, and acute death (Hoare, 1972; Stephen, 1986; Olaho-Mukani and Mahamat, 2000). However, the chronic form of the disease is more common, with the animal showing progressive anaemia, emaciation, recurrent fever, enlarged lymph nodes,

oedema of the abdomen, brisket and legs, and abortion (Raisinghani *et al*., 1980; Stephen, 1986; Haroun *et al*., 2000). As a result, *T. evansi* causes great economic losses in the livestock industry in many countries including Australia (Reid *et al*., 2001), Egypt (Abdel-Rady, 2008) and Philippines (Dargantes *et al*., 2009). In Malaysia, the disease is endemic (Abas Mazni *et al*., 1985; 1987) and numerous outbreaks have been reported in cattle (Cheah, 1997), deer (Adrian *et al*., 2010), pigs (Arunasalam *et al*., 1995) and Sumatran Rhinoceros (Vellayan *et al*., 2004). In addition, *T. evansi* is now recognised as an emerging zoonosis with recent human infections in India (Joshi *et al*., 2005; World Health Organisation, 2005; Shegokar *et al*., 2006). In Malaysia, human infections with trypanosomes (possibly *T. evansi*) were reported in local natives (*Orang asli*) in the 70's (Dissanaike *et al*., 1974; Else *et al*., 1976).

With the current efforts of the government to increase local food sufficiency, there has been an influx in the importation and rearing of goats, and numerous goat farms have sprung up around the country. It is imperative therefore that investigation into the common diseases of these animals be conducted in order to facilitate proper health management, husbandry and disease control measures. Trypanosomiasis in goats has been known to take on a different course of infection compared to other ruminant livestock. The chronic form of the disease with low levels of parasitaemia is common, to the extent that certain authors consider goats to be resistant to trypanosomal infection (Stephen, 1970; Boyt, 1971). Consequently, no obvious or typical clinical signs are observed, and acute trypanosomiasis is rare. It must be stressed however, that although *T. evansi* rarely appears fatal in goats, these animals do acquire the infection and suffer

loss in production and reduced weight gain (Reid, 2002; Dargantes *et al*., 2005), which are counterproductive for commercial livestock production. In addition, and more importantly, they serve as potential reservoirs of the parasite, and have been shown to have high levels of seroconversion in areas where the disease is endemic (Boid *et al*., 1981).

The extent and pathological effects of trypanosomiasis in goats is still poorly understood. In addition, there remains a dearth of published information on the disease among goats in Malaysia, and the possible negative effects it exerts on the expanding goat industry. Apart from clinical manifestation, these animals may also suffer secondary effects of the parasite, which may act as stressors on normal physiological functions (Mellor *et al*., 2005). It is well established that the Hypothalamic-pituitary-adrenal (HPA) axis is activated by exposure to stressful stimuli. The hypothalamus releases the corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP). CRH and AVP induce the secretion of adrenocorticotropic hormone (ACTH) from the pituitary gland and activate the secretion of glucocorticoids from the adrenal cortex (Whitnall, 1993). CRH plays an important role in the physiological and behavioural responses to stress in various species. While acute stress responses may be beneficial for fight or flight (Selye, 1950), chronic and prolonged stressful stimuli often prove to be detrimental (Nelson and Drazen, 2000). Parasites are known to act as stressors in various animals, and correlations between levels of parasitaemia and glucocorticoid secretion (Prichard *et al*., 1974) and elevation in the stress haemogram (Antoine-Moussiaux *et al*., 2008) have been demonstrated previously in laboratory animals. It is often postulated that trypanosomiasis in domestic animals is a

secondary infection and often a result of a parasite flare-up due to concurrent immunosuppression caused either by primary invaders or stressful stimuli. To date, the role of trypanosomes as primary disease agents in ruminants, and its potential to induce stress responses and immunosuppression, has not been well documented. The present study therefore, explores the effects of *T. evansi* infection in goats in relation to physiological stress responses and expression of stress proteins, with the following specific objectives:

1. To ascertain the stress response of the HPA axis to *T. evansi* infection in goats with reference to plasma cortisol levels.
2. To determine the alterations in putative plasma biochemical and haematological stress markers in response to *T. evansi* infection.

   and

3. To investigate the expression profiles of Acute Phase Proteins (Haptoglobin and Serum amyloid-A) in caprine *T. evansi* infection.

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