UNIVERSITI PUTRA MALAYSIA

EPIDEMIOLOGY, DIAGNOSIS AND CHEMOPROPHYLAXIS OF TRYPANOSOMA EVANSI INFECTION IN DAIRY CATTLE

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EPIDEMIOLOGY, DIAGNOSIS AND CHEMOPROPHYLAXIS OF *TRYPANOSOMA EVANSI* INFECTION IN DAIRY CATTLE

BY

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

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April 1997

Chairman : Associate Professor Dr. Rehana Abdullah Sani

Faculty : Veterinary Medicine and Animal Science

Trypanosomiasis caused by *Trypanosoma evansi* is considered to be one of the diseases of economic importance affecting dairy cattle in Malaysia. An investigation into the epidemiology, diagnosis and chemoprophylaxis of *T. evansi* infection in these animals was carried out. Four studies were conducted in this investigation.

In the first study, random blood samples were collected from animals of different age/ physiological status for parasitological, serological, haematological and biochemical tests between August 1995 and July 1996 The mean prevalence was highest in the lactating animals (13 4%) followed by those in the dry herd (8 8%), late pregnant animals (8 1%), early pregnant animals (4 7%), calves (0 3%) and yearlings (0 2%) The mean prevalence was significantly different ($p<0\ 05$) between cows and the other groups (calves and yearlings) Among the cows there was significant difference in the mean prevalence between the early pregnant and lactating animals ($p<0\ 05$) The association between the prevalence in lactating cows and raindays was significant ($p< 0\ 05$) Infected dry cows had a significant decrease in the PCV, Hb and albumin levels ($p< 0\ 05$) The antigen-detection ELISA was a useful diagnostic tool for detection of *T. evansi* infections as it was capable of detecting 91% of trypanosome-positive animals

In the second study, fifteen two-three weeks old calves were selected, tagged and bled monthly for 12 months All these animals were negative for *T. evansi* throughout the study Maternal antibodies were detected in three animals Twelve out of 15 animals were positive for antigen while six out of the 12 antigen positive animals had antibodies when the study ended Non infected calves had significantly higher PCV and RBC values ($p< 0\ 05$) The CATT was a useful diagnostic kit as the test had a sensitivity of 99 1%, specificity of 85% and an accuracy of 96 9%

Six adult cattle with natural *T. evansi* infection were treated with isometamidium chloride (0.25 mg/kg) in the third study, and five animals became negative after treatment. In the six untreated controls, one had swollen knee joints while another aborted a fully developed foetus.

In the fourth study, isometamidium chloride was given to 18 early pregnant and 19 lactating animals at the recommended prophylactic dose (0.5 mg/kg). The drug protected 33% and 22% of the early pregnant animals against *T. evansi* infection for two months and three months respectively. In the lactating cows the drug protected 63% and 53% of the animals for two and three months respectively.

In conclusion, firstly, patent parasitaemia appeared to be related to the physiological status of the animal; secondly, infection increased with increase in age of the calf; third, the combined use of Ag-ELISA and QBC were effective in diagnosis of infections; fourth, isometamidium chloride was curative in the treatment of infection at the recommended dose rate and fifth, a higher dose than recommended was probably required for chemoprophylaxis.

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EPIDEMIOLGI, KAEDAH DIAGNOSIS DAN KEMOPROFILAKSIS UNTUK JANGKITAN *TRYPANOSOMA EVANSI* PADA LEMBU TENUSU

Oleh

CHEAH TONG SOON

April 1997

Pengerusi : Professor Madya Dr. Rehana Abdullah Sani

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Penyakit trypanosomiasis yang disebabkan oleh *Trypanosoma evansi* adalah satu penyakit penting dari segi ekonomi bagi lembu tenusu di Malaysia. Penyiasatan dari segi epidemiologi, kaedah diagnosis dan kemoprofilaksis untuk jangkitan *T. evansi* telah dijalankan. Untuk ini, empat kajian telah dijalankan.

Dalam kajian pertama, sampel darah secara rawak telah diambil daripada lembu pelbagai umur/status fisiologi untuk ujian-ujian parasitologi, serologi, hematologi dan biokimia dari bulan Ogos 1995 hingga Julai 1996. Min prevalen tertinggi telah dikesan dalam kumpulan sedang menyusu (13.4%), diikuti oleh lembu-lembu kering susu (8.8%) lembu-lembu bunting lewat (8.1%), lembu-lembu bunting awal (4.7%), anak-anak lembu (0.3%) dan lembu-lembu dara (0.2%). Min prevalen berbeza yang bererti didapati di antara kumpulan lembu betina dan kumpulan lain (anak lembu dan lembu dara) dan juga di antara lembu bunting awal dan lembu sedang menyusu ($p<0.05$). Variasi secara bulanan untuk kadar prevalen bagi lembu sedang menyusu bergantung kepada hari-hari hujan ($p<0.05$). Lembu-lembu kering susu yang berjangkit menunjukkan penurunan yang bererti didalam nilai-nilai PCV, Hb dan tahap albumin ($p<0.05$). Satu alat yang berguna untuk mengesani jangkitan *T. evansi* adalah antigen-detection ELISA yang berupaya mengesani 91% lembu yang positif jangkitan tripanosom.

Untuk kajian kedua, limabelas ekor anak-anak lembu yang berumur diantara dua hingga tiga minggu telah dipilih, ditag dan diambil darah setiap bulan selama 12 bulan. Kesemua anak- anak lembu ini negatif untuk *T. evansi*. Antibodi maternal telah dikesan daripada tiga ekor anak lembu. Duabelas daripada limabelas ekor anak lembu itu telah didapati positif untuk antigen tripanosom sementara enam daripada duabelas ekor anak lembu yang positif itu ada antibodi pada akhir penyiasatan. Anak-anak lembu yang tidak dijangkiti telah menunjukkan nilai PCV dan RBC yang bererti yang lebih tinggi ($p<0.05$). Ujian CATT adalah satu alat diagnostik yang

berguna memandangkan ia mempunyai sensitiviti 99 1%, kadar spesifisiti 85% dan kadar ketepatan 96 9%

Dalam kajian ketiga, lima daripada enam lembu dewasa yang dijangkiti secara semulajadi dengan *T. evansi* setelah diberi rawatan dengan isometamidium klorida (0 25 mg/kg) telah didapati negatif Bagi enam lembu yang tidak dirawat, satu ekor telah menunjukkan tanda klinikal bengkak sendi lutut dan sekor lagi keguguran

Bagi kajian keempat, rawatan dengan isometamidium klorida (0 5 mg/kg) untuk 18 ekor lembu bunting awal dan 19 ekor lembu sedang menyusu telah menunjukkan kadar ketahanan 33% selama dua bulan dan 22% selama tiga bulan untuk lembu bunting awal Untuk lembu sedang menyusu, ubat tersebut telah memberi kadar ketahanan sebanyak 63% selama dua bulan dan 53% selama tiga bulan

Rumusan dari penyelidikan ini adalah, pertama, keadaan parasitemia ada kaitan dengan status fisiologikal ternakan, kedua, kadar jangkitan anak-anak lembu bertambah dengan peningkatan umur, ketiga, penggunaan bersama ujian-ujian Ag-ELISA dan QBC sangat berkesan dalam mendiagnosa jangkitan, keemapat, ubat isometamidium klorida paling berkesan dalam rawatan jangkitan pada dos yang ditetapkan dan kelima dos yang lebih tinggi mungkin perlu untuk kemoprofilaksis

CHAPTER 1

INTRODUCTION

Trypanosoma evansi has the widest geographical distribution among the pathogenic trypanosomes described. It can affect camels, horses, donkeys, cattle, buffaloes, pigs and dogs and wild animals like Asiatic elephants , tapirs and deer. Trypanosomiasis in livestock has received little attention in Malaysia with only occasional reports in horses (Ng and Vanselow, 1978). However, outbreaks of clinical "surra" in the eighties were recognised as a health problem in buffalo and cattle herds on institutional farms and heightened awareness to the potential importance of trypanosomiasis in livestock in Malaysia (Abas Mazni and Zainal-Abidin, 1985).

In Peninsular Malaysia the population of large ruminants was estimated at 744,015 in 1990 (Malaysia,1990). This figure comprised 614,498 heads of cattle and 129,517 heads of buffaloes. The cattle population consisted of 523,992 heads of beef type and 90,506 heads of dairy cattle respectively. The dairy development was started in 1974 based on the requirements of the New Economic Policy, and the aims were to increase the income of farmers through dairy production activities

and local production of milk towards meeting the liquid milk market demand (Ahmah Mustaffa, 1994). Trypanosomiasis caused by *T. evansi* may be of economic importance to the dairy industry in this country. Despite the importance of this disease in limiting the productivity of cattle, the epidemiology of the disease remains unknown. The urgent need to study the epidemiology of *T. evansi* in cattle, buffaloes and other animals in Malaysia has also been suggested by Ikede *et al.* (1983) and Abas-Mazni *et al.* (1987). Epidemiological data is vital in the design of effective control measures while the rapidity and accuracy of any one diagnostic technique or combination of techniques is important in determining the efficacy of chemotherapy and monitoring the effectiveness of chemoprophylaxis.

The objectives of this study were to determine the following :

1. prevalence of *T. evansi* in cross bred dairy cattle with respect to age and physiological status of animals, and in relation to weather,
2. age when the calf acquired infection,
3. sensitivity and specificity of laboratory tests used in diagnosis and
4. efficacy of isometamidium chloride in animals naturally infected with *T. evansi.*

CHAPTER II

LITERATURE REVIEW

History and Distribution of *Trypanosoma evansi*

A complete historical account of the discovery of *T. evansi* and its geographical distribution was given by Hoare (1972). The causative agent of surra was first described by Griffith Evans in 1880 after he observed motile spirillum - like organisms in the blood of equines and camels affected by this disease in Punjab, India. Different names had been given to the organism causing the disease by various workers, however according to Hoare (1956) , Balbiani was the first person to refer the flagellates to the genus *Trypanosoma*. The parasite was finally classified by Doeflein in 1901 as *Trypanosoma evansi* (Hoare, 1972). Surra , under various local names was more widespread than previously thought and its occurrence had been reported in most of the tropical and subtropical regions of the world. Their occurrence in various localities gave rise to new names for *T. evansi*, and these trypanosomes were considered to be new species or subspecies in early reports based on, host restriction, geographical distribution, clinical differences of the disease in various hosts or minor morphological differences. These were

exemplified by the creation of *T. hippicum* and *T. venezuelense* for trypanosome isolated from horses in Central and South America, *T. camelli* from camels in Somalia, *T. evansi* var *su-auru* from camels in Russia, *T. kirdani* from a tiger in Sumatra, and *T. evansi* var *rayi* from buffaloes in India However most of these trypanosomes are morphologically indistinguishable from typical *T. evansi*

Cross-immunity tests had also been employed to differentiate some of these variants of *T. evansi* and based on these studies the trypanosome that causes mborii in camels was named *T. evansi* var *mborii*, that causing "el debab" in North Africa was termed *T. soudanese*, and the trypanosome in horses in Indochina was called *T. annamense.*

The absence of kinetoplast had been used as a criterion in distinguishing the two evansi-like trypanosomes found in America, *T. equinum* and *T. venezuelense* from the *T. evansi* that occurred in the Old World However, electron microscope studies on the kinetoplasts of these two trypanosomes showed that the dominants of dykinetoplastic populations does not merit the creation of different species, and it merely represents a morphological variant of *T. evansi* which appears to be most common in South America According to Hoare (1954) spontaneous transformation of typical *T. evansi* into dyskinetoplastic forms occurred during animal passage under laboratory conditions and can also be produced by exposure to certain dyes Dyskinetoplastic forms had been reported in *T evansi* and various other species of trypanosomes after treatment of these organisms with a variety of

different trypanocides including Berenil (diminazene aceturate) (Killick- Kendrick, 1964) and Prothidium (phenanthridium) (Ray and Malhotra, 1960).

After extensive morphological studies of these various organisms, Hoare (1972) concluded that they are all synonyms of *T. evansi*. Further investigation on the intraspecies biochemical strains of *T. evansi* by electrophoresis of isoenzymes and by amino acid analysis showed that there were no isoenzyme differences, but differences were recorded in some of the proteins and polypeptides (Gibson *et al*., 1978).

Typical strains of *T. evansi* is practically monomorphic and this morphological feature together with some of its biological characteristics such as the parasite was not transmissible by *Glossina* spp., were used to differentiate it from *T. brucei* which is pleomorphic. There are reports of stumpy forms being observed in camel and horse (Godfrey and Killick-Kendrick,1962; Killick-Kendrick, 1964). Hoare (1956) conducted examinations of blood smears from naturally infected animals from different localities and the results showed that stumpy forms were present in small number and Hoare (1972) concluded that pleomorphism in *T. evansi* is not a consistent feature and appears sporadically.

Hoare (1972) postulated that *T. evansi* originated in tropical Africa from *T. brucei* infections. In Africa camels may have originally been infected with *T. brucei* when these animals employed as transport animals were in the marginal northern

zones of tsetse fly -infested areas When these animals returned to tsetse fly-free areas the trypanosome may have been mechanically transmitted to other animals by blood sucking diptera The parasite most probably lost its pleomorphic character and also its ability to develop in *Gossina* and behaved like present day blood - passage *T. brucei* in the laboratory The trypanosome then spread northwards throughout Northern Africa to Asia probably by camel caravans and through military campaigns. At the end of the last and the beginning of the present century, India was considered to be a centre from which surra was spread with infected livestock throughout the continent of Asia and the islands in the Indian Ocean, where the presence of tabanid vectors ensure the propagation of the disease In South- East Asia *T. evansi* was first recorded from mules in North Vietnam in 1888 (reviewed by Luckins, 1988) In Malaysia surra was first detected in an Australian mare in 1903 (Fraser and Symonds 1909) Spread of *T. evansi* into the western Hemisphere may have taken place resulting from infected horses being introduced by the Spaniards in the sixteenth century

Recent isoenzyme studies on *T evansi* stocks from South America, Nigeria, Sudan, Kenya and Kuwait showed that they are a homogeneous group resembling west African rather than east African *T. brucei* stocks which are further evidence for the origin of *T. evansi* from *T. brucei* (Gibson *et al* , 1983)

Classification of *T. evansi*

Correct identification of the agent causing disease is one of the prerequisites in the study of the epidemiology and control A system of classification of trypanosomes is therefore necessary in the identification of the parasites Differentiation of species of trypanosomes is based on their structural and behavioural characteristics Hoare (1972) divided the genus *Trypanosoma* into two groups, Salivaria and Stercoraria , according to the development in the vectors and transmission by either saliva or faecal contamination of the wound through the bite of vectors

a) Salivaria - The trypanosomes complete their development in the mouth parts or salivary glands of the vector (except in mechanical inoculator) with the formation of metatrypanosomes which are inoculated into the new host by blood sucking diptera (except in *T. equiperdum*)

b) Stercoraria - The development of the parasite is completed in the hind gut of the vector with the formation of metatrypanosomes which are excreted in the faeces and infect the new host through mucous membrane or minor abrasions on the skin