EPIDEMIOLOGY OF BOVINE TRYPANOSOMOSIS AND ITS ECONOMIC IMPACT IN TSETSE-INFESTED AND TSETSE-FREE AREAS OF AMHARA REGION, NORTH-WEST ETHIOPIA

By

THOMAS CHERENET ASFAW

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

TO MY CHILDREN DAGMAWI, HENOK AND YARED, TO MY WIFE MULU,

TO MY LATE MOTHER TIGAE AND FATHER CHERENET FOR THEIR

LOVE, AFFECTION AND ENCOURAGMENT

THAT ELEVATED MY SPIRITS

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

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Chairman: Associate Professor Rehana Abdullah Sani, Ph.D.

Faculty: Veterinary Medicine

The epidemiology of bovine trypanosomosis was studied in tsetse-infested and tsetse-free areas of Amhara Regional State, North-West Ethiopia. A multidisciplinary work was undertaken to elucidate the key factors determining the presence of tsetse flies and magnitude of bovine trypanosomosis. Cattle were selected from tsetse-infested and tsetse-free areas for monthly monitoring of trypanosome infection by blood sampling on glass slides, dried blood spot and buffy coat spot on filter papers. In this study, parasitological, serological (Ab-ELISA) and PCR methods were used to characterize trypanosomes infecting the vector and the host.

A total of 795 blood samples from cattle were examined from the tsetse-infested and tsetse-free areas; 13.5% of wet blood film and 15.6% of thin blood film were found positive for trypanosomes while PCR detected 18 % trypanosome infections. The dominant species was *T.vivax*, followed by *T.congolense* and *T.brucei*. *T.vivax* was

higher in tsetse-free areas, whereas *T.congolense* was more in tsetse-infested areas, *T.brucei* and mixed infection of *T.congolense* and *T.vivax* were only present in tsetse-infested areas. The monthly trypanosome prevalence in tsetse-infested areas varied from 15 to 28% and in tsetse-free areas was 9 to 27%, with December having the highest prevalence in both study areas. Sera and dried blood spot on filter paper in Ab-ELISA showed strong correlation, providing for the use of the more convenient dried blood spot.

In the retrospective study 7079 samples and accompanying data were analyzed for 5 consecutive years from the year 1997 till 2001. The prevalence of trypanosome infections was highest during the early dry season (September to December). Trypanosome infections were mainly due to *T. vivax. T*rypanosomosis reduced significantly the average packed cell volume and the body condition of the host. The monthly prevalence of infection was correlated with the density of biting flies suggesting their important role in transmission of trypanosomosis in the tsetse-infested and tsetse-free areas of the Amhara Region of north-west Ethiopia.

Flies were trapped for 3 days each month in the study period using 4 types of traps (`Biconical', `NGU', `Pyramidal' and hand net). A total of 5652 tsetse and other biting flies were captured; 3532 flies from tsetse-infested areas and 2120 flies from tsetse-free areas. PCR amplification analyses for trypanosome identification were carried out on 3751 flies, with primer sets specific for *Trypanosoma (Duttonella) vivax, T. (Nannomonas) congolense and T. (Trypanozoon) brucei.* Of 3751 flies; 699 (18.64%) were positive in PCR analysis with 132 (12.13 %) from tsetse-free areas and 567 (21.29

%) from tsetse-infested areas. Comparing within the type of flies, out of 1314 tsetse flies *(Glossina morsitans submorsitans* and *Glossina tachinoides*) 366 (27.85 %) were positive and out of 2437 other biting flies 333 (13.66 %) were found positive ($P<0.01$). Therefore PCR can be used to generate baseline data for fly infection.

From the 15 risk factors identified for trypanosome infection, multivariable logistic regression model produced the final logistic model containing seven variables (biting fly density, season, transhumance grazing, traction oxen, origin of the herd, area (tsetse-infested and tsetse-free) and Packed Cell Volume). Their significance was $P=0.05$ which showed a significant association with trypanosome infection.

The socio-economic effect of trypanosomosis was evaluated using Participatory Rural Appraisal (PRA) technique. Selected districts with three different trypanosomosis risk (high, medium and low) demonstrated much overlap between the farmers' perception of the disease (*gendi)* and bovine trypanosomosis. For example, '*gendi'* was associated with chronic weight loss, abortion, calf mortality and decrease milk yield in the high trypanosomosis risk area compared to the other two areas.

In conclusion, this study reported for the first time the use of PCR based assay on sample optained from Amhara Region, North West Ethiopia to detect trypanosome from naturally infected biting flies (dried blood meal residue) and dried buffy coat spot on filter paper from cattle. The dominant species noted was *T.vivax.* The epidemiology of trypanosomosis in cattle is also dependent on other biting flies apart from tsetse flies.

Consequently, the eradication of tsetse flies alone will not necessarily lead to eradication of trypanosomosis.

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

EPIDEMIOLOGI TRIPANOSOMOSIS BOVIN DAN IMPAK TERHADAP EKONOMIK DI KAWASAN TSETSE DAN KAWASAN BEBAS TSETSE DI NEGERI AMHARA, UTARA-BARAT ETHIOPIA

Oleh

THOMAS CHERENET ASFAW

Julai 2004

Pengerusi: Profesor Madya Rehana Abdullah Sani, Ph.D.

Fakulti: Perubatan Veterinar

Epidemiologi tripanosomosis bovin telah di kaji di kawasan tsetse dan kawasan bebas tsetse di negeri Amhara, Utara-Barat Ethiopia. Kajian berbagai disiplin telah dilakukan bagi menyiasat faktor utama yang menentukan kehadiran lalat tsetse serta keluasan tripanosomosis bovin. Lembu dipilih dari kawasan tsetse dan kawasan bebas tsetse untuk tinjauan bulanan bagi mengesan jangkitan tripanosom dengan sampel darah atas slaid kaca, titik darah kering dan titik lapisan buf atas kertas turas. Dalam kajian ini tripanosom yang menjangkiti vektor dan perumah di kenalpasti mengunakan teknik parasitologi, serologi dan PCR.

Sejumlah 795 sampel darah lembu di kaji dari kawasan tsetse dan kawasan bebas tsetse; 13.5% calitan darah basah dan 15.6% filem darah nipis didapati positif dan PCR mengesan 18% jangkitan tripanosom. Spesis yang dominan adalah *T.vivax*, diikuti

T.congolense dan *T.brucei*. *T.vivax* lebih banyak di kawasan bebas tsetse, *T.congolense* lebih di kawasan tsetse, *T.brucei* dan jangkitan campuran *T.vivax*-*T.congolense* hanya ujud di kawasan tsetse. Prevalens bulanan di kawasan tsetse antara 15-28% dan di kawasan bebas tsetse 9-27%, dengan prevalens tertinggi pada bulan Disember di kedua kawasan. Sera dan titik darah kering atas kertas turas dalam teknik Ab-ELISA menunjukkan korelasi yang kuat. Ini menunjukkan kegunaan titik darah kering atas kertas turas, suatu sampel yang lebih mudah diambil.

Dalam kajian retrospektif 7079 data sampel dianalisa selama lima tahun dari 1997 hingga 2001. Prevalens jangkitan tripanosom tertinggi dalam awal musim kering (September hingga December). Jangkitan tripanosom kebanyakkannya disebab oleh *T.vivax,* dan menurunkan secara ketara PCV purata serta keadaan badan perumah. Prevalens jangkitan bulanan ada korelasi dengan ketumpatan lalat menggigit. Ini membuktikan peranan penting lalat menggigit dalam transmisi tripanosomosis di kawasan tsetse dan kawasan bebas tsetse di negeri Amhara, Utara-Barat Ethiopia.

Lalat di tangkap selama 3 hari setiap bulan dalam jangkamasa kajian dengan menggunakan 4 jenis perangkap (`Biconical', 'NGU', 'Pyramidal' dan pukat tangan). Sebanyak 5652 lalat tsetse dan lalat menggigit lain ditangkap; 3532 lalat dari kawasan tsetse dan 2120 lalat dari kawasan bebas tsetse. Analisis PCR bagi mengenalpasti tripanosom dijalankan atas 3751 lalat, mengguna set primer khusus untuk *Trypanosoma vivax*, *T. congolense* dan *T.brucei*. Dari 3751 lalat; 699 (18.64%) positif dalam analisis PCR dengan 132 (12.13%) dari kawasan tsetse dan 567 (21.29%) dari kawasan bebas

tsetse. Perbandingan antara lalat menunjukkan dari 1314 lalat tsetse (*Glossina morsitans submorsitans* dan *Glossina tachinoides*), 366 (27.85%) didapati positif dan dari 2437 lalat menggigit lain, 333 (13.66%) didapati positif ($P<0.01$). Maka PCR boleh digunakan untuk menjana data asas bagi jangkitan lalat.

Dari 15 faktor risiko yang dikenalpasti untuk jangkitan tripanosom model `multivariable logistic regression' telah mengeluarkan model logistik terakhir yang mengandungi tujuh `variable' (kepadatan lalat menggigit, musim, ragutan `transhumance', lembu bertenaga kerja, asal-usul lembu, kawasan dan PCV). Ketaraan sebanyak $P=0.05$ menunjukkan pertalian kuat antara faktor risiko tersebut dengan jangkitan tripanosom. Kesan sosio-ekonomi tripanosomosis dinilai dengan teknik `Participatory Rural Appraisal' (PRA). Daerah terpilih yang mempunyai tiga tahap risiko bagi tripanosomosis (tinggi, sederhana dan rendah) menunjukkan ketindihan antara persepsi peladang mengenai penyakit `gendi dan tripanosomosis bovin. Sebagai contoh, `gendi' dikait dengan penurunan berat badan jangka panjang, kematian anak lembu dan penurunan produksi susu di kawasan tripanosomosis berisiko tinggi berbanding dengan kedua kawasan yang lain.

Kesimpulanya kajian ini melaporkan buat pertama kali perkembangan asai PCR bagi mengesan tripanosom dari lalat menggigit yang dijangkiti secara asli (dari darah kering yang dihisap) dan titik kering lapisan buf atas kertas turas. Spesis tripanosom yang dominan ialah *T.vivax*. Epidemiologi tripanosomosis pada lembu juga bergantung kepada lalat menggigit selain lalat tsetse. Akibatnya, permusnahan lalat tsetse sahaja tidak bermakna permansuhan penyakit tripanosomosis.

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Professor
Faculty of Veterinary Medicine
Addis Abeba University
Ethiopia

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Professor
Institute of Tropical Medicine
Veterinary Department
Belgium

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I certify that an Examination Committee met on 8th July 2004 to conduct the final Examination of Thomas Cherenet Asfaw on his Doctor of Philosophy thesis entitled "Epidemiology of bovine trypanosomosis and its economic impact in tsetse-infested and tsetse-free areas of Amhara Region, North-west Ethiopia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Abdul Rani Bahaman, Ph.D.
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Shaik Mohd Amin Babjee, Ph.D.
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Latiffah Hassan, Ph.D.
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

S. Geerts, Ph.D.
Professor
Prins Leopold Instituut
Institute of Tropical Medicine
Antwerp, Belgium
(Independent Examiner)

GULAM RASUL RAHMAT ALl, Ph.D.
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Rehana Abdullah Sani, Ph.D.
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Jothi M Panandam, Ph.D.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Nadzri Salim, MPVS.
Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

_____

AINI IDERIS, Ph.D.
Professor/Dean
School of Graduate Studies
Unversiti Putra Malaysia

Date:

DECLARATION

I hereby declare that the thesis 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 other degree at Universiti Putra Malaysia or other institutions.

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THOMAS CHERENET ASFAW

Date:

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