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KAMPALA, UGANDA

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INTERNATIONAL SCIENTIFIC COUNCIL FOR TRYPANOSOMIASIS RESEARCH AND CONTROL ISCTRC

CONSEIL SCIENTIFIQUE INTERNATIONAL POUR LA RECHERCHE ET LA LUTTE CONTRE LES TRYPANOSOMIASES CSIRLT



Thirtieth Meeting
30^{ème} Réunion

Kampala, Uganda
2009

AU-IBAR
UA-BIRA

PUBLICATION No. 125

**International Scientific Council for Trypanosomiasis Research and
Control
(ISCTRC)**

**Conseil Scientifique International pour la Recherche et la
Lutte contre les Trypanosomiasés
(CSIRLT)**

**THIRTIETH MEETING
30^{ème} REUNION**

**KAMPALA, UGANDA
2009**

**Edited by AU-IBAR
Edité par UA-BIRA**

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THE KAMPALA DECLARATION

RESOLUTION

The International Scientific Council for Trypanosomiasis and Research and Control (ISCTRC),

NOTING that in 1948 the European governments administering African colonial territories in Africa set up the Commission for Technical Cooperation for Africa south of Sahara (CCTA) for advising and recommending to them on technical development matters;

NOTING further that under the Commission were the Inter-Africa Bureau of Epizootic diseases and Tsetse and Trypanosomiasis (T&T) from which emerged the present International Scientific Council for Trypanosomiasis and Research and Control (ISCTRC) for advising on technical development in that field;

NOTING that in 1965 the African Heads of states and Governments of OAU resolved to take over the functions of the CCTA to operate under the Organization of Africa Unity (OAU) Science Technology and Research Commission (ISCTRC);

NOTING that in the implementation of above resolution the inter-African Bureau for Epizootic Disease was changed to Inter-African Bureau for Animal Resources to encompass T&T programme which covered ISCTRC;

RECOGNISING that in 1971 the ISCTRC Constitution gave the Council the mandate to stimulate, encourage and co-ordinate research in the field of T&T, including mobilization of funds and regular meetings;

NOTING that the ISCTRC mandate that has been implemented mainly through the holding of the regular meetings;

NOTING that a 2006 consultant report on strengthening of ISCTRC has been approved by the Executive Committee of ISCTRC and the African Union Department of Rural Economy and Agriculture (AU/DREA), all the recommendations be implemented including its structure within AU/IBAR and the provision of necessary funding and staffing without further delay;

RESOLVED that the AU Commissioner for Rural Economy and Agriculture be requested to attend to all the outstanding recommendations of the 2006 consultancy report including the inclusion of ISCTRC Secretariat within the AU/IBAR structure and to provide the necessary funding and staffing.

LA DECLARATION DE KAMPALA

RÉSOLUTION

Le Conseil Scientifique International pour la Recherche et la Lutte contre les Trypanosomiase (CSIRLT):

NOTANT qu'en 1948, les gouvernements européens administrant les territoires coloniaux d'Afrique ont mis en place la Commission pour la Coopération Technique en Afrique au Sud du Sahara (CCTA) pour les conseiller et leur prodiguer des recommandations sur les questions techniques liées au développement;

NOTANT en outre que sous cette Commission, était logé le Bureau Interafricain des Maladies Epizootiques, de la Tsé-tsé et la Trypanosomiase d'où a émergé l'actuel Conseil Scientifique International pour la Recherche et la Lutte contre les Trypanosomiase (CSIRLT) chargé de donner des conseils techniques liés au développement dans ce domaine ;

NOTANT qu'en 1965, les Chefs d'Etats et de Gouvernements de l'Organisation de l'Unité Africaine (OUA) ont résolu de reprendre les fonctions du CCTA pour le faire opérer sous le couvert de la Commission Science, Technologie et Recherche (CSIRLT) de l'Organisation de l'Unité Africaine (OUA) ;

NOTANT qu'au cours de la mise en œuvre de la résolution ci-dessus citée, le Bureau Interafricain pour les Maladies Epizootiques a été changé en Bureau Interafricain des Ressources Animales pour englober les programmes Tsé-tsé et Trypanosomiase que couvrait le CSIRLT;

RECONNAISSANT qu'en 1971, la Constitution du CSIRLT a donné au Conseil le mandat de stimuler, encourager et coordonner la recherche dans le domaine de la mouche tsé-tsé et de la trypanosomiase, y compris la mobilisation des fonds et la tenue de réunions régulières ;

NOTANT que le mandat du CSIRLT a été mis en œuvre principalement par le biais de la tenue de réunions régulières ;

NOTANT que le Rapport 2006 d'un Consultant sur le renforcement du CSIRLT a été approuvé par le Comité Exécutif du CSIRLT et le Département Economie Rurale et Agriculture de l'Union Africaine (UA-

DERA), toutes les recommandations devant être appliquées sans délai, y compris l'inclusion de sa structure au sein de l'UA-BIRA ainsi que la provision du financement et les effectifs nécessaires ;

A RÉSOLU qu'il soit demandé au Commissaire de l'UA pour l'Economie Rurale et l'Agriculture, de veiller à l'exécution de toutes les recommandations du rapport 2006 du consultant, y compris l'inclusion du Secrétariat CSIRLT dans la structure de l'UA-BIRA et la provision de financement et de ressources humaines nécessaires.

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REPORT OF THE ISCTRC SECRETARY ON THE 30TH CONFERENCE

Opening ceremony

The 30th ISCTRC Meeting was held at the Speke Commonwealth Resort in Kampala, Uganda between the 21st and 25th of September 2009. The meeting coincided with the 60th anniversary of the ISCTRC and the theme for the meeting was “Towards consolidating strategies to manage trypanosomiasis in Africa”. It was opened by the Rt. Hon. First Deputy Prime Minister and Minister for East African Federation Hon. Enya Kategaya who read a speech on behalf of the President of the Republic of Uganda, His Excellency, Yoweri Kaguta Museveni. The Deputy Prime Minister highlighted the impact of T&T on rural development and human welfare on the African continent and urged scientists to focus on research areas that would contribute to the effective management of T&T. The opening ceremony was also graced by the presence of Hon. Hope Mwesigye Minister for Agriculture, Animal Industry and Fisheries and the Director AU-IBAR, Prof Ahmed Elsayalhy who also read a speech on behalf of the Commissioner for Rural Economy and Agriculture, Madame Rhoda Peace Tumusiime.

Conference participants and presentations

The conference was attended by 312 participants from 36 tsetse infested countries and 12 international organisations.

Presentations focused on the following thematic areas: The Pan African Tsetse and Trypanosomiasis Campaign (PATTEC), Human African Trypanosomiasis, Animal African Trypanosomiasis, *Glossina* Biology and Control, Socio-Economics, Environment and Land Use. Key note papers were presented under each of the thematic areas.

A total of ninety seven (97) scientific presentations were made. Seventy one (71) papers were presented in plenary while twenty six (26) were presented as posters. Twenty (20) African countries presented their reports on progress of T&T activities since the last meeting held in Luanda, Angola in the year 2007.

Distribution of the proceedings of the 29th Conference

The proceedings of the 29th Conference in Luanda were distributed to the participants in both hard and soft copies. ISCTRC visibility materials including caps and T-shirts were also distributed to the conference participants.

Side meetings

During the meeting two international organisations, DNDi and FIND held side meetings and used the opportunity to explain their activities and to launch new products, in the market, for management of Trypanosomiasis. DNDi launched a combination therapy for stage two human African trypanosomiasis in the form of NECT (Nifurtimox-Eflornithine combination Therapy) to address emerging drug resistance to Melarsoprol, which has been in use for a long time with resistance having been reported in Angola, DRC and Uganda. FIND launched and demonstrated a field fluorescent microscope.

Training and mentoring of young scientists

The Yale University of Public health trained 30 young scientists on vector biology and scientific report writing skills. The meeting noted that the training was timely as previously trained scientists in T&T had reduced in numbers and hence such more trainings are encouraged. An appeal for other organizations to support capacity building was made.

Strengthening of ISCTRC

The mandate and functions of the ISCTRC were discussed and the Council passed a resolution which called for the strengthening of the council to enable it to respond to emerging challenges. The council resolved that the Commissioner of AU/DREA be requested to initiate the implementation of inclusion of ISCTRC Secretariat within the structure of AU/IBAR and the provision of budget line and staffing.

Commemoration of the 60th Anniversary and Award of medals and certificates to outstanding scientists

Ten medals and certificates were awarded to 10 scientists who had made significant contribution to research and control of T&Ts in the last 10 years up to 2009. Five other medals that would have been awarded posthumously during the 50th ISCTRC anniversary were also awarded to their next of kin. The medals were for Mr Jack Lancien, Dr G L Kasyumba, Prof E Bursell, Dr E Freidheim and Dr A van der Vloedt and were in recognition for their contributions in T&T management.

The medals and certificates of Lancien and Kazyumba were passed to Dr Pere Simarro of WHO to pass to the family. Similarly the one for van de Vloedt were passed to Dr Udo Feldmann of IAEA who was in contact with his family. The list of posthumous beneficiaries is contained in PAAT Newsletter, Issue No. 4 of October 1999.

The 34th Executive Committee meeting in Kampala decided to award a medal and a certificate to Dr John Kabayo, the Coordinator of PATTEC for his contribution in creating awareness and mobilising resources for Tsetse eradication.

Certificate awards were also given to the best five posters as a means of encouraging the production of high quality and educative posters in future meetings.

34th ISCTRC Executive Committee meeting

The 34th Executive committee meeting of ISCTRC was held in Speke Resort Kampala on 20th September 2009. It was attended by 7 out of 9 regional members and the Institutional members from AU-IBAR, FAO, WHO, PAAT, PATTEC, ILRI, ICIPE, and CIDRES. The meeting recommended the use of standardised format for country reports and establishment of a database, support the evaluation of PAAT, support PATTEC as a technical committee on Tsetse eradication activities. The committee also held a meeting on 25th September 2009 to review the 30th ISCTRC conference and several recommendations to improve the management of future ISCTRC meetings were made.

Members of the Executive Committee 2009-2011

The Executive committee appointed Dr. Kalinga Chilongo, Principal Biologist, T&T Control, Department of Veterinary Services, Zambia to replace Dr. Philemon Motsu as the representative of the Southern Africa Region. Dr. Nickolas Kauta became the chairperson and Dr. Mamadou Dia was elected Vice Chairperson. Prof Theophile Josenando, the immediate Chairperson was to serve another term on advisory capacity. The other regional members of the Executive committee were retained.

New regional member:

Southern Africa	Dr. Kalinga Chilongo, Zambia

Continuing regional members:

Central Africa	Dr Louis Banipe, Cameroon
	Dr Victor Kande, DRC
Eastern Africa	Dr Ahmed H A Rahman, Sudan
	Ms Joyce Daffa, Tanzania
Southern Africa	Prof Josenando Théophile, Angola
West Africa	Dr Charles Mahama, Ghana
	Dr Issa Degoga, Mali
Northern Africa	Dr Lamine Dia Mamadou, Mauritania

International organisation membership:

FAO, IAEA, PAAT, PATTEC, WHO, ICIPE, ILRI, CIRDES.

Reception dinner and cocktail

A grand dinner was organised by the Government of Uganda on 21st September 2009 and CEVA, DNDi, and FIND organised cocktails for the conference participants.

Closing ceremony

The Closing Ceremony was officiated by Hon, Minister of Primary Health Care, and Hon. James Kakooza. He observed that the acute and chronic forms of sleeping sickness in Uganda remain a challenge to his Ministry and the Country as they continue spreading north-eastwards, due largely to increase human and cattle movements in the sub-region.

He observed that a combination therapy in the form of NECT to address emerging drug resistance to the traditional Melarsoprol reported in Angola, DRC and Uganda had been launched during the Conference. The Closing ceremony was also graced by the Minister of state for animal industry, Hon Bright Rwamirama and the director of AU-IBAR who represented the Commissioner for Rural Economy and Agriculture.

In Her closing Remarks, the Commissioner of Rural Economy and Agriculture congratulated the participants for their contribution and patience during the conference. She reiterated the need to translate scientific innovations into usable services and products for the benefit of the society and she in particular recognised the tremendous efforts being made by partners to develop new drugs and diagnostics against Trypanosomiasis such as the new products for the management of the disease that were launched during the conference.

She thanked the Government and people of Uganda for their generous contribution and for hosting the conference.

In a vote of thanks the participants expressed their gratitude to the Government and people of Uganda for accepting to host the meeting and for the warm hospitality that they received.

Dr. James K. Wabacha
ISCTRC Secretary, AU-IBAR
Nairobi, Kenya
October 2009

RAPPORT DU SECRÉTAIRE DU CSIRLT SUR LA 30ÈME CONFERENCE

Cérémonie d'ouverture

La 30^{ème} réunion du CSIRLT a eu lieu à Speke Commonwealth Resort à Kampala, en Ouganda du 21 au 25 Septembre 2009. La réunion coïncidait avec le 60^{ème} anniversaire du CSIRLT et le thème de la réunion était «Vers la consolidation des stratégies de gestion des trypanosomiasés en Afrique". Elle a été ouverte par Son Excellence M. le Premier Vice-Premier Ministre et Ministre Chargé de la Fédération de l'Afrique de l'Est, l'Honorable Enya Kategaya qui a lu un discours au nom du Président de la République de l'Ouganda, Son Excellence Yoweri Kaguta Museveni. Le Vice-Premier Ministre a souligné l'impact de la mouche Tsé-tsé et des Trypanosomiasés sur le développement rural et le bien-être des populations sur le continent Africain et a également exhorté les chercheurs à se concentrer sur les domaines de recherche susceptibles de contribuer à la gestion efficace de la mouche Tsé-tsé et des Trypanosomiasés. La cérémonie d'ouverture a été également rehaussée par la présence de l'Honorable Hope Mwesigye, Ministre de l'Agriculture, de l'Elevage et de la Pêche ainsi que du Directeur de l'UA-BIRA, Prof Ahmed Elsalwaly qui a également lu un discours au nom de la Commissaire du Département de l'Economie Rurale et Agriculture de l'Union Africaine, Mme Rhoda Peace Tumusiime.

Participants à la conférence et présentations

La conférence a été honorée par la présence de 312 participants venant de 36 pays infestés de glossines et de 12 organisations internationales.

Les présentations ont porté sur les domaines thématiques suivants: La Campagne Panafricaine contre la Mouche Tsé-tsé et les Trypanosomiasés (PATTEC), la Trypanosomiase humaine africaine, les Trypanosomiasés animales africaines, la biologie et le contrôle de la glossine, la socio-économie, l'environnement et l'aménagement du territoire. Des notes liminaires ont été présentées pour chacun des domaines thématiques.

Un total de quatre vingt dix sept (97) présentations scientifiques ont été faites. Soixante et onze (71) communications ont été présentées en séance plénière tandis que vingt six (26) ont été présentés sous forme de

posters. Vingt (20) pays africains ont présenté leurs rapports sur le progrès des activités relatives la tsé-tsé et aux trypanosomiasés depuis la dernière réunion qui s'est tenue à Luanda, en Angola en 2007.

Distribution du compte-rendu de la 29^{ème} Conférence

Le compte-rendu de la 29^{ème} Conférence, aussi bien en copies papier qu'électroniques, a été distribué aux participants. Les documents et articles de promotion du CSIRLT (chapeaux et T-shirts) ont également été distribués aux participants de la conférence.

Réunions parallèles

Au cours de la réunion, deux organisations internationales, DNDi (l'Initiative pour les Médicaments des Maladies Négligées) et FIND (Fondation pour des Outils Diagnostiques Nouveaux et Novateurs) ont tenu des réunions parallèles pendant desquelles elles ont profité pour expliquer leurs activités et lancer sur le marché de nouveaux produits contribuant à la gestion de la Trypanosomiasé. DNDi a lancé une thérapie de combinaison pour la deuxième phase de la Trypanosomiasé humaine Africaine, sous une forme appelée NECT (Nifurtimox-Eflornithine Combinaison Therapy) et qui empêche les nouvelles résistances au Mélarsozol, un produit utilisé depuis longtemps avec aujourd'hui des résistances signalées en Angola, en RDC et en Ouganda. FIND a lancé en démontrant l'usage, un microscope de terrain à fluorescence.

Formation encadrement des jeunes scientifiques

L'Université de Santé Publique de Yale a formé 30 jeunes scientifiques en biologie des vecteurs et en rédaction scientifique. Les participants à la réunion ont noté que cette formation venait à point nommé puisque le nombre de scientifiques formés dans le domaine de la Tsé-tsé et des Trypanosomiasés était en réduction, raison pour laquelle de telles formations sont encouragées. Un appel a été lancé aux autres organisations pour appuyer le renforcement des capacités.

Renforcement du CSIRLT

Le mandat et les fonctions du CSIRLT ont été examinés et le Conseil a adopté une résolution qui appelle au renforcement du Conseil afin de lui permettre de répondre aux défis émergents. Le Conseil a décidé que la Commissaire de l'UA-DERA soit conviée à entamer la mise en œuvre de l'inclusion du Secrétariat du CSIRLT au sein des structures de l'UA-BIRA ainsi que sa dotation d'une ligne budgétaire avec les effectifs en ressources humaines nécessaires.

Commémoration du 60ème anniversaire et remise de médailles et certificats à d'éminents scientifiques

Dix médailles et certificats ont été décernés à 10 scientifiques qui ont apporté une contribution significative à la recherche et au contrôle de la mouche Tsé-tsé et des Trypanosomiasés au cours des 10 dernières années, jusqu'en 2009. Cinq autres médailles qui auraient dû être décernées à titre posthume au cours du 50^{ème} anniversaire du CSIRLT ont également été attribuées aux proches des défunts. Les médailles ont été attribuées à M. Jack Lancien, Dr GL Kasyumba, Prof E Bursell, Dr E Freidheim et Dr A. van der Vloedt en reconnaissance de leurs contributions à la gestion de la mouche Tsé-tsé et des Trypanosomiasés.

Les médailles et les certificats de Lancien et Kasyumba ont été transmis au Dr. Pere Simarro de l'OMS pour les retransmettre à leurs familles respectives. De même ceux de van der Vloedt ont été transmis au Dr Udo Feldmann de l'AIEA qui a été en contact avec sa famille. La liste des bénéficiaires à titre posthume est contenue dans le bulletin d'information du PLTA, dans le fascicule N°4 d'octobre 1999.

La 34^{ème} réunion du Comité Exécutif du CSIRLT à Kampala a également décidé de décerner une médaille et un certificat à Dr John Kabayo, le Coordonnateur du PATTEC pour sa contribution dans la sensibilisation et la mobilisation des ressources pour éliminer la mouche Tsé-tsé en Afrique.

Des certificats et des prix ont aussi été décernés aux cinq meilleurs posters afin d'encourager la production de posters éducatifs de haute qualité pour les futures réunions.

34^{ème} réunion du Comité Exécutif du CSIRLT

La 34^{ème} réunion du Comité Exécutif du CSIRLT a eu lieu à Speke, Resort à Kampala le 20 Septembre 2009. Elle a connu la participation de 7 des 9 membres régionaux et tous les membres des institutions internationales, en particulier l'UA-BIRA, la FAO, l'OMS, le PLTA, PATTEC, l'ILRI, l'ICPE, et le CIRDES. La réunion a recommandé l'utilisation d'un format standardisé pour les rapports nationaux et la mise en place d'une base de données. Elle a aussi recommandé d'appuyer l'évaluation du PLTA et de soutenir le PATTEC en tant que comité technique pour les activités d'éradication de la mouche Tsé-tsé. Le comité a également tenu une réunion le 25 Septembre 2009 pour examiner le déroulement de la 30^{ème} conférence du CSIRLT et plusieurs recommandations ont été formulées pour améliorer la gestion des futures réunions.

Les membres du Comité Exécutif 2009-2011

Le comité exécutif a nommé Dr Kalinga Chilongo, Biologiste Principal Mouche Tsé-tsé et Lutte contre les Trypanosomiasés, Département des Services Vétérinaires de Zambie pour représenter la région Afrique Australe en remplacement du Dr Philémon Motsu. Dr Nickolas Kauta a été élu Président et Dr Mamadou Dia Vice-président. Prof Théophile Josenando, le Président en exercice a été reconduit pour un nouveau mandat en qualité de Conseiller. Les autres membres régionaux du comité exécutif ont été reconduits.

Nouveau membre régional:

Afrique du Sud M. Kalinga Chilongo, Zambie

Les membres régionaux reconduits:

Afrique centrale	Dr Louis Banipe, Cameroun
	Dr Victor Kande, RDC
Afrique de l'Est	Dr Ahmed H A Rahman, Soudan
	Mme Joyce Daffa, Tanzanie
Afrique Australe	Prof Josenando Théophile, Angola
Afrique de l'Ouest	Dr Charles Mahama, Ghana
	Dr Issa Degoga, Mali

Afrique du Nord

Dr Lamine Dia Mamadou, Mauritanie

Les organisations internationales membres sont la FAO, l'AIEA, le PLTA, le PATTEC, l'OMS, l'ICIPE, l'ILRI et le CIRDES.

Réceptions, dîners et cocktail

Un dîner de gala a été organisé par le Gouvernement Ougandais le 21 Septembre 2009. De même, CEVA, DNDi et FIND ont organisés des cocktails pour les participants à la conférence.

Cérémonie de clôture

La cérémonie de clôture a été présidée par Mr le Ministre chargé des Soins de Santé Primaires, l'Honorable James Kakooza. Le Ministre a fait observer qu'en Ouganda, les formes aiguës et chroniques de la maladie du sommeil continuent de poser un défi à son ministère et au pays car ces maladies ont été propagées vers le nord-est, principalement en raison de l'accroissement des mouvements d'hommes et de bétail dans la sous-région. Il a fait observer qu'une thérapie combinée sous la forme de NECT, permettant de combattre les nouvelles résistances au traditionnel Mélarsozol signalées en Angola, en RDC et en Ouganda, a été lancée durant la Conférence. La cérémonie de clôture a été également honorée par la présence de Mr le Ministre d'État à l'Industrie Animale, l'Honorable Bright Rwamirama ainsi que le Directeur de L'UA-BIRA et la Commissaire à l'Economie Rurale et à l'Agriculture de l'UA.

Dans ses remarques de clôture, la Commissaire à l'Economie Rurale et à l'Agriculture a félicité les participants pour leurs contributions et leur patience au cours de la conférence. Elle a réitéré la nécessité de traduire les innovations scientifiques en des services et produits utilisables pour le bénéfice de la société, et elle a souligné en particulier les efforts considérables déployés par les partenaires pour développer de nouveaux médicaments et diagnostics contre les Trypanosomiasés tels que les produits nouveaux lancés lors de cette conférence pour la gestion de la Trypanosomiasé humaine.

Elle a remercié le Gouvernement et le peuple Ougandais pour sa généreuse contribution et pour avoir accueilli la conférence.

Dans une motion de remerciement, les participants ont exprimé leur gratitude au Gouvernement et au peuple Ougandais pour avoir accepté d'accueillir la réunion et pour la chaleureuse hospitalité dont ils ont fait l'objet.

M. James K. Wabacha
Secrétaire du CSIRLT, UA-BIRA
Nairobi, Kenya
Octobre 2009

**SPEECHES/
DISCOURS**

A SPEECH BY THE HON. MINISTER OF AGRICULTURE, ANIMAL INDUSTRY & FISHERIES

Your Excellency, the Vice President of The Republic Of Uganda,
Your Excellencies, the Ambassadors Present,
The Commissioner for Rural Economy and Agriculture at the African Union,
The Hon. Ministers Present,
The Director of the Inter-African Bureau for Animal Resources
The Resident Representative of African Development Bank
The Country Representative of the Food and Agricultural Organization in Uganda

The Representative of the World Health Organization in Uganda,
Representatives of Development Partners,
Chairman for the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC),

Members of ISCTRC Executive Committee,
Members of Parliament Present
Conference Participants
Ladies and Gentlemen

I am profoundly honoured to be with you today and with great humility, I would like to welcome you all to Uganda for this 30th Conference of the International Scientific Council for Trypanosomiasis Research and Control.

As you are aware, Uganda is one of the 37 tsetse infested countries in Sub-Saharan Africa. It is estimated that more than two thirds of the country are Tsetse infested which puts approximately 70% of the national herd at risk of contracting Nagana. About 9 million people leave at risk of contracting sleeping sickness.

I am reliably informed that there are three major species of Tsetse flies that are of economic importance in Uganda namely *Glossina fuscipes fuscipes*, *Glossina pallidipes* and *Glossina morsitans morsitans*. I am also reliably informed that there are two forms of sleeping sickness; the acute form found in South Eastern Uganda and chronic form found in

North Western Uganda; and that Uganda is the only country where both forms occur. Approximately 700 new cases of sleeping sickness are reported each year. On the other hand 70% of cattle live at the risk of contracting Nagana and the prevalence of the disease ranges from 5% to 40%.

Since the early part of the last century, the Government of Uganda and development partners have made attempts to control the disease and its vector, the Tsetse fly. While these attempts have had significant impact on reducing on the prevalence/ incidence of the disease in both animals and humans, they have not been sufficient to eradicate the disease because they were not based on an area-wide approach.

Uganda through the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) adopted the strategy on area-wide integrated approach for the Tsetse eradication which is currently being implemented in six AU member countries. This approach is expected in the long run to free the continent from the effects of T&T. The approach is in consonance with the decision made in Lome in 2000 urging member states to act collectively to eradicate Tsetse from the African continent.

As you are aware, there is no vaccine against this disease and no new drugs are being developed and currently there is a limited range of drugs in use. These drugs are highly toxic and increasingly becoming ineffective due to drug resistance. There is need to invest in research for development of more effective drugs against the disease. In this connection, Uganda has been participating in combination therapy trials to improve on treatment of sleeping sickness.

Let me take this opportunity on behalf of the Government of Uganda and on my own behalf to extend our sincere thanks to the PATTEC secretariat at the African Union who are spear heading the coordination of the campaign against Tsetse flies.

I also wish to thank Partners in Development especially the African Development Bank, World Health Organization, and the International Atomic Energy Agency who are assisting us technically and financially in this struggle to eradicate T&T in our country.

It is my privilege to invite H.E. the Vice President of the Republic of Uganda to address the participants and open the 30th Conference of the

International Scientific Council for Trypanosomiasis Research and Control.

For God and My Country

DISCOURS DE L'HONORABLE MINISTRE DE L'INDUSTRIE ANIMALE, DE L'AGRICULTURE, ET DE LA PECHE

Votre Excellence, M. le Vice-président de la République de l'Ouganda,
Excellences, Mesdames et Messieurs les Ambassadeurs ici présents,
Mme la Commissaire à l'Economie Rurale et Agriculture de l'Union
Africaine,

Honorables Ministres ici présente,

M. le Directeur du Bureau Interafricain des Ressources Animales,

M. Le Représentant Résident de la Banque Africaine de Développement,

M. le Représentant Résident de l'Organisation pour l'Alimentation et
l'Agriculture (FAO) en Ouganda,

M. le Représentant de l'Organisation Mondiale de la Santé en Ouganda,
Mesdames et Messieurs les Représentants des partenaires au
développement,

M. le Président du Conseil Scientifique International pour la Recherche
et la Lutte contre la Trypanosomiase (CSIRLT),

M. les membres du Comité Exécutif du CSIRLT,

Mesdames et Messieurs les Membres du Parlement ici présents,

Mesdames et Messieurs les participants à la Conférence,

Mesdames et Messieurs.

Je suis profondément honoré d'être avec vous aujourd'hui et c'est avec
beaucoup d'humilité que je vous souhaite la bienvenue à tous en
Ouganda pour cette 30^{ème} Conférence du Conseil Scientifique
International pour la Recherche et la Lutte contre Trypanosomiase.

Comme vous le savez, l'Ouganda est l'un des 37 pays infestés par les
glossines en Afrique sub-saharienne. On estime que plus des deux tiers
du pays est infesté de mouches Tsé-tsé ce qui met environ 70% du
cheptel national au risque de contracter le Nagana. Environ 9 millions de
personnes vivent avec le risque de contracter la maladie du sommeil.

Je sais de source sûre qu'il y a trois principales espèces de mouches tsé-
tsé qui sont d'une importance économique en Ouganda nommément
Glossina fuscipes fuscipes, *Glossina pallidipes* et *Glossina morsitans*
morsitans. Je suis aussi sûr qu'il y a deux formes de la maladie du
sommeil, la forme aiguë retrouvée dans le Sud-Est de l'Ouganda et la
forme chronique trouvée au Nord-Ouest de l'Ouganda, et que l'Ouganda
est le seul pays où les deux formes se retrouvent. Environ 700 nouveaux
cas de maladie du sommeil sont signalés chaque année. D'autres parts,

70% des bovins vivent au risque de contracter le Nagana et la prévalence de la maladie est variable, allant de 5% à 40%.

Depuis le début du siècle dernier, le gouvernement de l'Ouganda et les partenaires au développement ont tenté de mettre sur pieds des actions pour contrôler la maladie et son vecteur, la mouche Tsé-tsé. Bien que ces tentatives aient eu un impact significatif sur la réduction de la prévalence ou l'incidence de la maladie chez les animaux et les humains, elles n'ont pas été suffisantes pour éradiquer la maladie parce qu'elles n'étaient pas fondées sur une approche à grande échelle.

L'Ouganda, par la Campagne Panafricaine d'Eradication de la mouche Tsé-tsé et la Trypanosomiose (PATTEC) a adopté la stratégie d'approche à grande échelle pour l'éradication de la tsé-tsé, qui est actuellement mis en œuvre dans six pays membres de l'UA. Cette approche devrait, à long terme, libérer le continent des effets de la mouche Tsé-tsé et la Trypanosomiose. L'approche est en accord avec la décision prise à Lomé en 2000 invitant les Etats membres à agir collectivement pour éradiquer la mouche Tsé-tsé sur le continent africain.

Comme vous le savez, il n'existe aucun vaccin contre cette maladie, il n'y a pas de nouveaux médicaments développés et il n'existe actuellement qu'une gamme limitée de médicaments en cours d'utilisation. Ces médicaments sont très toxiques et de plus en plus inefficaces en raison de la résistance- développée par les parasites aux médicaments. Il est donc nécessaire d'investir dans la recherche pour le développement de médicaments plus efficaces contre la maladie. À cet égard, l'Ouganda a participé à des essais de thérapie de combinaison visant à améliorer le traitement de la maladie du sommeil.

Permettez-moi de saisir cette occasion au nom du Gouvernement de l'Ouganda et en mon nom propre pour exprimer nos sincères remerciements au Secrétariat de la Campagne Panafricaine d'Eradication de la mouche Tsé-tsé et la Trypanosomiose de l'Union Africaine qui est le moteur de la coordination de la lutte contre la mouche Tsé-tsé.

Je tiens également à remercier les partenaires au développement, notamment la Banque Africaine de Développement, l'Organisation Mondiale de la Santé, et l'Organisation Internationale de l'Energie Atomique qui nous aident techniquement et financièrement dans cette lutte pour éradiquer la mouche Tsé-tsé et la Trypanosomiose dans notre pays.

J'ai le privilège d'inviter son Excellence M. le Vice-président de la République de l'Ouganda à s'adresser aux participants et prononcer l'ouverture de la 30^{ème} Conférence du Conseil Scientifique International pour la Recherche et la Lutte contre la Trypanosomiase.

Pour Dieu et pour mon pays

SPEECH FOR H.E. THE PRESIDENT OF THE REPUBLIC OF UGANDA

Your Excellences, The Ambassadors;
The Commissioner for Rural Economy and Agriculture at the African Union;
Hon. Ministers of Agriculture, Animal Industry & Fisheries and Health
The Director of the Inter-African Bureau for Animal Resources
The Resident Representative of African Development Bank
The Resident Representative of the Food and Agricultural Organization in Uganda
The Representative of the World Health Organization in Uganda,
Representatives of Development Partners,
Chairman for the International Scientific Council for Trypanosomiasis Research and Control
Members of ISCTRC Executive Committee,
Members of Parliament Present
Conference Participants
Ladies and Gentlemen

I wish to take this opportunity to welcome you all to Uganda. I hope you will enjoy your stay in Kampala and spare some time to see the countryside.

Africa is lagging behind in economic development partly due to the high burden of preventable diseases. Uganda like other developing countries is affected by many tropical diseases, which fall into the category of Neglected Tropical Diseases (NTDs) that affect the rural poor populations. The Republic of Uganda recognizes the Public health importance of the NTDs, which include Trypanosomiasis, River blindness, Bilharzia, etc.

The tsetse transmitted sleeping sickness and Nagana are a major hindrance to the achievement of the Millennium Development Goals. Historically, Uganda has been affected by a number of sleeping sickness outbreak cycles, the most severe of which occurred at the turn of the last century (1901) where an estimated 300,000 people lost their lives and others had to be evacuated from their homes to the disease free areas. The consequence was the negative socio-economic impact (Social disruption, large expanses of fertile land were abandoned, human productivity was severely undermined).

Similarly, between 1900-07, the great western fly belt of Northern Tanganyika expanded northwards into southwestern Uganda and by

1930s tsetse infestations had occupied more than 9000 square kilometers of grazing land and livestock were moved further north to prevent more deaths.

The eradication of T&T is a priority area to my Government and as a result, many National Programmes have been developed to control and eliminate Nagana and sleeping sickness. The development and implementation of these programmes have been as a result of close partnership with international collaborating partners, donors and bilateral agencies that I wish to recognize.

Dear Participants, you will recall that at the 36th assembly of Heads of State and Government held in Lome, Togo in 2000, a decision was taken to eradicate T&T from the African continent. Member countries affected by this problem were urged to mobilize the necessary human, financial and material resources required to render Africa Tsetse free within the shortest time possible. As a result of this, 2001 was declared the year to mark the beginning of renewed efforts in the campaign for the eradication of Tsetse flies in Africa.

I note that the theme for this conference is ***‘Towards consolidating strategies to manage Trypanosomiasis in sub-Saharan Africa.’*** There is now a renewed global interest for integrated control with eventual elimination of Nagana and sleeping sickness and Uganda has chosen to take this line. Uganda has developed a national policy on eradication of Tsetse and Trypanosomiasis whose vision is a ***‘Healthy and prosperous people free from Nagana and Sleeping sickness’***.

Uganda has successfully halted the merger of the two forms of sleeping sickness through a public private partnership to implement a stamp out sleeping sickness (SOS) campaign.

In this conference, scientific papers will be presented making suggestions on the approaches towards achieving eradication of the Tsetse flies and elimination of sleeping sickness and Nagana. It is my belief that this scientific conference will come up with recommendations on methodologies to be used for the attainment of the Lome 2000 decision of the Heads of State and Government to eradicate Tsetse and eliminate Sleeping Sickness and Nagana from the African continent within the shortest time possible.

I am also reliably informed that the ISCTRC is celebrating 60 years of existence, in which there have been major achievements in research and

control of Nagana and sleeping sickness. For example Botswana has managed to eradicate Tsetse from the country and am sure that the rest of the countries are following the same trend. I take this opportunity to wish you good commemoration.

Lastly, I wish to thank the organizers of this conference for the job well done.

I now declare the 30th International Scientific Council for Trypanosomiasis Research and Control Open.

For God and My Country

DISCOURS DE SON EXCELLENCE MONSIEUR LE PRÉSIDENT DE LA RÉPUBLIQUE DE L'UGANDA

Excellence, Messieurs les Ambassadeurs;
Madame la Commissaire à l'Economie Rurale et Agriculture de l'Union Africaine;
Excellence, Messieurs les Ministres de l'Agriculture, de l'Elevage et des Pêches et de la Santé ;
Monsieur le Directeur du Bureau Interafricain des Ressources Animales ;
Monsieur le Représentant Résident de la Banque Africaine de Développement ;
Monsieur le Représentant Résident de l'Organisation pour l'Alimentation et l'Agriculture en Ouganda ;
Monsieur le Représentant de l'Organisation Mondiale de la Santé en Ouganda ;
Mesdames Messieurs les Représentants des partenaires au développement ;
Monsieur le Président du Conseil Scientifique International pour la Recherche et la Lutte contre la Trypanosomiase ;
Messieurs les Membres du Comité Exécutif du CSIRLT ;
Messieurs les Membres du Parlement présents ;
Mesdames, Messieurs les Participants à la Conférence ;
Mesdames et Messieurs.

Je tiens à saisir cette occasion pour vous souhaiter la bienvenue en Ouganda. J'espère que vous apprécierez votre séjour à Kampala et que vous trouverez un peu de temps pour découvrir nos campagnes.

L'Afrique est en retard dans son développement économique, en partie en raison de la forte charge de maladies évitables. L'Ouganda comme d'autres pays en développement est affecté par de nombreuses maladies tropicales qui entrent dans la catégorie des maladies tropicales négligées (MTN) affectant les populations pauvres des zones rurales. La République de l'Ouganda reconnaît en matière de la santé l'importance des MTN que sont la Trypanosomiase, l'Onchocercose, la Bilharziose, etc...

La maladie du sommeil et le Nagana transmis par la mouche Tsé-tsé sont un obstacle majeur à la réalisation des Objectifs de Développement du Millénaire. Historiquement, l'Ouganda a été affecté par un certain nombre de cycles d'éruption de la maladie du sommeil, la plus grave

d'entre elles étant celle qui a eu lieu vers la fin du siècle dernier (1901), où environ 300.000 personnes ont perdu la vie et où d'autres ont dû être évacuées de leur domicile vers des zones exemptes de maladies. La conséquence a été un impact socio-économique négatif (perturbation sociale, de grandes étendues de terres fertiles abandonnées, la productivité humaine gravement compromise).

De même, entre 1900 et 1907, la grande ceinture occidentale de mouches située au nord du lac Tanganyika s'est élargie vers le nord pour englober le sud-ouest de l'Ouganda et en 1930 des infestations de mouche Tsé-tsé avaient occupé plus de 9000 kilomètres carrés de terres de pâturage, ce qui a conduit au déplacement du bétail plus au nord pour éviter davantage de pertes.

L'éradication de la mouche Tsé-tsé et de la Trypanosomiose est une priorité pour mon gouvernement et par conséquent, de nombreux programmes nationaux ont été développés pour contrôler et éliminer le Nagana et la maladie du sommeil. L'élaboration et la mise en œuvre de ces programmes ont été le résultat d'un partenariat étroit avec les partenaires internationaux, les donateurs et des agences bilatérales que je tiens à saluer ici.

Chers participants, vous vous souviendrez que lors de la 36^{ème} assemblée des chefs d'État et de Gouvernement tenue à Lomé, au Togo en 2000, une décision a été prise pour éradiquer la mouche Tsé-tsé et la Trypanosomiose sur le continent Africain. Les Etats membres touchés par ce problème ont été invités à mobiliser les ressources humaines, financières et matérielles nécessaires pour rendre l'Afrique libre de mouche Tsé-tsé dans les plus brefs délais. À la suite de cela, 2001 a été déclarée l'année pour marquer le début des efforts renouvelés dans la campagne pour l'éradication de la mouche Tsé-tsé en Afrique.

Je note que le thème de cette conférence est *«Vers la consolidation des stratégies de gestion de la Trypanosomiose en Afrique sub-saharienne.»* Il y a maintenant un regain d'intérêt mondial pour la lutte intégrée pour l'élimination éventuelle du Nagana et la maladie du sommeil et l'Ouganda a choisi de prendre cette ligne. L'Ouganda a élaboré une politique nationale sur l'éradication de la mouche Tsé-tsé et la Trypanosomiose dont la vision est *«Un peuple en bonne santé et prospère sans Nagana et sans la maladie du sommeil»*.

L'Ouganda a réussi à interrompre la fusion des deux formes de la maladie du sommeil grâce à un partenariat public-privé qui a mis en œuvre la campagne d'élimination (stamping-out) de la maladie du sommeil (SOS).

Au cours de cette conférence, des articles scientifiques seront présentés faisant des suggestions sur les approches en vue d'atteindre l'éradication de la mouche Tse-tsé et l'élimination de la maladie du sommeil et le Nagana. Je reste convaincu que cette conférence scientifique va formuler des recommandations sur les méthodes à utiliser pour atteindre la décision des chefs d'État- et de Gouvernement- de Lomé 2000 pour éradiquer la mouche Tsé-tsé et éliminer la maladie du sommeil et le Nagana du continent Africain dans les plus brefs délais possibles.

Je suis également informé de source sûre que le CSIRLT fête ses 60 ans d'existence, au cours desquelles il ya eu des succès importants dans la recherche et le contrôle du Nagana et de la maladie du sommeil. Par exemple le Botswana a réussi à éradiquer la mouche Tsé-tsé de son territoire et je suis sûr que les autres pays suivent la même tendance. Je saisis cette occasion pour vous souhaiter une bonne commémoration.

Enfin, je tiens à remercier les organisateurs de cette conférence pour le travail bien fait.

Sur ce, je déclare la 30^{ème} conférence du Conseil Scientifique International pour la Recherche et la Lutte contre la Trypanosomiase ouverte.

Pour Dieu et pour mon pays.

REMARKS BY HER EXCELLENCY MRS RHODA PEACE TUMUSIIME, COMMISSIONER FOR RURAL ECONOMY AND AGRICULTURE

Your Excellency, The Vice-President of the Republic of Uganda.
The Minister of Agriculture, Animal Industry and Fisheries
Executive Committee members of the ISCTRC
Representatives of International Organizations
Distinguished guests
The National Organizing Committee members
Ladies and Gentlemen

It is with great pleasure and honour, that I welcome you to the 30th conference of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) on behalf of the Chairperson of the African Union Commission, His Excellency Dr. Jean Ping. He requested that I convey his greetings and best wishes to the Government and the people of the Republic of Uganda, delegates and participants attending this meeting. He wishes all of you very successful deliberations.

Your Excellencies

On behalf of the African Union Commission, I take this opportunity to thank the Government of Uganda for having accepted to host the 34th Executive Committee meeting and the 30th ISCTRC conference following a request by the Executive Committee of ISCTRC. Your kind gesture is highly appreciated. The African Union Commission also notes with appreciation your support for the ISCTRC conference in the past during the 10th and 22nd conferences of 1964 and 1993, respectively.

Ladies and Gentlemen

I also take this opportunity to thank the Executive Committee of ISCTRC, the Scientific Committee and the National organising committee for working tirelessly to make this event possible. Special thanks also go to the participants, who left their busy schedules to attend this conference and on behalf of the African Union Commission please accept our appreciation.

Your Excellencies

The Department of Rural Economy and Agriculture (DREA) of the African Union Commission (AUC) which I head has several technical agencies which deliver on specific mandates. One of the specialised agencies of DREA is the AU-IBAR. The mission of AU-IBAR is to provide leadership in the development of animal resources for Africa through supporting and empowering AU Member States and Regional Economic Communities. One of the mandate areas of AU-IBAR is to improve public and animal health through the control and eradication of trans-boundary animal diseases and zoonoses including Human and Animal African Trypanosomiasis.

I am pleased to inform you that AUC, DREA and AU-IBAR are undergoing strategic reforms to adjust to the changing environment. The anticipated reforms are expected to strengthen the operations of the AUC including those of the Council in regard to approaching Trypanosomiasis research and control through the new six strategic thrusts of AU-IBAR namely; natural resources management, investment and competitiveness, reducing impacts of trans-boundary animal diseases and zoonoses, standards and regulation, knowledge management and policies and capacity building.

Ladies and Gentlemen

The International Scientific Council for Trypanosomiasis Research and Control operates under the auspices of the African Union and the secretariat is managed by AU-IBAR. The Council was established in 1948 and held its first conference in 1949.

The International Organisations, which hitherto were coordinating the activities of the Council handed over the Council to African Union in 1965 to be funded by member states. It was then transformed into Scientific Technical Research Committee (STRC) which later became to be known as International Scientific Council for Trypanosomiasis Research and Control (ISCTRC). Since then, the Council has continued to promote research and control of Trypanosomiasis in the 37 Tsetse infested countries in the African continent

In running the activities of the Council, AU/IBAR works closely with international organizations and programmes including FAO, WHO, IAEA, PAAT, PATTEC, NGOs and development partners in order to support efforts to manage T&T control in Africa. The activities carried

out by the Council include organising trainings and conferences as fora that provide member countries and scientists with opportunities for interaction and exchanging ideas on T&T research and control and identify priority areas of research and collaboration.

Your Excellencies:

Tsetse transmitted African Trypanosomiasis that affects both human and livestock is one of the most severe constraints to human and animal health and to the agricultural economy, especially in rural areas of Africa. Sixty five million people and 50 million cattle are at high risk in nearly 9 million square kilometres in the Sub-Saharan Africa. The disease causes many human deaths in Africa and leads to annual agricultural production loss of US\$ 4.5 billion annually.

Under the guidance and advocacy of the ISCTRC the AU Member states have implemented successful projects and programmes to control T&T and they include:

AU supported Pan African Tsetse and Trypanosomiasis Campaign (PATTEC) with phase one being implemented in six African countries: Ethiopia, Kenya and Uganda in Eastern Africa and Burkina Faso, Ghana and Mali in Western Africa with loan from African Development Bank. The Multinational Project: Creation of sustainable T&T free areas in six sub-Saharan African Countries which is now halfway in its implementation targeted to clear 130,000 square kilometres of Tsetse flies by the end of the project in year six.

The 10 year coordinated programme by SADC countries

The Farming in Tsetse Controlled Areas of Eastern Africa, 1999-2004 which Uganda has continued to implement with funding from European Community (EC) up to date.

Some of the success stories from the above projects include:

Increased awareness about T&T.

More countries and regions are developing Tsetse eradication programmes

Reduction in cases of animal and human African Trypanosomiasis among others

It is with great appreciation that following advocacy and awareness creation by PATTEC Secretariat. This was the first time governments took bank loan to eradicate T&T.

Ladies and Gentlemen:

It is my pleasure to note that we are celebrating the 60th anniversary of ISCTRC and this is an appropriate time to reflect about past achievements, challenges and lessons learnt in order to plan for the future. The theme of this year Conference '**Towards Consolidating Strategies to Manage Trypanosomiasis in Africa**' is very fitting as it reflects the strategy that ISCTRC and its various stakeholders need to adopt in the research and control of both human and animal African Trypanosomiasis. The consolidation of strategies will allow International Organisations, NGOs, development partners and members states to work together and avoid duplication, overlaps, gaps and provide synergy that will be necessary to manage the Trypanosomiasis menace in Africa.

In addition to consolidating the technologies, there will be need to increase the numbers, commitment and capacity of young scientists, field officers and beneficiary communities to cope with demands for the Trypanosomiasis disease control programmes.

Ladies and Gentlemen:

May I take this opportunity to thank all the organisations represented here for their willingness and commitment to work with AU-IBAR and the Member States. I also thank the distinguished guests who have put aside their busy schedules to attend this very important conference. I also thank all those institutions, men and women who have contributed immensely in the area of Tsetse and Trypanosomiasis research, and control.

Finally, Your Excellencies, Ladies and Gentlemen:

I wish to thank the Government of Uganda and the People of the Republic of Uganda yet again for hosting the 30th ISCTRC conference. We sincerely appreciate the hospitality and the arrangements that you have made for this important occasion including provision of this magnificent conference facilities. Let me take this opportunity, on behalf

of the Chairperson of the African Union Commission, and on my own behalf, to thank you all and wish you successful deliberations.

Thank you

**ALLOCUTION DE SON EXCELLENCE MADAME
TUMUSIIME RHODA PEACE, COMMISSAIRE A
L'ECONOMIE RURALE ET AGRICULTURE DE L'UNION
AFRICAIN**

Votre Excellence, Monsieur le Vice-Président de la République de l'Ouganda ;

Monsieur le Ministre de l'Industrie Animale, de l'Agriculture et des Pêches ;

Mesdames et Messieurs les Membres du Comité Exécutif du CSIRLT ;

Mesdames et Messieurs les Représentants des Organisations Internationales ;

Mesdames et Messieurs les Invités de marque ;

Mesdames et Messieurs les Membres du Comité National d'Organisation de la Conférence ;

Mesdames et Messieurs.

C'est avec grand plaisir et honneur que je vous souhaite la bienvenue à la 30^{ème} conférence du Conseil Scientifique International pour la Recherche et la Lutte contre la Trypanosomiase (CSIRLT) au nom du Président de la Commission de l'Union Africaine, Son Excellence M. Jean Ping. Il a demandé que je transmette ses salutations et ses meilleurs vœux au Gouvernement et au peuple de la République de l'Ouganda et aux délégués et participants à cette réunion. Il vous souhaite à tous des délibérations très fructueuses.

Excellences,

Au nom de la Commission de l'Union Africaine, je saisis cette occasion pour remercier le Gouvernement de l'Ouganda pour avoir accepté d'abriter la 34^{ème} Réunion du Comité Exécutif et la 30^{ème} Conférence du CSIRLT suite à la demande du Comité Exécutif du CSIRLT. Votre aimable geste est très apprécié. La Commission de l'Union Africaine prend également note avec satisfaction de votre soutien pour la Conférence du CSIRLT dans le passé lors des 10^{ème} et 22^{ème} conférences organisées respectivement en 1964 et 1993.

Mesdames et Messieurs,

Je saisis également cette occasion pour remercier le Comité Exécutif du CSIRLT, le Comité Scientifique et le Comité National d'Organisation pour le travail sans relâche grâce auquel cet événement est possible. Des remerciements spéciaux vont aussi aux participants, qui ont quitté leur emploi du temps chargé pour assister à cette conférence et au nom de la Commission de l'Union Africaine, Veuillez accepter nos remerciements.

Excellences,

Le Département d'Economie Rurale et Agriculture (DERA) de la Commission de l'Union Africaine que je dirige dispose de plusieurs organismes techniques qui travaillent sous des mandats spécifiques. L'une de ces institutions spécialisées du DERA est l'UA-BIRA. La mission de l'UA-BIRA est d'assurer un leadership dans le développement des ressources animales pour l'Afrique en soutenant et en habilitant les États membres de l'UA et les Communautés Economiques Régionales. L'un des secteurs du mandat de l'UA-BIRA est d'améliorer la santé publique et la santé animale à travers le contrôle et l'éradication des maladies animales transfrontalières et les zoonoses, y compris les trypanosomiasés africaines humaines et animales.

Je suis heureuse de vous informer que la Commission de l'Union Africaine, la DERA et l'UA-BIRA font actuellement l'objet de réformes stratégiques pour s'adapter à l'évolution de l'environnement. Les réformes prévues devraient permettre de renforcer les opérations de la CUA, y compris celles du Conseil en ce qui concerne l'approche de la recherche et la lutte contre les trypanosomiasés grâce aux six nouveaux axes stratégiques de l'UA-BIRA, notamment, la gestion des ressources naturelles, les opportunités d'investissement et la compétitivité, la réduction de l'impact des maladies animales transfrontalières et des zoonoses, les normes et réglementations, la gestion des connaissances et l'appui à l'élaboration des politiques et au renforcement des capacités.

Mesdames et Messieurs,

Le Conseil Scientifique International pour la Recherche et la Lutte contre la Trypanosomiasé fonctionne sous les auspices de l'Union Africaine et son Secrétariat est géré par l'UA-BIRA. Le Conseil a été créé en 1948 et a tenu sa première conférence en 1949.

Les organisations internationales, qui jusqu'alors assuraient la coordination des activités ont remis le Conseil de l'Union Africaine en

1965 pour qu'il soit financé par les Etats Membres. Il a ensuite été transformé en Comité Scientifique et Technique pour la Recherche (CSTR), qui devait plus tard être connu sous le nom de Conseil Scientifique International pour la Recherche et la lutte contre la Trypanosomiase (CSIRLT). Depuis lors, le Conseil a continué à promouvoir la recherche et le contrôle de la Trypanosomiase dans les 37 pays infestés de glossines sur le continent africain.

En exécutant les activités du Conseil, l'UA-BIRA travaille en étroite collaboration avec les organisations internationales et les programmes y compris la FAO, l'OMS, l'AIEA, le PLTA, le PATTEC, les ONG et les partenaires au développement afin de soutenir les efforts visant à gérer les efforts de lutte contre la mouche Tsé-tsé et les Trypanosomiasés en Afrique. Les activités menées par le Conseil comprennent l'organisation de formations et de conférences, des forums qui fournissent aux pays membres et aux scientifiques des possibilités d'interaction et l'échange d'idées sur la recherche et la lutte contre la mouche Tsé-tsé et les Trypanosomiasés ainsi que l'identification des domaines prioritaires de recherche et de collaboration.

Excellences,

Les Trypanosomiasés africaines transmises par la mouche Tsé-tsé qui affectent les humains et le bétail est l'une des contraintes les plus graves pour la santé humaine et animale et pour l'économie agricole, en particulier dans les zones rurales d'Afrique. Soixante-cinq millions de personnes et 50 millions de bovins sont exposés à un risque élevé sur près de 9 millions de kilomètres carrés en Afrique sub-saharienne. La maladie provoque de nombreux décès humains en Afrique et conduit à des pertes de production agricole annuelle de 4,5 milliards \$ US.

Sous la direction et le plaidoyer du CSIRLT, les Etats Membres de l'UA ont mis en œuvre des projets et programmes efficaces de lutte contre les glossines et les Trypanosomiasés qui comprennent,

La Campagne Panafricaine contre la mouche tsé-tsé et la trypanosomiase (PATTEC) appuyée par l'UA avec sa phase I mis en œuvre dans six pays africains: l'Ethiopie, le Kenya et l'Ouganda en Afrique de l'Est et le Burkina Faso, le Ghana et le Mali en Afrique de l'Ouest avec un prêt auprès de la Banque Africaine de Développement ;

Le Projet multinational: Le projet de création d'aires durablement libres de mouche Tsé-tsé et de Trypanosomiase dans six pays d'Afrique subsaharienne qui est maintenant à mi-chemin dans sa mise en œuvre avec en cible le nettoyage des mouches Tsé-tsé sur 130.000 kilomètres carrés d'ici la fin du projet, dans six ans ;

Le programme de 10 ans coordonné par les pays de la SADC ;

L'Agriculture en Zones Tsé-tsé contrôlées en Afrique de l'Est entre 1999 et 2004 que l'Ouganda a continué à mettre en œuvre jusqu'à ce jour avec un financement de la CE.

Certaines expériences réussies par les projets ci-dessus mentionnés comprennent,

La prise de conscience accrue par rapport à la mouche Tsé-tsé et les Trypanosomiasés ;

D'avantage de pays et régions développent actuellement des programmes d'éradication de la mouche Tsé-tsé ;

La réduction, entre autres, des cas animaux et humains de la Trypanosomiase africaine ;

C'est une grande satisfaction que, suite au plaidoyer et à la sensibilisation faite par le Secrétariat du PATTEC, pour la première fois, les Gouvernements contractent un prêt bancaire pour éradiquer la mouche Tsé-tsé.

Mesdames et Messieurs,

Il me plaît de constater que nous célébrons le 60^{ème} anniversaire du CSIRLT et c'est un moment opportun pour réfléchir sur les réalisations passées, les défis et leçons apprises afin de planifier pour l'avenir. Le thème de cette conférence cette année « Vers la Consolidation des Stratégies pour Gérer les Trypanosomiasés en Afrique" est très approprié car il reflète la stratégie que le CSIRLT et ses différentes parties prenantes doivent adopter dans la recherche et le contrôle des deux Trypanosomiasés humaine et animale en Afrique. La consolidation des stratégies va permettre aux organisations internationales, ONG, partenaires au développement et les Etats Membres de travailler ensemble pour éviter les doubles emplois, les chevauchements, les lacunes et créer des synergies qui seront nécessaires pour gérer la menace des Trypanosomiasés en Afrique.

En plus de consolider les technologies, il y aura besoin d'augmenter le nombre, l'engagement et la capacité des jeunes scientifiques, agents de terrain et les communautés bénéficiaires pour faire face aux exigences des programmes de lutte contre la maladie de la Trypanosomiase.

Mesdames et Messieurs,

Je voudrais saisir cette occasion pour remercier toutes les organisations représentées ici pour leur volonté et leur engagement à travailler avec l'UA-BIRA et les États Membres. Je remercie également les invités de marque qui ont mis de côté leurs emplois du temps chargé pour participer à cette conférence très importante. Je remercie également toutes les institutions, les hommes et les femmes qui ont contribué énormément dans le domaine de la recherche et la lutte contre la mouche Tsé-tsé et les Trypanosomiasés.

Enfin, Excellences, Mesdames et Messieurs,

Je tiens à remercier, une fois de plus, le Gouvernement de la République de l'Ouganda et le peuple Ougandais pour avoir accueilli la 30^{ème} conférence du CSIRLT. Nous apprécions sincèrement l'hospitalité et les arrangements faits pour cet important événement, y compris la mise à disposition de cette magnifique salle de conférence. Permettez-moi de saisir cette opportunité, au nom du Président de la Commission de l'Union Africaine, et en mon nom propre, pour vous remercier tous et vous souhaiter des délibérations fructueuses.

Je vous remercie.

**REPORTS AND RECOMMENDATIONS/
RAPPORTS ET RECOMMANDATIONS**

INTERNATIONAL ORGANISATIONS

Moderator: Dr. Prof. Ahmed Elsayalhy

Rapporteur: Dr. Raffaele Mattioli

The International Organisations presented their activities over the last two years and came up with the following recommendations

FAO

The meeting acknowledges the complexity of the T&T problem and notes that the various sectors of the livestock-agriculture development, including land use and natural resources, and socio-economic development are affected by the presence of the disease.

The meeting welcomes the work of FAO in:

- Generating standardized information pertaining to animal health, livestock and agricultural production systems, environment and agro-ecology of areas infested by Tsetse fly;
- Providing assistance to Tsetse affected countries in the formulation of policies, strategies and guidelines for T&T interventions.

The meeting RECOMMENDS

- To increase efforts for greater and wider adoption of developed policies and guidelines for planning T&T field programmes;
- To pursue endeavours for increased efficacy and impact at the field and country levels and enhance synergies with national, regional and international entities and initiatives.

WHO

The meeting acknowledges the achievements made in the area of sleeping sickness control. It is however concerned by the lack of appropriate tools to develop adapted control methodologies and the weaknesses of health systems to integrate sleeping sickness control and surveillance to sustain current results.

The meeting RECOMMENDS:

- WHO to continue its support to countries to adapt control strategies taking into consideration the evolution of the disease;
- Research and Development (R&D) groups to develop new diagnostic tools and drugs to ensure a cost effective, adapted and sustainable control strategies for sleeping sickness;
- To increase awareness and advocacy on decision makers and donors to ensure that sleeping sickness is kept on their agenda.

The meeting notes that transmission rate of sleeping sickness is still high in areas of Central African Republic and Democratic Republic of Congo where security constraints hamper control activities.

The meeting RECOMMENDS

- NGOs that have previously been showing commitment and success in sleeping sickness control to continue providing support and maintain their efforts and assistance.

The meeting recognizes leadership and the efforts of WHO on mapping sleeping sickness distribution and acknowledges the assistance and support provided by FAO/PAAT and welcomes the outcomes already obtained on the development of this tool.

The meeting RECOMMENDS

- Countries to continue providing necessary inputs to WHO to complete the Atlas of human African Trypanosomiasis;
- WHO to provide countries with the necessary equipment and training to own the Atlas and be able to continue its update and to use it as a tool for planning control activities and monitoring disease evolution.

PAAT

The meeting notes with appreciation the progress made by PAAT after the last ISCTRC conference, in the continuous production and wide

distribution of the TTI, PAAT Technical and Scientific Series and decision support information on PAAT website for the T&T control family. It welcomes the annual organization of the meetings of the Panel of PAAT Advisory Group Coordinators and of the Programme Committee. ISCTRC also welcomes the decision to review PAAT and its structures.

The meeting **URGES**

- That this review will result in greater consultation, coordination and concertation with project activities in countries implementing large scale T&T projects in Africa;
- The use of the agreed harmonized guidelines / criteria for the selection of areas intended for T&T intervention to ensure the achievement of sustainable agriculture and rural development (SARD), improved human and animal livelihood, and poverty alleviation.

DNDi

The meeting notes the unbalanced ratio between the global disease burden and the development of new drugs for tropical diseases, including human African Trypanosomiasis, and acknowledges the achievements and actions taken by DNDi in addressing the need for novel treatments of neglected tropical diseases.

The meeting **RECOMMENDS**

DNDi to further progress its strategy and enlarge its strategic partnership with the private sector and WHO for joint development of new drugs and/or treatment protocols for sleeping sickness.

FIND

The meeting commends the actions undertaken by FIND in the development of diagnostic tools for poverty related diseases of public health importance as sleeping sickness and recognizes the need to develop accurate diagnostic tools applicable in endemic (rural) areas. The meeting also notes the Memorandum of Understanding (MoU) between FIND and PATTEC for advocacy.

The meeting RECOMMENDS

To pursue the process of development, evaluation, demonstration and implementation of diagnostic tests for human African Trypanosomiasis

PATTEC

The meeting notes with pleasure the establishment of the monitoring and evaluation (M&E) Unit in the PATTEC Coordination Office. This unit has been able to monitor and evaluate the performance of the six countries currently implementing the AfDB-funded projects, thus providing information on their levels of individual project achievements in implementation.

The meeting commends this step and hopes that by this process, these projects will be challenged so that a success story will be reported and recorded.

The meeting also notes that those countries currently implementing PATTEC projects and the second batch of countries have pledged funds for the next phase. Based on the experience gained in the implementation by the current beneficiaries of the AfDB loans, the meeting URGES: these countries to immediately release part of their pledges to be used for the collection of standardized baseline data, crucial to the success of the projects.

RESEARCH CENTRES/INSTITUTIONS

The meeting notes with appreciations the research themes developed by the international and regional research centres/institutions (CIRDES, ICIPE, ILRI) leading to provide new insights and increased scientific knowledge supporting planners in conceiving T&T intervention projects and translate them into field application of research results for improved efficiency of control techniques. However, the meeting also notes that there is no silver bullet and magic solutions for the creation of T&T free zone. An array of tools has been developed and used with different degree of success, in order to achieve greater impact and cost-effective disease management, eventually leading to disease elimination.

The meeting RECOMMENDS

- To further continue the research with impact focus research on the development of cost-effective control measures based also on the principles of integrated pest and disease management and using a phased conditional approach;
- The technology to be developed and used be environmentally acceptable and economically justified;
- Proper, detailed baseline surveys be conducted, data collected and analyzed as necessary pre-requisite actions supporting the formulation of field T&T intervention campaigns;
- To use the international / regional research centres / institutions as entities for coordinating, harmonizing and collating national and regional research data on T&T and related matters, and establishing data banks publicly accessible.

RAPPORTS ET RECOMMANDATIONS

ORGANISATIONS INTERNATIONALES

Modérateur, Ahmed Elsaywalhy

Rapporteur: Raffaele Mattioli

Les organisations internationales ont présenté les activités qu'elles ont menées au cours des deux dernières années et les recommandations suivantes ont été élaborées,

La FAO

La réunion prend acte de la complexité du problème des mouches Tsé-tsé et de la Trypanosomiase et note que les différents secteurs de développement de l'élevage-agriculture, y compris l'utilisation des terres et des ressources naturelles ainsi que le développement socio-économique sont affectés par la présence de la maladie.

La réunion se félicite du travail accompli par la FAO notamment à:

Générer des informations normalisées relatives à la santé animale, l'élevage et les systèmes de production agricole, l'environnement et l'agro-écologie des zones infestées par la mouche Tsé-tsé.

Fournir une assistance aux pays touchés par la mouche Tsé-tsé dans la formulation des politiques, stratégies et directives pour les interventions sur la mouche Tsé-tsé et la Trypanosomiase.

La réunion RECOMMANDE,

D'accroître les efforts pour une plus grande et plus large adoption des politiques et directives développées pour la planification de programmes Tsé-tsé et Trypanosomiase de terrain;

De poursuivre leurs efforts pour une plus grande efficacité et impact de terrain dans les pays, et renforcer les synergies avec les entités nationales, régionales et internationales et les différentes initiatives.

L'OMS

La réunion prend acte des progrès réalisés dans le domaine de la lutte contre la maladie du sommeil. Elle est toutefois préoccupée par le manque d'outils appropriés pour développer des méthodes de contrôle adaptées et par les faiblesses des systèmes de santé à intégrer la lutte contre la maladie du sommeil et la surveillance afin de maintenir les résultats actuels.

Le CSIRLT RECOMMANDE que,

L'OMS poursuive son appui aux pays pour adapter les stratégies de contrôle en tenant compte de l'évolution de la maladie ;

Les groupes de R&D développent de nouveaux outils de diagnostic et des médicaments pour assurer un rapport coût-efficacité adapté ainsi que des stratégies de lutte durable contre la maladie du sommeil;

La sensibilisation et le plaidoyer soient accrus auprès des décideurs et bailleurs de fonds pour s'assurer que la maladie du sommeil est maintenue dans leurs agendas.

La réunion note que le taux de transmission de la maladie du sommeil est encore élevé dans des régions de la République Centrafricaine et de la République Démocratique du Congo, où les contraintes de sécurité entravent les activités de contrôle.

La réunion RECOMMANDE que,

Les ONG qui ont déjà fait preuve d'engagement et de réussite dans la lutte contre la maladie du sommeil continuent à soutenir et à maintenir leurs efforts et leur assistance ;

La réunion RECONNAIT le leadership et les efforts de l'OMS sur la cartographie de la distribution de la maladie du sommeil et reconnaît l'aide et le soutien fournis par la FAO / PLTA et se félicite des résultats déjà obtenus sur le développement de cet outil.

La réunion RECOMMANDE que,

Les pays continuent à fournir les contributions nécessaires à l'OMS pour qu'elle achève l'Atlas de la trypanosomiase humaine africaine ;

L'OMS fournisse aux pays l'équipement et la formation nécessaires pour qu'ils s'approprient l'Atlas et soient en mesure de poursuivre sa mise à jour et l'utiliser comme outil de planification des activités de contrôle et de suivi de l'évolution de la maladie.

Le PLTA

La réunion note avec satisfaction les progrès accomplis par le PLTA après la dernière conférence du CSIRLT, dans la production continue et la large diffusion du TTI, la série d'informations techniques et scientifiques et outil d'aide à la décision du PLTA, publiée sur le site Internet du PLTA pour la communauté scientifique travaillant sur la mouche Tsé-tsé et les Trypanosomiasés. Elle se félicite de l'organisation annuelle des réunions du Panel Consultatif du Groupe des

Coordonnateurs du PLTA et du Comité du Programme. Le CSIRLT se félicite également de la décision de réviser le PLTA et ses structures.

La réunion EXHORTE

Cette révision à donner lieu à une meilleure consultation, coordination et concertation avec les activités du projet dans les pays mettant en œuvre des projets Tsé-tsé et Trypanosomiase de grande échelle en Afrique;

A l'utilisation des lignes directrices harmonisées et acceptées ainsi que les critères de sélection des aires envisagées dans les interventions Tsé-tsé et Trypanosomiase afin d'assurer la réalisation d'une agriculture durable et le développement rural (ADDR), l'amélioration des conditions de vie des hommes et des animaux, et la réduction de la pauvreté.

La DNDi

La réunion prend note du ratio déséquilibré entre le poids global des maladies et le développement de nouveaux médicaments pour lutter contre les maladies tropicales, y compris la Trypanosomiase humaine africaine, et reconnaît les réalisations et les mesures prises par la DNDi pour répondre aux besoins de nouveaux traitements des maladies tropicales négligées.

La réunion RECOMMANDE

A la DNDi de progresser davantage dans sa stratégie et d'élargir son partenariat stratégique au secteur privé et à l'OMS pour le développement conjoint de nouveaux médicaments et / ou protocoles de traitement de la maladie du sommeil.

La FIND

La réunion se félicite des mesures prises par la FIND dans le développement d'outils de diagnostic pour les maladies importantes en santé publique et liées à la pauvreté comme la maladie du sommeil et reconnaît la nécessité de développer des outils précis de diagnostic applicables dans les régions endémiques (rurales). La réunion prend également acte du protocole d'accord de plaidoyer entre la FIND et la PATTEC.

La réunion RECOMMANDE

De poursuivre le processus de développement, d'évaluation, de démonstration et de mise en œuvre des tests de diagnostic de la trypanosomiase humaine africaine.

La PATTEC

Le CSIRLT note avec plaisir la création d'une unité de suivi et évaluation (M&E) au sein du Bureau de coordination de la PATTEC. Cette unité a été en mesure de suivre et d'évaluer les performances des six pays mettant actuellement en œuvre des projets financés par la BAD, fournissant ainsi des informations sur les niveaux de réalisation des projets individuels en cours.

Le CSIRLT se félicite de cette étape et espère que par ce procédé, ces projets seront mis au défi de sorte que des expériences réussies et meilleures pratiques soient rapportés et consignés.

Le CSIRLT note également que les pays mettant actuellement en œuvre les projets de la PATTEC de même que ceux du deuxième lot, ont promis des fonds pour la phase suivante, sur la base de l'expérience acquise dans la mise en œuvre par les bénéficiaires actuels des prêts de la BAD.

La réunion EXHORTE,

Ces pays à immédiatement libérer la partie de leurs engagements destinés à la collecte des données normalisées de référence qui sont cruciales pour la réussite desdits projets.

REPORT AND RECOMMENDATION ON PATTEC

Theme 1: PATTEC

Moderator: Issa Sidibe

Rapporteur: Solomon Hale Mariam

Twenty-three (23) papers were presented in the second session, a special session that was for the first time devoted entirely to reports on activities within the PATTEC Initiative. Presenting the overall progress report on PATTEC, Dr John Kabayo, the AU PATTEC Coordinator, presented a summary of the activities undertaken within the PATTEC Initiative during the past 2 years, since the ISCTRC Conference in Angola. He recounted the progress made, including the successful eradication of tsetse and trypanosomiasis in Botswana and Namibia; and the various activities that have been carried out aimed at consolidating the full extent and purposes of the campaign. These activities include the progress in the execution of T&T s eradication projects in Burkina Faso, Kenya, Ethiopia, Ghana, Mali and Uganda, using support in form of soft loans from the African Development Bank; initiation of self-funded tsetse eradication activities in Angola and Zambia, using the sequential Aerosol Technique; the development of several multi-national bankable project proposals (including: Mozambique, South Africa, and Swaziland; Sudan and Ethiopia; Uganda and Sudan; Chad, Central Africa Republic (CAR), Cameroon and Nigeria; Tanzania, Burundi and Rwanda; Burkina Faso, Niger, Nigeria and Togo); extensive consultations with several affected countries; resources mobilization; monitoring and evaluation and training. He appreciated the financial and technical support provided to the PATTEC Initiative by various partners and urged those willing to help to liaise with the PATTEC Coordination Office in planning intended interventions, for maximum synergy, harmony and coordination. He expressed his gratitude for sense of commitment shown by affected countries; his satisfaction for the cooperation and sprit of the African Union in the planning and execution of PATTEC projects; and shared his feelings of optimism and hope that the objective of the PATTEC Initiative will be realized.

Papers were also presented on the progress made by the PATTEC Coordination Office in the area of strengthening advocacy activities, monitoring and evaluation efforts, the development of the PATTEC websites and dynamic database systems for PATTEC. It was also reported that PATTEC had signed MoUs with FIND to strengthen

advocacy activities; with WHO on cooperation in the training and capacity building activities was gratefully acknowledged. The PATTEC Coordination office is in the process of establishing regional PATTEC Coordination Offices to enhance cooperation of PATTEC activities in response to increasing intervention within the PATTEC Initiative.

From the 23 papers, 18 papers give more details on countries on going activities including the 6 first phase countries which are sponsored by ADB, the national programs started with the own government funds, and the multinational regional project draft to be submitted for financing. Many countries express their commitment to support PATTEC initiatives and to start their own activities.

Following the presentations of the 23 papers, a brief discussion ensued and the following recommendations were also made:

Recommendations

The 30th ISCTRC Conference commends with satisfaction the progress and achievements so far made by PATTEC and calls upon other tsetse-affected countries to join the PATTEC Initiative if they have not already done so.

While PATTEC welcomes partners willing to provide support in the implementation of PATTEC it is essential that those partners discuss the area in which support is anticipated with the PATTEC Coordination Office for Purpose of effective coordination.

The conference noted with satisfaction that all projects which were presented under the umbrella of the PATTEC Initiative demonstrated the strength and consolidation of the PATTEC Programme. The PATTEC session should be a permanent feature of future ISCTRC Conference and should include Country reports.

PATTEC programmes should also put emphasis on Non-Tsetse transmitted Trypanosomiasis in future.

Noting that the decision of the African Heads of State and Government will only end when Trypanosomiasis is eliminated from Africa, and considering that researching institutions are engaged in activities to support the eradication process, it is recommended that the AU Commissioner for Rural Economy and Agriculture consider the appropriateness of ISCTRC as the technical advisory Council to PATTEC.

Considering the trans-boundary nature of Tsetse distribution and the divergence of T&T management systems between countries, it is recommended that project proposals for creation of Tsetse free areas be developed and operated independently for Tsetse belts that occupy more than one country as a sub-regional project.

RAPPORT ET RECOMMANDATION SUR LA PATTEC

Thème 1: PATTEC

Modérateur: Issa Sidibé

Rapporteur: Salomon Hailé Mariam

Vingt trois (23) exposés ont été présentés à la deuxième session, une session spéciale qui pour la première fois a été entièrement consacrée aux rapports sur les activités dans le cadre de l'initiative PATTEC. Dans sa présentation du rapport d'activité global de la PATTEC, Dr John Kabayo, Coordonnateur de l'UA-PATTEC, a présenté un résumé des activités menées au sein de l'initiative PATTEC au cours des deux dernières années, depuis la Conférence du CSIRLT en Angola. Il a relaté les progrès accomplis, y compris l'éradication réussie de la Tsé-tsé et la Trypanosomiase au Botswana et en Namibie, et les diverses activités qui ont été menées visant à consolider l'étendue et les objectifs de la campagne. Ces activités comprennent les progrès accomplis dans l'exécution des projets d'éradication de la Tsé-tsé et la Trypanosomiase au Burkina Faso, au Kenya, en Ethiopie, au Ghana, au Mali et en Ouganda, en utilisant un soutien financier sous forme de prêts bonifiés de la Banque Africaine de Développement ; le lancement d'activités autofinancées d'éradication des glossines ; l'Angola et la Zambie utilisant la technique d'aérosols séquentiels; le développement de plusieurs propositions de projets multinationales bancables (y compris: le Mozambique, l'Afrique du Sud et le Swaziland, le Soudan et l'Éthiopie, l'Ouganda et le Soudan, le Tchad, la RCA, le Cameroun et le Nigeria, la Tanzanie, le Burundi et le Rwanda, le Burkina Faso, le Niger, le Nigéria et le Togo); les consultations approfondies entre plusieurs pays touchés; la mobilisation des ressources; le suivi et l'évaluation ; la formation. Il a apprécié le soutien financier et technique apporté à l'initiative PATTEC par les différents partenaires et a exhorté ceux qui sont prêts à aider, d'entrer en relation avec le Bureau de Coordination de la PATTEC avant la planification d'interventions pour une synergie, harmonie et coordination maximale. Il a exprimé sa gratitude pour le sens d'engagement manifesté par les pays touchés; sa satisfaction pour la coopération et l'esprit de l'Union Africaine dans la planification et l'exécution des projets de la PATTEC et a partagé ses sentiments d'optimisme et d'espoir que l'objectif de l'initiative PATTEC sera réalisé.

Des exposés ont également été présentés sur les progrès réalisés par le Bureau de Coordination de la PATTEC dans le domaine du renforcement des activités de plaidoyer, les efforts de suivi et d'évaluation, le développement du site Internet de la PATTEC ainsi que les systèmes de base de données dynamiques pour la PATTEC. Il a également été signalé que la PATTEC a signé un protocole d'accord avec la FIND pour renforcer les activités de plaidoyer; le protocole d'accord avec l'OMS sur la coopération dans les activités de formation et de renforcement des capacités a été apprécié. Le Bureau de Coordination de la PATTEC est en train d'établir des bureaux régionaux de coordination de la PATTEC pour renforcer la coopération des activités de la PATTEC, en réponse aux interventions croissantes au sein de l'initiative PATTEC.

Des 23 documents présentés, 18 donnent plus de détails sur les activités en cours dans les pays, y compris les 6 pays de la première phase, qui ont été financés par la BAD, les programmes nationaux ayant commencé avec les fonds propres des gouvernements, et les projets multinationaux régionaux devant être soumis pour financement. De nombreux pays ont exprimé leur engagement à soutenir les initiatives de la PATTEC et de lancer leurs propres activités.

Après les présentations des 23 articles, une brève discussion a suivi et les recommandations suivantes ont également été formulées,

Recommandations

La 30^{ème} Conférence du CSIRLT félicite avec satisfaction les progrès et les réalisations accomplis par la PATTEC à ce jour et demande aux autres pays touchés par la mouche Tsé-tsé de se joindre à l'initiative de la PATTEC, si elles ne l'ont déjà fait.

Bien que la PATTEC accueille les partenaires prêts à fournir leur appui à la mise en œuvre de la PATTEC, il est essentiel que ces partenaires discutent des zones dans lesquelles le soutien est prévu avec le Bureau de Coordination de la PATTEC dans le but d'une coordination efficace ;

La Conférence a noté avec satisfaction que tous les projets qui ont été présentés dans le cadre de l'Initiative PATTEC ont démontré la solidité et la consolidation du Programme. La session consacrée à la PATTEC devrait ainsi revêtir un caractère permanent à l'avenir lors des conférences CSIRLT et devrait inclure les rapports des pays ;

Les programmes de la PATTEC devraient également à l'avenir mettre l'accent sur les Trypanosomiasés non- transmises par la mouche Tsé-tsé ;

Notant que la Décision des Chefs d'Etats et de Gouvernements ne prendra fin que lorsque la Trypanosomiase aura été éliminée d'Afrique, et considérant que les Institutions de Recherche sont engagées dans des activités pour soutenir le processus d'éradication, il est recommandé que la Commissaire de l'UA à l'Economie Rurale et Agriculture considère la pertinence du CSIRLT à être le Conseil Consultatif Technique de la PATTEC ;

Compte tenu de la nature transfrontalière de la distribution de la mouche Tsé-tsé et de la divergence des systèmes de gestion des mouches Tsé-tsé entre les pays, il est recommandé que les propositions de projet de création de zones exemptes de Tsé-tsé soient développées et exploitées de façon indépendante pour les ceintures de glossines qui occupent plus d'un pays, sous la forme de projets sous-régionaux.

REPORT AND RECOMMENDATION ON COUNTRY REPORTS

Moderator: Theophile Josenando

Rapporteur: Louis Banipe

Given the fact that most countries are involved in the PATTEC programme, this session was devoted to some aspects which were not sufficiently highlighted during the previous PATTEC presentations.

Six countries and the East-African Trypanosomiasis Control Network presented their activities. Those are:

Angola

Democratic Republic of Congo

Tanzania

Uganda

South Sudan

Guinea (Conakry)

All reports highlighted the increasingly important consideration given to Trypanosomosis and their vectors control. The meeting also welcomed the increasing commitment of NGOs in the control and activities, and drew attention that a lot remains to be done.

Angola presented the massive efforts granted by the Government and various partners not only for identifying the disease but also to control it. Angola expressed concerns over the proposed delimitation area designed by PATTEC within the ongoing sub-regional project with the DRC "Cleaning-up of pastoral areas of Kasai and Luanda provinces". Indeed, Angola pointed that the delimitation does not take the reality into account and requested that henceforth, extensive studies should be carried out to design consistent proposals.

The Democratic Republic of Congo underlined once more that three quarters of the recently reported cases of African Human Trypanosomiasis in Africa are from DRC. This requires further sustained attention. Welcoming the multifaceted support, DRC wished that the support be maintained and hoped that the control could be incorporated into traditional structures in charge of health aiming at improvement of cases' care.

Sudan made a special presentation on the situation in the South. Faced to the war situation prevailing in that locality, NGOs are progressively abandoning the area leaving a vacuum difficult to be filled. This worrisome situation which prevents the production of reliable reports of disease cases is not likely to reassure. In addition, efforts are being made to conduct general census on which field activities should be based.

Uganda, presenting all the gift nature offered to the country, namely the rich hydrographical net, pointed out that this remains the heart of many diseases, in particular Trypanosomosis and its vectors. Thanking the partners, Uganda pleaded that the support is sustained to help achieving the effective control of the plague.

Tanzania indicated that the wildlife case remains a concern with an important infestation of livestock at the interface of protected areas. The African Human Trypanosomiasis (ATH) centre of Serengeti, formerly extinct is now revived. The personnel training needs should be pursued and even backed up by various actors.

Guinea (Conakry) provided an update on ongoing studies and expressed difficulties to access targeted sites.

The Eastern Africa Network of Trypanosomosis (EANETT)

During the EANETT's presentation, the Network was presented to the audience. It came out that the network was founded in 1999, but its activities only started in 2000. It is in the sub-region, the only research network on African Human Trypanosomiasis (AHT). The founding institutions are: The Swiss Tropical Institute (STI), KETRI (currently KARI-TRC), TTRI (Tanzania), TMRI (Sudan) and LIRI (currently NaLIRRI, Uganda). Three other countries joined the network. These are: Malawi, Zambia and the Democratic Republic of Congo. The Network organizes scientific conferences once a year and also offers opportunity for young scientists to intervene, share and be backed up in research field on Trypanosomiasis and Tsetse flies. Young scientists in postgraduate education are encouraged to seize the opportunity.

Following the presentations which did not raise a lot of questions, the Conference formulated the following recommendations:

Country reports should strictly follow the outline developed by the ISCTRC Secretariat;

Reports should only focus on the specified period.

RAPPORT ET RECOMMANDATION SUR LES RAPPORTS PAYS

Modérateur, Theophile Josenando

Rapporteur, Louis Banipe

Compte tenu du fait que la plupart des pays sont engagés dans le programme PATTEC, cette session a été consacrée à quelques aspects occultés lors des présentations de la PATTEC.

Six pays et le réseau est-africain de lutte contre les trypanosomoses ont donc fait des présentations. Il s'agit de:

- l'Angola
- la République Démocratique du Congo
- la Tanzanie
- l'Ouganda
- Sud Soudan
- la Guinée (Conakry)

Tous ces rapports ont souligné la prise en compte de plus en plus importante de la lutte contre les trypanosomoses et leurs vecteurs. La réunion a également accueilli favorablement l'engagement croissant des ONGs dans les activités de contrôle et a attiré l'attention sur le fait que beaucoup reste à faire.

L'Angola a présenté les efforts énormes consentis par le gouvernement et les divers partenaires non seulement dans l'identification de la maladie mais aussi dans le contrôle. L'Angola a exprimé quelques préoccupations sur la zone de délimitation proposée par la PATTEC au sein du projet sous-régional en cours d'exécution avec la RDC "Assainissement des zones pastorales du Kasai et les provinces de Luanda". Concrètement, l'Angola a souligné que la délimitation ne prend pas soin de la réalité du terrain et a demandé que, dorénavant, des études approfondies soient menées avant l'élaboration des propositions cohérentes.

La République démocratique du Congo a souligné une fois de plus que les trois quarts des cas de trypanosomiase humaine africaine déclarés récemment en Afrique provenaient de la RDC. Cela nécessite une attention particulière. Se félicitant de l'appui multiforme reçu, la RDC a souhaité que cet appui soit maintenu et a suggéré que le contrôle de la

maladie soit intégré dans les structures traditionnelles de santé afin d'améliorer la prise en charge des cas.

Le Soudan a fait une présentation spéciale de la situation au Sud. Face à la situation de guerre qui prévaut dans cette localité, les ONGs quittent de plus en plus la région laissant un vide qui n'est pas forcément comblé. Cette situation préoccupante qui ne permet pas la production des rapports fiables des cas de maladies n'est pas de nature à rassurer. Par ailleurs, des efforts sont faits pour la conduite d'un recensement général, base de toute activité de terrain.

L'Ouganda, en présentant les facilités que lui offre la nature en l'occurrence le riche réseau hydrographique, signale qu'il reste le centre de nombreuses maladies et en particulier les trypanosomoses et leurs vecteurs. Remerciant les différents partenaires, il a plaidé pour un appui afin de parvenir au contrôle effectif du fléau.

La Tanzanie a indiqué que le cas de la faune sauvage reste une préoccupation avec une infestation importante des animaux domestiques vivants à l'interface des aires protégées. Le foyer de trypanosomiase humaine africaine (THA) dans le Serengeti autrefois éteint s'est aujourd'hui ravivé. La formation du personnel doit être poursuivie et même soutenue par les différents acteurs.

La Guinée (Conakry) a fait le point des études en cours et les difficultés pour accéder aux sites ciblés.

Le Réseau Est- Africain de lutte contre les Trypanosomoses (EANETT)

Le Réseau Est- Africain de lutte contre les Trypanosomoses a été présenté à l'auditoire. Il en ressort que le réseau a été fondé en 1999, et que ses activités n'ont débuté qu'en 2000. C'est un réseau de recherche uniquement sur la trypanosomiase humaine africaine (THA) dans la sous-région. Les institutions fondatrices sont: L'Institut Tropical Suisse (ITS), le KETRI (actuellement KARI-TRC), le TTRI (Tanzanie), le TMRI (Soudan) et le LIRI (actuellement NaLIRRI, Ouganda). Trois autres pays ont rejoint le réseau. Ce sont: le Malawi, la Zambie et la République Démocratique du Congo. Le Réseau organise des conférences scientifiques une fois par an et offre également l'occasion aux jeunes scientifiques d'intervenir, de partager et d'être appuyé dans le domaine de la recherche sur la Trypanosomiase et la mouche Tsé-tsé.

Les jeunes scientifiques en formation post-universitaire sont encouragés à saisir cette opportunité.

A la suite des présentations qui n'ont pas beaucoup soulevé des questions, la conférence a fait des recommandations suivantes,

- Que les rapports des pays respectent strictement le canevas élaboré par le secrétariat du CSIRLT
- Que les rapports se focalisent uniquement sur la période indiquée

REPORT AND RECOMMENDATIONS ON HUMAN AFRICAN TRYPANOSOMOSIS (HAT)

Moderator: Pere Simarro

Rapporteurs: Dawson Mbulamberi

Session 6 & 7: Basic Research on trypanosome and Diagnostics:

By Dawson Mbulamberi & Jose Ramon Franco

Sessions 6 and 7 focused on the diagnosis of Human African Trypanosomiasis (HAT), including some basic research on Trypanosomes. Ten papers were presented during these sessions

The first three presentations were related to basic research on the parasite (population genetics of *T. b. Gambiense* improved in vitro medium for bloodstream form of *T. b. gambiense* and comparative genomic analysis of procyclic *T. b. r* with DNA microarrays), all these presentations addressed the improvement of knowledge on the genetic characteristics of the Trypanosome and the search for an improved culture media.

The subsequent presentations tried to demonstrate the impact of HAT on the sensitivity of HIV diagnostic tests and the public health implications of this impact. A study performed with samples coming from Mbuji-Mayi, (DRC) revealed a decrease in the sensitivity of the usual HIV rapid tests.

The presentation on immune trypanolysis revisited this test as described in 1995, tried to explore the possibility of using it as tool for epidemiological decisions, with examples in some countries in West Africa. This presentation raised different questions and comments about the interpretations of seropositive individuals without parasitological confirmation, particularly those who spontaneously turn seronegative.

The presentations 3.06 and 3.07 were about research on new staging markers. The use of some proteins showed promising preliminary results, thus emphasizing the need to combine several markers such as CXCL10, CXCL8 and H-FABP.

Presentations 3.08 and 3.09 dealt with molecular methods of sleeping sickness diagnosis (NASBA and Trypanozoon OligoC-strip), they gave a review of the efforts to standardize and simplify these tools, highlighting the use of olicromatography as a simpler amplicon lecture method.

The last presentation of the session addressed the use of algorithms combining different field criteria (presence of Trypanosomes and White Blood cell count in CSF) with the aim of shortening the follow up period. The result seemed to support the possibility of shortening the follow up period of second stage *gambiense* HAT patients. However, the application of these results was limited because of the peculiarity of the cohort studied. The other limiting factor for the application of these results was the unusually high failure rate of treatment with Melarsoprol. There was a possibility of these results being different if another treatment had been used.

Recommendations:

- Further evaluation of the impact of HAT on HIV rapid tests, coordinating with HIV programs the assessment of HIV tests in HAT patients;
- To assess the utility of the immune trypanolysis tests in other different epidemiological situations;
- To comparatively evaluate and standardize the different PCR tests available, to highlight its real value in the diagnosis process;
- To encourage the efforts made to shorten the follow up period and recommend the application of the proposed algorithms in different cohorts (including stage 1 cases), considering always the variability according to the drug used for treatment.

Session 8: Epidemiology of HAT

By Dawson Mbulamberi & Diarra Abdoulaye

Four presentations were made during this session; two presentations dealt with epidemiology, one with long term follow up of sleeping sickness patients and one with the impact of interventions targeting the control animal reservoir of *T.b.rhodesiense* within the framework of Public /Private Partnership and community involvement.

The presentations on disease epidemiology attempted to describe the current trends of the disease, the ongoing control activities and the perspectives in terms of surveillance and control. In general, there seems to be an increase in control activities leading to the decrease of the number of reported cases over the past ten years. However, it was observed that there was still low coverage of endemic areas.

Parameters underlying the epidemiology of *rhodesiense* sleeping sickness, as well as the role of domestic reservoir hosts were described.

The effectiveness of PPP (public-private partnership) and the very important role of communities in controlling the animal reservoir were described as an important factor for sustainability of activities and their expansion.

Sleeping Sickness patients who refused to be treated were regularly followed up by using serological and parasitological methods to monitor the evolution of the disease without treatment linked to the individual susceptibility (human trypanotolerance or self cure). Some of them died after developing second stage disease while some cured spontaneously

and a few remained positive as asymptomatic carriers with low parasitemia.

Recommendation:

The meeting acknowledged the complex epidemiological features of the Rhodesiense form of sleeping sickness and its zoonotic component and recommended that:

T. b. rhodesiense endemic countries should develop integrated control approaches including human, animal and vector components, fostering PPP and community involvement

Session 9: Disease distribution and Treatment of HAT

By Dawson Mbulamberi & Jose Postigo

Seven presentations were made during Session 9. Two presentations dealt with epidemiology and five with treatment.

One presentation on epidemiology in West Africa showed how the spatial evolution of HAT over the past 100 years, seems to have moved from the North to the South and that it has disappeared from savannah areas and is currently occurring in forest and mangrove areas.

The second presentation on epidemiology showed the current status of the Atlas of HAT which constitutes three quarters of the cases reported during the period 2000-2008 already mapped at the village level. Representatives from Angola and DRC expressed their full support for this initiative and committed themselves to share their data with WHO to finalize the mapping exercise. The final outcome of the Atlas will be made available in the public domain through WHO and FAO/PAAT websites.

Out of the five presentations on the subject of treatment, the first one dealt with the implications of the nifurtimox-efornithine combination therapy clinical study and its implications for the national sleeping sickness programme in Uganda. The second presentation showed the results of a study using the 10-day melarsoprol schedule for second stage *T.b. rhodesiense* patients. The study concluded that the 10-day schedule does not expose patients to a higher risk of serious adverse events or death and it has high efficacy. The third presentation dealt with Phase III trial on the safety and efficacy of pafuramidine maleate (DB289) for the

treatment of first stage *T.b. gambiense* sleeping sickness. The study had to be stopped due to severe adverse events associated with the new medicine. The fourth and fifth presentations dealt with molecular parasitological studies carried out in relapsed *T.b. gambiense* patients. The studies did not yield conclusive results on the molecular mechanisms leading to relapses.

Recommendations:

1. Recalling the recommendation of the 27th ISCTRC held in Pretoria, South Africa in 2003 to adopt the 10-days course of melarsoprol for the treatment of the late stage *T.b. gambiense* sleeping sickness and requested to undertake similar studies for *T.b. rhodesiense* sleeping sickness.

On the basis of the results of the clinical trials conducted in Tanzania and Uganda, the meeting recommends that Disease-endemic countries to adopt the abridged 10-day melarsoprol schedule as the new regimen for the treatment of the late-stage *T. brucei rhodesiense* sleeping sickness.

2. Considering that nifurtimox-eflornithine combination treatment for the second stage of *T. brucei gambiense* infections has been included in the WHO Essential List of Medicines in May 2009 after a successful clinical trial developed by a collaborative partnership carried out in Congo and the Democratic Republic of the Congo and preliminary results of a similar clinical trial in Uganda also showing positive results of this combination treatment; the meeting recommends:

- Countries to include nifurtimox-eflornithine combination as a treatment option in their national protocols to treat sleeping sickness and initiate the process of ordering this combination treatment through WHO.

- WHO to make available to countries all necessary training, information and support to implement this combination treatment.

RAPPORTS ET RECOMMANDATIONS SUR LA TRYPANOSOMIASE HUMAINE AFRICAINE (THA)

Modérateur: Simarro Pere

Rapporteurs: Dawson Mbulamberi

Session 6 & 7: Recherche de base sur les trypanosomes et les diagnostics:

Rapporteurs: Dawson Mbulamberi et Jose Ramon Franco

Les sessions 6 et 7 se sont focalisées sur le diagnostic de la Trypanosomiase Humaine Africaine, incluant les recherches de base sur les trypanosomes. Dix communications ont été présentées au cours de ces deux sessions.

Les trois premiers exposés sont relatifs à la recherche fondamentale sur le parasite (la génétique des populations de *T. b. gambiense* ; un milieu in vitro amélioré pour la forme sanguine de *T. b. gambiense* ; et l'analyse génomique des formes procycliques de *T. b. rhodesiense* à l'aide de puces à ADN). Tous ces exposés ont porté sur l'amélioration des connaissances sur les caractéristiques génétiques du trypanosome et la recherche d'une amélioration des milieux de culture.

Les présentations subséquentes ont tenté de démontrer l'impact du portage de la THA sur la sensibilité des tests de diagnostic du VIH et les implications de cet impact en santé publique. Une étude réalisée avec des échantillons provenant de Mbuji-Mayi (RDC), a en effet révélé la diminution de la sensibilité des tests rapides usuels du VIH.

La présentation sur la trypanolyse immunitaire a revisité ce test tel qu'il a été décrit en 1995, et a tenté d'explorer la possibilité de l'utiliser comme outil de décision épidémiologique, avec des exemples dans certains pays d'Afrique de l'Ouest. Cette présentation a soulevé différentes questions et commentaires à propos des interprétations des tests sur les personnes séropositives sans confirmation parasitologique, en particulier ceux qui redeviennent spontanément séronégatifs.

Les présentations 3.06 et 3.07 étaient consacrées à la recherche sur de nouveaux marqueurs de stades. L'utilisation de certaines protéines a montré des résultats préliminaires prometteurs, soulignant ainsi la

nécessité de combiner plusieurs marqueurs tels que le CXCL10, CXCL8 et H-FABP.

Les présentations 3.08 et 3.09 ont traité des méthodes moléculaires de diagnostic de maladie du sommeil (NASBA et Trypanozoon Bande-OligoC). Elles ont fait le point des efforts investis pour normaliser et simplifier ces outils, en soulignant l'utilisation de l'oligomatographie comme méthode d'amplification de lecture plus simple.

La dernière présentation de la session portait sur l'utilisation d'algorithmes combinant des critères différents de terrain (présence de trypanosomes et de globules blancs dans le LCR) dans le but de raccourcir la période de suivi. Le résultat semblait favorable à la possibilité de raccourcir la période de suivi de patients de deuxième stade de THA à *T. b. gambiense*. Toutefois, l'application de ces résultats a été limitée en raison de la particularité de la cohorte étudiée. L'autre facteur limitant pour l'application de ces résultats a été le taux d'échecs anormalement élevé des traitements avec le Melarsoprosol. La probabilité d'obtenir des résultats différents si un autre traitement avait été utilisé était élevée.

Recommandations:

La réunion recommande,

Une évaluation plus poussée de l'impact de la THA sur les tests de dépistage rapide du VIH en collaboration avec les programmes sur le VIH grâce à l'évaluation des tests VIH chez les patients à THA ;

Une évaluation de l'utilité des tests de trypanolyses immunitaire dans des situations épidémiologiques différentes ;

Une évaluation comparative et une standardisation des différents tests PCR disponibles pour mettre en évidence leur valeur réelle dans le processus de diagnostic ;

Un renfort des efforts investis dans le raccourcissement de la période de suivi et l'application des algorithmes proposés dans les différentes cohortes (y compris pour les cas de stade 1), en considérant toujours la variabilité liée aux médicaments utilisés pour le traitement.

Session 8: Epidémiologie de la THA

Rapporteurs: Dawson Mbulamberi et Diarra Abdoulaye

Quatre communications ont été présentées au cours de cette session.

Deux présentations traitaient de l'épidémiologie, l'une du suivi à long terme des patients atteints de maladie du sommeil et l'autre de l'impact des interventions ciblant le réservoir animal dans le contrôle de *T. b. rhodesiense* dans un cadre de partenariat public / privé en tenant compte de la participation communautaire.

Les présentations sur l'épidémiologie de la maladie ont tenté de décrire les tendances actuelles de la maladie, les activités de contrôle en cours et les perspectives en termes de surveillance et de contrôle. En général, il semble y avoir une augmentation des activités de contrôle conduisant à la diminution du nombre de cas signalés au cours des dix dernières années. Toutefois, il a été observé qu'il y avait encore une faible couverture des zones endémiques.

Les paramètres sous-tendant l'épidémiologie de la maladie du sommeil à *T. b. rhodesiense*, ainsi que le rôle des réservoirs domestiques ont été décrits.

L'efficacité du PPP (partenariat public-privé) et le rôle très capital des communautés dans la lutte contre le réservoir animal ont été décrits comme des facteurs importants de durabilité et d'expansion des activités.

Les patients atteints de la maladie du sommeil qui refusaient d'être traités ont été suivis régulièrement en utilisant des méthodes sérologiques et parasitologiques pour observer l'évolution de la maladie en l'absence de traitement et en fonction de la susceptibilité individuelle (trypanotolérance humaine ou auto-guérison). Certains d'entre eux sont morts après avoir développé le deuxième stade de la maladie alors que d'autres ont guéri spontanément, quelques-uns par contre sont restés positifs, porteurs asymptomatiques avec une parasitémie faible.

Recommandations:

La réunion a reconnu les caractéristiques épidémiologiques complexes de la forme à *T. b. rhodesiense* de la maladie du sommeil et sa composante zoonotique et a ainsi recommandé que:

les pays endémiques à *T. b. rhodesiense* élaborent des approches de lutte intégrée incluant l'homme, l'animal et les composantes vectorielles ainsi que la promotion du PPP et la participation communautaire.

Session 9: Distribution et traitement des maladies de la THA

Rapporteurs: Dawson Mbulamberi & José Postigo

Sept présentations ont été faites au cours de cette session. Deux d'entre elles traitaient de l'épidémiologie et les cinq autres du traitement.

Un exposé sur l'épidémiologie de la maladie en Afrique de l'Ouest a montré comment l'évolution spatiale de la THA s'est faite au cours des 100 dernières années. Elle semblerait avoir migré du nord vers le sud et avoir disparu des zones de savane, mais se retrouverait actuellement dans les zones forestières et dans les zones de mangrove. Le deuxième exposé sur l'épidémiologie a montré l'état actuel de l'Atlas de la THA couvrant déjà les trois quarts des cas déclarés au cours de la période 2000-2008, tous les cas sont insérés sur les cartes jusqu'au niveau-village. Les représentants de l'Angola et de la RDC ont exprimé leur plein appui à cette initiative et se sont engagés, avec l'OMS, à partager leurs données afin de finaliser l'exercice de cartographie. Les résultats finaux de l'Atlas seront mis à disposition dans le domaine public sur les sites Internet de l'OMS et de la FAO/PLTA.

Sur les cinq présentations ayant pour sujet le traitement, le premier portait sur les implications de l'étude clinique de la thérapie de combinaison nifurtimox-efornithine et ses implications sur le programme national de la maladie du sommeil en Ouganda. Le deuxième exposé a présenté les résultats d'une étude utilisant le méfarsoprol sur un programme de 10 jours chez des patients atteints de *T. b. rhodesiense* en deuxième phase. L'étude a conclu que le programme de 10 jours n'expose pas davantage les patients aux effets indésirables graves ou la mort et qu'il a une forte efficacité. La troisième présentation a traité de la phase III des essais d'innocuité et d'efficacité du maléate de pafuramidine (DB289) sur le traitement du premier stade de la maladie du sommeil à *T. b. gambiense*. L'étude a dû être stoppée en raison d'effets indésirables graves associés au nouveau médicament. Les quatrième et cinquième présentations ont traité d'études parasitologiques moléculaires effectuées sur des patients en rechute de trypanosomiose à *T. b. gambiense*. Les études n'ont pas donné de résultats concluants sur les mécanismes moléculaires conduisant à ces rechutes.

Recommandations:

Le Conseil rappelle la recommandation de la 27^e session du CSIRLT tenue à Pretoria, Afrique du Sud en 2003, suggérant l'arrêt du traitement au mélarsoprol au bout de 10 jours lors du traitement des stades avancés de la maladie du sommeil à *T. b. gambiense* et recommandant d'entreprendre des études similaires pour la maladie du sommeil à *T. b. rhodesiense*. Sur la base des résultats des essais cliniques menés en Tanzanie et en Ouganda, le Conseil Scientifique International pour la Recherche et la Lutte contre la Trypanosomiase recommande,

- aux pays endémiques, d'adopter la version abrégée du programme thérapeutique de 10 jours au mélarsoprol comme nouveau schéma thérapeutique dans le traitement des stades avancés de la maladie du sommeil à *T. brucei rhodesiense*.
- Considérant que, le traitement combiné nifurtimox-éflornithine pour le deuxième stade des infections à *T. brucei gambiense* a été positif et que les deux produits sont inclus dans la liste des médicaments essentiels de l'OMS en mai 2009, après un essai clinique réussi développé dans un cadre de partenariat collaboratif mené au Congo et en République Démocratique du Congo, et que les résultats préliminaires d'un essai clinique similaire en Ouganda montrent également des résultats positifs de ce traitement combiné, le Conseil Scientifique International pour la Recherche et la Lutte contre la Trypanosomiase recommande,
- aux pays d'inclure la combinaison nifurtimox-éflornithine comme une option thérapeutique dans leurs protocoles nationaux de traitement de la maladie du sommeil et de lancer le processus de commande de cette association médicamenteuse curative à travers l'OMS.
- à L'OMS de mettre à la disposition des pays toute la formation, l'information et le soutien nécessaires pour mettre en œuvre ce traitement combiné.

REPORT AND RECOMMENDATIONS AFRICAN ANIMAL TRYPANOSOMOSIS

Moderator: A. Ilemobade

Rapporteur: A. H. A. Rahman

A key note address was given by Prof. Eli Katunguka-Rwakishaya who gave a historical perspective of the disease and the efforts exerted into its control and he stressed the need for concerted multidisciplinary approach to control the disease. Out of the 15 papers accepted for oral presentation only 12 were presented. The papers addressed five major areas, epidemiology and baseline data collection, trypanocides resistance, trypanotolerance, drug quality and safety assurance and chemotherapy.

In the area of trypanotolerance, there is one paper under the title:

- Effect of N'Dama origin marker alleles on trypanotolerance in a backcross cattle population under natural Tsetse and Trypanosomosis challenge.

In the area of animal trypanosomosis epidemiology, surveys and baseline data collection 4 papers were presented:

- Baseline survey on bovine trypanosomosis and chemo-resistance in the Sikasso Cercle of Mali as a preamble to a vector control intervention.
- Bovine antibody response directed against *Glossina* saliva: An epidemiological marker of cattle exposure to Tsetse bites
- Donkey trypanosomiasis, their vectors, Helminthiasis, in Pate Island of Lamu District, Kenya.
- Sero-epidemiology of dourine in Bale Highlands of Oromyia Region, Ethiopia.

In the area of drug resistance the following papers were presented:

- Occurrence of diminazene, homidium and isometamidium resistant *T. congolense* strains isolated from cattle in Ghibe Valley and Lake Abaya localities, South-west Ethiopia.
- Field detection and evaluation of trypanocidal drug resistance in the Sissala East District of Northern Ghana.
- Field detection of chemo-resistance to isometamidium and diminazene in the region of Boucle Du Mouhoun, Burkina Faso.

In the area of drug quality and safety assurance two papers were presented:

- Determination of diminazene aceturate in animal tissues by Enzyme-Linked Immunosorbent Assay (ELISA).
- Poor quality and fake trypanocidal drugs, a real threat for a sustainable and profitable livestock production in sub-Saharan Africa.

In the area of chemotherapy two papers were presented:

- Cymelarsan effectively cures trypanosomes in dourine infections with no relapses
- Identification and experimental validation of potential drug targets in *Trypanosoma brucei*.

The baseline data reported in the presentations included Trypanosomiasis prevalence and Tsetse densities. Also the surveys included the non-tsetse transmitted *T. equiperdum*. The antibody responses directed against *Glossina* saliva was suggested to be used as a marker of the exposure of cattle to Tsetse flies. Drug resistance against trypanocidals was reported from Ethiopia, Burkina Faso and Ghana, while the paper from Ethiopia recommended the use of Cymelarsan in the treatment of dourine. Methionine synase and Homocysteine methyltransferase were reported to be potential drug targets in *T. brucei* in one of the presentations. Two presentations warned from the presence of residues of diminazene and other trypanocides in addition to the fake drugs in the different tissues of animals and from their severe implication on both animal health and food safety.

Recommendations

1- Recognizing the alarming and long lasting problem of the presence of substandard veterinary drugs in the free market, in specific trypanocides which is among the causes of drug resistance in chemotherapy, the ISCTRC recommends to the Food and Drug Agencies in the African countries that they are to take action of inspection, follow up and take legal action to manufacturers and importers who do their business against Pharmaceutical SOPs and rules of quality and safety assurance.

2- The ISCTRC recognizes with appreciation the success WHO /FIND has achieved in developing diagnostics for HAT requesting them to respond similarly to the diagnostic needs in AAT.

3- Referring to the reports on the wide spread resistance to all the available trypanocidals, FAO is requested to assist in developing a standard protocol that is to be applied in the different countries to detect the presence of the resistance,

4- Management of this problem requires concerted efforts, using parasitological and molecular tools, to quantify the level of resistance, its distribution and where possible, conduct remedial actions.

RAPPORT ET RECOMMANDATIONS SUR LA TRYPANOSOMIASE ANIMALE AFRICAINE (TAA)

Modérateur: A. Ilemobade

Rapporteur: A. H. A. Rahman

Une note liminaire a été prononcée par le professeur Eli Katunguka-Rwakishaya qui a donné une perspective historique de la maladie et les efforts déployés dans son contrôle. Il a souligné la nécessité d'une approche pluridisciplinaire concertée pour lutter contre la maladie. Sur les 15 articles acceptés, seulement 12 ont été présentés oralement. Les articles portaient sur cinq domaines majeurs, l'épidémiologie, la collecte de données de base afin d'établir une ligne de référence pour les comparaisons, l'efficacité des trypanocides, la trypanotolérance, la qualité des médicaments et l'assurance d'innocuité et la chimiothérapie.

Dans le domaine de la trypanotolérance, l'article présenté portait le titre, « Effet des allèles de marqueur d'origine N'Dama sur la trypanotolérance dans une population de bovins issue d'une première génération de croisement en retour (BC1) sous une pression naturelle des glossines et de la trypanosomose ».

Dans les domaines de l'épidémiologie de la trypanosomiose animale et des enquêtes de collecte de données afin d'établir un point de référence pour les comparaisons, 4 communications ont été présentées:

1. « Etude préliminaire sur la trypanosomiose bovine et la chimio-résistance dans le Cercle de Sikasso, au Mali en prélude à une opération de lutte anti-vectorielle » ;
2. « Evaluation chez les bovins de la réponse anticorps dirigés contre les antigènes salivaires de glossines comme marqueur d'exposition des bovins aux piqures de mouches tsé-tsé » ;
3. « Les trypanosomioses de l'âne, leurs vecteurs et l'helminthiase dans l'île de Pate du district de Lamu, au Kenya ».
4. « La séro-épidémiologie de la dourine dans les montagnes de Bale, Région d'Oromyia, en Ethiopie ».

Dans le domaine de la résistance aux médicaments les communications suivantes ont été présentées:

1. « Apparition des souches de *T. congolense* résistantes au diminazene, l'homidium et à l'isométnidium isolées chez des bovins dans la vallée de Ghibe et dans les localités du lac Abaya, au Sud-Ouest de l'Ethiopie ».
2. « Détection sur le terrain et évaluation de la résistance aux trypanocides dans le District de Sissala Est au nord du Ghana ».
3. « Détection de terrain de la chimio-résistance à l'isométnidium et au diminazène dans la région de la Boucle du Mouhoun au Burkina Faso ».

Dans le domaine de l'assurance qualité et de l'innocuité des médicaments, deux communications ont été présentées,

1. « Détermination de l'acéturate de diminazène dans les tissus animaux par dosage immuno-enzymatique (ELISA) ».
2. « Mauvaise qualité et faux trypanocides, une réelle menace pour une production animale stable et fructueuse en Afrique subsaharienne ».

Dans le domaine de la chimiothérapie deux communications ont été présentés:

1. « Le Cymelarsan élimine efficacement les trypanosomes dans la dourine sans crainte de rechutes »
2. « Identification et validation expérimentale des cibles potentielles de médicaments chez *Trypanosoma brucei*. »

Les données présentées sur l'établissement des points de référence pour des comparaisons incluaient la prévalence de la trypanosomiase et les densités de mouche Tsé-tsé. Les enquêtes couvraient également la trypanosomiase *T. equiperdum* non transmise par la mouche Tsé-tsé. L'utilisation de la réponse anticorps dirigés contre les antigènes salivaires de *Glossina* a été suggérée comme marqueur de l'exposition des bovins aux mouches tsé-tsé. Les résistances aux trypanocides ont été signalées en Ethiopie, au Burkina Faso et au Ghana, et la présentation de l'Ethiopie recommande l'utilisation du Cymelarsan dans le traitement de la dourine. La Méthionine synthase et homocystéine méthyltransférase ont été signalés comme des cibles potentielles de médicaments chez *T. brucei* dans l'une des présentations. Deux présentations ont attiré l'attention sur la présence de résidus de diminazène et autres trypanocides dans différents tissus animaux en plus des médicaments

contrefaits et de leur implication graves sur la santé animale et la salubrité des aliments.

Recommandations

1. Reconnaissant le caractère alarmant et durable du problème de circulation des médicaments vétérinaires non conformes sur le marché, et de façon spécifique celui des trypanocides qui comptent entre autres parmi les causes de résistance aux médicaments en chimiothérapie, le CSIRLT recommande aux agences africaines en charge de la qualité des aliments et des médicaments,

Que ces agences prennent des mesures d'inspection et de suivi et qu'elles prennent des mesures juridiques contre les importateurs et les fabricants de produits pharmaceutiques qui devraient faire fonctionner leurs affaires sur la base de manuels standardisés de procédures et des règles d'assurance qualité et d'innocuité.

2. Le CSIRLT reconnaît avec satisfaction le succès accompli par l'OMS / FIND dans l'élaboration des outils de diagnostic de la THA et les sollicite pour pourvoir aux besoins similaires de diagnostic pour les TAA.

En se référant aux rapports sur la chimio-résistance étendue à tous les trypanocides disponibles, la FAO est sollicitée pour aider à l'élaboration de protocoles standardisés applicables dans les différents pays pour assurer l'efficacité des traitements malgré le problème de résistance.

3. La gestion de ce problème requiert des efforts concertés, utilisant des outils parasitologiques et moléculaires, permettant de quantifier le niveau de la résistance, sa distribution, et là où il est possible de conduire des actions correctives.

REPORT AND RECOMMENDATIONS ON *GLOSSINA* BIOLOGY

Moderator: Ambrose Gidudu

Rapporteur: Joyce Daffa

There were 11 papers presented in this session. We noted that from the lead paper there is no easy single method that can be applied alone, no much work has been done on savannah *Glossina spp.* Due to the feeding behaviour of tsetse flies insecticide application in cattle can be restricted to legs only. There was a proposition that the target size can be reduced from 1x1 m to 0.5 x 0.5 m as the efficacy is almost the same. There are new advances in Tsetse genomics and bioinformatics, molecular biology specifically on fungus and bacteria towards improvement of Tsetse control. Symbionts have expressed trypanocidal agents. It has been noted that due to technical problems in rearing and colonization of different species Sterilized Insect Technic (SIT) is not available for the current eradication programme period. Other papers were on Detection of Salivary Gland Hypertrophy Virus (SGHV), Developments on research for Tsetse and host attractants, Nutritional stress in Tsetse for Trypanosome susceptibility, Improved visual baits, Genetic diversity among geographically separated Tsetse populations, Management and control of vector borne for improved productivity. Furthermore, spatial analytical tools and mathematical models can be used in baseline data collection to develop a stratified entomological sampling protocol. Finally there are old areas whereby they were once announced free of Tsetse but are currently infested by Tsetse flies.

Recommendations drawn from these presentations and discussions were:

- It has been noted that due to technical problems in rearing and colonization of different species SIT is not available for the current eradication programme period; therefore countries have been urged to undertake area-wide integrated Tsetse suppression and eradication techniques until when the SIT is available for field use.
- Insecticide treated target / screens size may be reduced in to tiny size however, this should further be investigated

- Undertake further investigation on the fungus and bacterial for future use in Tsetse control programmes
- Conduct surveys to monitor Tsetse fly in Tsetse free countries to ensure its current status areas.
- Undertake further investigation on the fungus for use in tsetse control programs
- Undertake area-wide integrated Tsetse control/eradication until when the SIT is available for use.

RAPPORT ET RECOMMANDATIONS SUR LA BIOLOGIE DE *GLOSSINA*

Modérateur: Ambrose Gidudu

Rapporteur: Joyce Daffa

Onze communications ont été présentées au cours de cette session. Il faut retenir de la communication principale qu'il n'existe aucune méthode développée pouvant être appliquée singulièrement, et que très peu de travaux ont été conduits sur *Glossina spp* émanant de la savane. En raison du comportement alimentaire de la mouche Tsé-tsé, l'application d'insecticides sur les bovins pourrait être limitée strictement aux pattes. Il a également été proposé que la taille des pièges à glossine soit réduite de 1 x 1 m à 0,5 x 0,5 m puisque l'efficacité est quasiment identique. La session a noté de nouvelles avancées en matière de génomique de la mouche Tsé-tsé, de bio-informatique et de biologie moléculaire plus spécifiquement sur les champignons et des bactéries pouvant améliorer les techniques de lutte contre les glossines. Il a été montré que des symbiontes ont exprimé des qualités trypanocides. La session a également noté qu'en raison de quelques problèmes techniques d'élevage et de la colonisation de zones écologiques par différentes espèces de glossines, la « La Technique des Insectes Stériles » (SIT) n'est pas encore disponible présentement pour le programme d'éradication. D'autres communications ont abordées la détection du virus de l'hypertrophie des glandes salivaires (VHGS), l'évolution de la recherche sur les glossines et agents attractifs, le stress nutritionnel chez la mouche Tsé-tsé en relation avec sa susceptibilité aux trypanosomes, l'amélioration des appâts visuels, la diversité génétique au sein de populations de mouches Tsé-tsé géographiquement séparées, la gestion et le contrôle vectoriel pour l'amélioration de la productivité. En outre, les outils d'analyse spatiale et des modèles mathématiques peuvent être utilisés dans la collecte des données de références pour élaborer un protocole d'échantillonnage entomologiques stratifié. Enfin, il y a des zones autrefois déclarées libres de mouches Tsé-tsé qui sont actuellement ré-infestées par les glossines.

Recommandations,

- En raison de problèmes techniques d'élevage des mouches et de la colonisation de zones écologiques par différentes espèces de glossines, la SIT n'est pas encore disponible présentement pour le programme d'éradication des mouches Tsé-tsé et, par conséquent les pays ont été instamment invités à mettre en œuvre les techniques intégrées d'élimination des mouches Tsé-tsé en attendant que la SIT soit disponible pour usage sur le terrain ;
- La taille des écrans imprégnés d'insecticides pourraient être réduite. Toutefois, cette possibilité devrait être davantage étudiée ;
- La session recommande que des études plus approfondies soient menées sur les champignons et les bactéries en vue de leur utilisation future dans les programmes de lutte contre les glossines ;
- La session recommande que des enquêtes soient entreprises dans les zones déclarées libres de mouche Tsé-tsé afin d'évaluer la situation et mener des actions pour préserver leur statut actuel.

REPORT AND RECOMMENDATIONS ON SOCIO-ECONOMICS

Moderator: Hippolyte Affognon

Rapporteur: Cecchi Giulian

During this session five presentations were given. The first was entitled “Community-based Livestock Health Delivery Services: The Case of the medium to high Tsetse and Trypanosomiasis challenge areas of the Ghibe Valley, South-west Ethiopia”. This presentation focused on an ILRI project in the Ghibe Valley of south western Ethiopia where pour-on insecticide treated-cattle was used and improved income and welfare of farmers was achieved. The method was well accepted by the community which was willing to pay for the treatment. ILRI facilitated a series of consultation workshops with farmers’ representatives and service providing institutions with a view towards institutionalizing sustainable service delivery. Farmers’ animal health service cooperatives were formed, which were successfully implemented for about five years. Such community-based animal health delivery was the first of its kind in Ethiopia and experiences from such institutional innovations could be scaled out/up to areas with similar challenges.

The second presentation entitled “Do social networks influence livestock keepers’ know-how on animal trypanosomiasis and its control?” concerned social networks and their contribution to the diffusion and improvement of cattle farmers’ knowledge on animal Trypanosomiasis and its control in Solenzo in Burkina Faso. A knowledge, attitude and practices (KAP) survey and a social network analysis were conducted in two villages where all cattle farmers in both villages were involved. A knowledge score was developed as a percentage point of total knowledge and a regression analysis conducted. Results suggest that besides other means of information dissemination, farmer-to-farmer information sharing should be promoted in order to improve farmers’ know-how on animal Trypanosomiasis and its control.

The third presentation was entitled “Livelihood strategies in endemic livestock breeds based production systems: Trends, tradeoffs and implications” and concentrated on trypanotolerant endemic ruminant livestock breeds, whose relative population is decreasing as a result of both increased crossbreeding with Zebu cattle and Sahelian sheep, and

degraded habitat due to forest conversion and bushfires. These trends suggest tradeoffs between livelihoods (cross-breeding and cotton cultivation) and ecosystem preservation (endemic ruminant genetic resources and their habitat). The paper used Participatory Rural Appraisal in selected communities to look into the current trends, tradeoffs and implications of observed breeding strategies and natural resource management. Results indicate that trends in habitat quality tend to drive changes in breed composition at the site levels but also changes (tradeoffs) in livelihoods (income generating activities). Habitat degradation as suggested by the results is related to an increase or decrease of particular animal breeds. Furthermore, the analysis revealed that livelihood options were largely defined by the assets base (resources), which were mostly common property in the study areas (in the Gambia). Community-based management of the natural resources was indicated as 'critical' to sustainable endemic ruminant livestock, natural resources and livelihoods.

The fourth presentation was entitled "Trypanocidals cost as an economic parameter in the socio-economic surveys of animal trypanosomiasis in the Sudan". For this study, surveys were conducted in two Tsetse-infested areas in the Sudan (one in the Blue Nile State and one in Central Equatoria State) to estimate the cost of Trypanosomiasis treatment. Both areas possess huge numbers of livestock, particularly cattle; and are infested with several species of Tsetse flies. Questionnaires and interview of community leaders and group discussions were used for data collection. Usage of Berenil (Diminazine aceturate) was found to be at an average rate of 2.15 doses per animal and year, Homidium (Ethidium bromide) at average rate of 1.9 and Antrycide (Quinopyramine sulphate) at average rate of 0.45. The cost of Trypanosomiasis treatment amounted to 7.9% and 6.3% of the gross livestock production cost in the two study areas respectively. The results of the study indicate that Trypanosomiasis treatment cost may be used as a tool for assessing the economic impact of African Animal Trypanosomiasis.

The last paper was entitled "Improving food security through facilitation of community-based management of Trypanotolerant cattle in the high disease challenge in Ghibe Valley". The study was aimed at facilitating community action-learning in the participatory screening and verification of allegedly trypanotolerant cattle breeds from traditional managed herds in four villages in the Ghibe valley. Animals were identified that had better tolerance to Trypanosomiasis, as measured in terms of less or even

no infection, maintenance of reasonably high PCV values after infection, and the need for only few trypanocidal treatments in a year. Participating communities also recognized these attributes of their animals, and accepted the results as true. They have expressed strong interest for continuation of activities initiated by this project. All the participating farmers recognized the genetic basis of trypanotolerance, and those farmers who own selected animals started to record pedigrees of these animals to help them in selecting replacement breeding animals. Series of village-level and regional consultation workshops were held. The reporting-back and policy dialogue workshop held discussed on the outcomes of the study, and recommended that the process of screening continue. Unanimous recommendations were finally made to try to encourage farmers to use the established farmers groups for enhanced breeding and reproduction of selected animals.

Recommendations

It was noted that socio-economic component in the ISCTRC meeting is getting weak and weak, few papers were presented and many participants were absent.

The meeting recommended that projects and programmes developed for Trypanosomiasis and its control put more emphasize on the socio-economic component and encourage participation in the ISCTRC meetings.

The meeting recognized the need to have countries flexible policies to allow the communities and their partners to organize themselves in groups and play active role in the sustainable management of Tsetse and Trypanosomiasis control.

The meeting recognized the need for appropriate support by projects and the communities and their partners engaging them into income generations activities using own resources.

The meeting encouraged and supported the dissemination of successful experiences to communities and their partners.

RAPPORT ET RECOMMANDATIONS SUR LA SOCIO-ECONOMIE

Modérateur: Hippolyte Affognon

Rapporteur: Cecchi Giulian

Au cours de cette session, cinq présentations ont été faites. Le premier était intitulé « Prestations des services de santé animale sur base communautaire: cas des zones de moyenne à haute pression glossinaire/trypanosomienne de la vallée du Ghibe, au Sud-ouest de l'Ethiopie » ". Cette présentation a porté sur un projet de l'ILRI dans la vallée du Ghibe au Sud-ouest de l'Ethiopie, où l'utilisation des insecticides pour-on sur des bovins a permis l'amélioration du revenu et du bien-être des agriculteurs. La méthode a été bien acceptée par la communauté qui était prête à payer pour les traitements. L'ILRI a animé une série d'ateliers de consultation avec les représentants des agriculteurs et des institutions prestataires de service en vue de l'institutionnalisation de prestations de services durables. Des coopératives d'agriculteurs prestataires de services en santé animale ont été formées, et elles ont fonctionné avec succès depuis environ cinq ans. Ces prestations communautaires de services de santé animale ont été les premières du genre en Ethiopie et les expériences de cette innovation institutionnelle pourraient être étendues à des zones à pression glossinaire/trypanosomienne similaire.

La deuxième présentation intitulée « Les réseaux sociaux influencent-ils le savoir-faire des éleveurs sur la trypanosomiose animale et son contrôle? » a traité des réseaux sociaux et de leur contribution à la diffusion des connaissances entre éleveurs de bovins sur la trypanosomiose animale et son contrôle à Solenzo au Burkina Faso. Une enquête sur les connaissances, attitudes et pratiques (CAP) et une analyse des réseaux sociaux ont été menées dans deux villages où tous les éleveurs de bovins dans les deux villages ont été impliqués. Une fiche de collecte de données sur le niveau de connaissance a été conçue appréciant le pourcentage total des connaissances, celle-ci était suivie d'une analyse de régression. Les résultats suggèrent qu'en plus des moyens de diffusion de l'information, le partage de l'information

d'agriculteur à agriculteur devrait être encouragé afin d'améliorer le savoir-faire de ceux-ci sur la trypanosomiase animale et de son contrôle.

Le troisième exposé était intitulé « Les stratégies de subsistance dans des systèmes de production fondées sur l'élevage des races de bétail endémique: tendances, compromis et implications » et s'est concentré sur les races locales trypanotolérantes, dont l'importance relative de la population est en baisse à la suite des croisements accrus, avec le zébu et les moutons du Sahel, ainsi qu'à la dégradation de l'habitat due à la conversion des forêts et des feux de brousse. Ces tendances suggèrent des compromis entre les moyens de subsistance (croisements de races et culture du coton) et la préservation des écosystèmes (ressources génétiques endémiques de ruminants et leur habitat). L'étude a utilisé une approche d'évaluation rurale participative dans des communautés sélectionnées pour examiner les tendances actuelles, les compromis et les implications des stratégies d'élevage observées et la gestion des ressources naturelles. Les résultats indiquent que concernant la qualité des habitats les tendances conduisent à des changements dans la composition des races au niveau des sites mais aussi à des changements (compromis) dans les moyens de subsistance (activités génératrices de revenus). La dégradation de l'habitat, telle que suggérée par les résultats, est liée à une augmentation ou une diminution de races particulières d'animaux. En outre, l'analyse a révélé que les moyens de subsistance étaient largement définis sur la base des ressources qui pour la plupart étaient des propriétés communes dans les zones d'étude (en Gambie). La gestion communautaire des ressources naturelles a été indiquée comme un facteur «critique» pour le développement durable du bétail ruminant endémique, des ressources naturelles et des moyens de subsistance.

La quatrième présentation était intitulée « Coût des trypanocides pris comme paramètre économique dans les enquêtes socio-économiques sur la trypanosomose animale au Soudan ». Pour cette étude, des enquêtes ont été menées dans deux zones infestées au Soudan (l'une dans l'État du Nil bleu et l'autre dans le centre de l'Etat d'Equatoria) pour estimer le coût du traitement contre la trypanosomiase. Les deux régions possèdent un très grand nombre de têtes de bétail, notamment les bovins, et sont infestées par plusieurs espèces de mouches tsé-tsé. Des questionnaires et interviews des dirigeants communautaires ainsi que des discussions de groupe ont été utilisés pour la collecte des données. Le Bérénil (acéturate de diminazene) est utilisé à un rythme moyen de 2,15 doses par animal et par an, l'homidium (bromure d'éthidium) au rythme moyen de 1,9 dose par animal et par an et l'Antrycide (Sulfate de quinapyramine) à 0,45

dose par animal et par an. Le coût du traitement contre la trypanosomiase a été de 7,9% et 6,3% du coût brut de production du bétail dans les deux zones d'étude. Les résultats de l'étude indiquent que le coût du traitement contre la trypanosomiase peut être utilisé comme un outil d'évaluation de l'impact économique de la trypanosomiase animale africaine.

Le dernier article était intitulé « Améliorer la sécurité alimentaire grâce à la facilitation de la gestion communautaire des bovins trypanotolérants dans vallée du Ghibe à haute pression de maladie ». L'étude visait à faciliter l'apprentissage-action communautaire dans le dépistage participatif et la vérification des races de bovins supposés trypanotolérants à partir de troupeaux gérés traditionnellement dans quatre villages de la vallée du Ghibe. Les animaux qui présentaient raisonnablement un faible pourcentage ou aucune infection en tenant compte du PCV après infection et nécessitaient quelques traitements trypanocides seulement par an ont été identifiés comme ayant une meilleure tolérance à la trypanosomiase. Les communautés ont également reconnu ces caractéristiques chez leurs animaux, et ont accepté les résultats comme justes. Elles ont exprimé leur vif intérêt pour la poursuite des activités initiées par ce projet. Tous les agriculteurs participants ont témoigné de la base génétique de la trypanotolérance, et les agriculteurs qui possédaient des animaux sélectionnés ont débuté l'enregistrement des pedigrees de ces animaux pour la sélection d'animaux reproducteurs pour le remplacement. Plusieurs séries d'ateliers de consultations aux niveaux villages et régionaux ont été organisés. L'atelier de restitution et de dialogue politique a permis de discuter les résultats de l'étude et a recommandé que le processus de dépistage se poursuive. Une recommandation unanime a finalement été faite pour tenter d'encourager les agriculteurs à utiliser les groupes d'agriculteurs mis en place pour améliorer l'élevage et la reproduction des animaux sélectionnés.

Recommandations

Il a été noté que la composante socio-économique à la réunion du CSIRLT devient de plus en plus faible, peu d'articles ayant été présentés et de nombreux absents.

La réunion a recommandé que,

- les projets et programmes élaborés pour la trypanosomiase et son contrôle mettent davantage l'accent sur la composante socio-

économique et que la participation aux réunions du CSIRLT soit encouragée.

- La réunion a reconnu la nécessité d'avoir des politiques nationales souples pour permettre aux communautés et leurs partenaires de s'organiser en groupes et jouer un rôle actif dans la gestion durable de la mouche tsé-tsé et la trypanosomiase.
- La réunion a reconnu la nécessité d'apporter un soutien approprié par des projets et que les collectivités et leurs partenaires participent à des pratiques génératrices de revenu utilisant leurs ressources propres.
- La réunion encourage et soutient la dissémination des expériences réussies aux communautés et leurs partenaires.

REPORT AND RECOMMENDATIONS ON LANDUSE AND ENVIRONMENT

Moderator: Okoth O. Josue

Rapporteur: Joseph Maitima

Recommendation for the land use Environmental section

1. Realizing the importance of climate change on the dynamics of ecosystems that determine the distribution of habitats, the meeting recommended for further work to show the impacts of climate change on future habitats of Tsetse and the implications on PATTEC activities.
2. The meeting recommended harmonization of socio-economic impacts assessment across regions so that PATTEC achievements can be compared across regions, across Tsetse belts and across countries.
3. The meeting recommended more training on communities in PATTEC working sites on proper use of chemicals to ensure that chemicals used in Tsetse control are used safely and that trypanocides are applied to animals using the correct procedures.

RAPPORT ET RECOMMANDATIONS SUR L'UTILISATION DES TERRES ET L'ENVIRONNEMENT

Modérateur: Dr. Okoth O. Josue

Rapporteur: Dr. Joseph Maitima

Recommandation pour l'utilisation des terres, section Environnement

1. Conscient de l'importance des changements climatiques sur la dynamique des écosystèmes qui déterminent la répartition des

habitats des mouches Tsé-tsé, la réunion a recommandé de poursuivre les travaux pour montrer l'impact des changements climatiques sur les comportements futures de la mouche Tsé-tsé et ses implications sur les activités de la PATTEC ;

2. La réunion a recommandé l'harmonisation de l'évaluation des impacts socio-économiques entre les régions pour que les réalisations de la PATTEC puissent être comparées entre régions, entre zones à glossines et entre pays.
3. La réunion a recommandé plus de formation pour les communautés dans les sites d'intervention de la PATTEC à propos du bon usage des produits chimiques pour s'assurer que les produits chimiques utilisés dans la lutte contre la mouche Tsé-tsé sont utilisés en toute sécurité et que les traitements trypanocides sont appliqués aux animaux en suivant les procédures correctes.

REPORT AND RECOMMENDATIONS ON POSTERS

Moderator: Dr. Mamadou Lamine Dia

Rapporteur: Dr. James Wabacha

1. Preamble

The announcement of the ISCTRC meeting and the call for papers required that the posters should be in English or French and should carry the following information:

- Title, author(s),
- Institutional affiliation, address (es) and email.
- Indicate the corresponding author with an asterisk after the name.
- The space allocated for posters is 100 x 150cm.
- Poster must be easy to read.
- The poster should bear the following sections:
 1. An introduction stating the purpose of the study,
 2. material and methods, results (text and illustrations),
 3. Conclusions/recommendations and
 4. References.

2. Observations/Comments

- No posters were posted on the first day as the poster stands were not ready.
- Out of the forty two **(42) posters** selected for presentation, only **26 posters** had been posted by the third day of the conference.
- The scientific content of all the posters was of high quality
- The visual quality of most posters was high
- However, for some posters the following were the shortcomings:
 - ✓ Some included the abstract/Summary
 - ✓ Some discussed the results at length rather than providing conclusions only

- ✓ Some had small font sizes and made them not visually attractive
- ✓ Some contained a lot of information and therefore made the posters clumsy
- ✓ Some did not contain the references
- ✓ Some did not carry the acknowledgements

Summary of Posters selected for presentation viz a viz those that were presented

THEME	Number of posters accepted	Number of posters presented
3. Human African Trypanosomiasis	15	12
4. Animal African Trypanosomiasis	9	5
5. <i>Glossina</i> Biology/Control	13	5
6. Socio-Economics	2	1
7. Land Use and Environment	3	2
Grand total	42	25

RAPPORT ET RECOMMANDATIONS SUR LES POSTERS

Modérateur: Mamadou Lamine Dia

Rapporteur: James Wabacha

1. PREAMULE

L'annonce pour la participation à la conférence a exigé que les posters soient en anglais ou en français et doivent porter les renseignements suivants,

- Titre, auteur (s),
- Affiliation institutionnelle, adresse (s) et e-mail.
- Indiquez l'auteur principal par un astérisque après le nom.
- L'espace alloué pour les posters est de 100 x 150 cm.
- Le poster doit être facile à lire.
- Le poster doit contenir les sections suivantes
 1. Une introduction indiquant l'objet de l'étude,
 2. Le matériel, les méthodes et les résultats (texte et illustrations)
 3. Les conclusions / recommandations et
 4. Les Références.

2. OBSERVATIONS / COMMENTAIRES

- Aucun poster n'a été affiché le premier jour puisque les stands d'affiche n'étaient pas prêts.
- Sur les **quarante-deux (42)** posters sélectionnés pour la conférence, **seulement 25** ont été affichés au troisième jour (23/9/2009) de la conférence.
- Le contenu scientifique de tous les posters a été de grande qualité
- La qualité visuelle de la plupart des posters était bonne
- Toutefois, pour certains posters les lacunes suivantes ont été identifiées,
 - ✓ Certains ont inclus le résumé / sommaire ;
 - ✓ Certains ont discuté les résultats en détails de manière très longue plutôt que de fournir seulement les grandes lignes ;
 - ✓ Certains avaient utilisé des polices de petite taille ce qui les a rendu visuellement non-attractives ;

- ✓ Certains contenaient beaucoup d'informations qui les ont rendu peu pratiques ;
- ✓ Certains ne contenaient aucune référence ;
- ✓ Certains n'ont pas fait cas des remerciements.

Récapitulatif des posters acceptés pour la conférence et présentés

THEME	Nombre de posters acceptés	Nombre de posters présentés
3. Trypanosomiase humaine africaine	15	12
4. Trypanosomiase animale africaine	9	5
5. Biologie des Glossines / contrôle	13	5
6. Socio-économie	2	1
7. Utilisation des terres et environnement	3	2
Total général	42	25

**REGIONAL AND COUNTRY REPORTS/
RAPPORTS NATIONAUX ET REGIONAUX**

MOZAMBIQUE

Résumé

Le Mozambique est situé en Afrique australe, entre 11 et 27 degrés de latitude Sud et 30 et 41 degrés de longitude Est (Annexe 1).

La végétation prédominante du plateau et des zones plus en altitude du Nord et de l'Ouest du pays, est un mélange de savane et de forêt ouverte ou forêt « miombo » qui est favorable à l'infestation par la mouche Tsétsé. Ces formations sont entrecoupées de portions de forêt plus dense à des latitudes plus élevées dans les provinces de Niassa, Nampula, Zambezia, Manica et Sofala.

La Trypanosomose animale transmise par la mouche Tsétsé constitue l'une des principales contraintes pour la production animale au Mozambique. Environ 75% de son territoire, soit 786 000 km², sont infestés par la mouche Tsétsé.

Quatre espèces, notamment *Glossina morsitans morsitans*, *G. pallidipes*, *G. brevipalpis* et *G. austeni* sont présentes au Mozambique (Annexe 2), les *G. morsitans* et *G. pallidipes* étant les espèces les plus répandues. Actuellement, seules les *G. brevipalpis* et *G. austeni* sont présentes dans la partie australe du pays. L'infestation par la mouche Tsétsé continue d'être l'un des principaux facteurs qui influent sur la répartition du bétail sur l'ensemble du pays, concentrant davantage la production animale dans les zones exemptes de mouche Tsétsé au sud de la rivière Save et dans certaines hautes terres de la région Nord et Centrale du pays.

Les *Trypanosoma congolense*, *T. vivax* et *T. brucei* sont les espèces les plus souvent déclarées, la *T. congolense* étant l'espèce la plus courante chez le bétail et les petits ruminants.

Concernant la Trypanosomose humaine, la maladie est liée aux sous espèces de *T. brucei rhodesiense*. Elle survient dans les provinces de Tete, Niassa, Nampula et Cabo Delgado. On ne connaît pas la situation actuelle réelle, car aucune activité surveillance n'a été effectuée depuis

plus de 30 ans. Entre-temps, quelques cas de maladie du sommeil ont été signalés dans les hôpitaux des provinces susmentionnées.

La population bovine réelle compte 1,6 million de têtes environ dont un tiers serait exposé. Les moutons et les chèvres sont au nombre de 4 639 433 environ, les porcs sont 1 349 502.

Summary

Mozambique is situated in Southern Africa between latitude 11 and 27 degrees South and longitude 30 and 41 degrees East. The predominant vegetation of the plateau and areas of higher elevation in the North and West of the country is a mixture of savannah and open woodland or miombo forest that is suitable for Tsetse infestation. This is interpolated with patches of denser forest at higher latitudes in the provinces of Niassa, Nampula, Zambezia, Manica and Sofala. Tsetse transmitted animal Trypanosomosis is one of the major constraints of livestock production in Mozambique. About 75% of its land area of 786,000 square kilometres is infested by Tsetse flies. Four species namely *Glossina morsitans morsitans* West., *G. pallidipes* Austen, *G. brevipalpis* Newst. and *G. austeni* Neswt. are present in Mozambique. The most widely distributed are *G. morsitans* and *G. pallidipes*. Currently only *G. brevipalpis* and *G. austeni* are present in the southern part of the country. The Tsetse infestation continues to be one of the major factors that influence cattle distribution all over the country, making livestock production more concentrated in the relatively Tsetse free areas in the South of the Save river and in some highlands in Northern and Central part of the country. *Trypanosoma congolense*, *T. vivax* and *T. brucei* are the species known to be present. However, *T. congolense* is the most prevalent in cattle and small ruminants.

Regarding Human Trypanosomosis, the disease is associated with infection of *T. brucei rhodesiense*. It occurs in the provinces of Tete, Niassa, Nampula and Cabo Delgado. The real current situation is unknown, because no active surveys have taken place in more than 30 years. Meanwhile few cases of sleeping sickness have been reported in the provincial hospitals of the provinces above mentioned. Cattle population number about 1.6 million heads of which one-third is estimated to be at risk. Sheep and goats make around 4,639,433. Swine are around 1,349,502 heads.

Introduction

Mozambique is situated in Southern Africa between latitude 11 and 27 degrees South and longitude 30 and 41 degrees East (**Annex 1**).

The predominant vegetation of the plateau and areas of higher elevation in the North and West of the country is a mixture of savannah and open woodland or miombo forest that is suitable for Tsetse infestation. This is interpolated with patches of denser forest at higher latitudes in the provinces of Niassa, Nampula, Zambezia, Manica and Sofala.

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Four species namely *Glossina morsitans morsitans*, *G. pallidipes*, *G. brevipalpis* and *G. austeni* are present in Mozambique (**Annex 2**) being *G. morsitans* and *G. pallidipes* the most widely distributed species. Currently only *G. brevipalpis* and *G. austeni* are present in the Southern part of the country. The Tsetse infestation continues to be one of the major factors that influence cattle distribution all over the country, making livestock production more concentrated in the relatively Tsetse free areas in the South of the Save river and in some highlands in Northern and Central parts of the country. *Trypanosoma congolense*, *T. vivax* and *T. brucei* are the species reported all over the time, being *T. congolense* the most prevalent specie in the cattle and small ruminants. Regarding Human Trypanosomosis, the disease is related to subspecies of *T. brucei rhodesiense*. It occurs in the provinces of Tete, Niassa, Nampula and Cabo Delgado. The real current situation is unknown, because no active systematic surveys have taken place in more than 30 years. Meanwhile few cases of sleeping sickness have been reported in the provincial hospitals of the provinces above mentioned.

The actual cattle population is about 1.6 million heads from which one-third is estimated to be at risk. Sheep and goats make around 4, 639, 433. Swine are around 1,349,502 heads.

Tsetse and Trypanosomosis control

Tsetse control

Extensive work on Tsetse distribution was undertaken before independence (1975). Very little has been done then as related to Tsetse. Nevertheless, surveys using traps baited with acetone and cocktail of phenols were carried out in the restocking and animal traction programme areas. Reduction of fly density has been noted in some known as infested areas due to game pouching and forest devastation for agricultural purposes, fire wood, building material industry, etc. Tsetse fly is widely distributed in the centre and north regions of the country although there is no region that can be considered as totally free of Tsetse fly.

Trypanosomosis control

Tsetse transmitted Trypanosomosis occurs in all infested areas and attention is given to its control. The animal Trypanosomosis control programme relays on applications of diminazene aceturate and isometamidium chloride as curative and prophylactic drugs respectively. However there is a significant reduction on the number of animals treated every year because since some years ago the government has been changing the policy with the introduction of the cost-recovery programme. Farmers are reluctant to buy trypanocides, most often due to lack of financial capacity.

All animals moving for breeding purposes are tested for trypanosomosis prior to their movement.

Trypanocidal drug resistance

Drug resistance was suspected and confirmed in the province of Zambezia (Annex 3). Because there are no other tested areas, we believe that the problem is not restricted to Zambezia province only and there is a plan to extend the work to the neighbours Sofala Province.

Training

Training on Tsetse and Trypanosomosis surveys as well as on Trypanosomosis control has been carried out with special emphasis on “on-the-job” training.

The Provincial Veterinary Services have the minimal capacity to handle Trypanosomosis problems. However, the main weakness is the high turnover of the trained staff being moved to other posts or activities not related to the Tsetse field.

PAN AFRICAN TSETSE AND TRYPANOSOMOSIS ERADICATION CAMPAIGN (PATTEC)

The program was launched during the 26th ISCTRC meeting held in Ouagadougou – Burkina Faso in 2001. However, in Mozambique the program is still in the embryonic phase. Only during the second semester of 2006, the PATTEC national focal point was appointed and the national technical team designated. Bilateral meetings with the Republic of South Africa and the Republic of Zimbabwe, two out of five neighbouring countries that are tsetse infested (Annex 4), were planned to discuss the implementation of the PATTEC initiative.

With the Republic of South Africa we have designed a Bi-National Project with technical support of the International Atomic Energy Agency which implementation started in the current year 2009 and it will last for 3 years. The main objective of the project is the collection of baseline data in order to test the methodology to be used later on the eradication of the Tsetse fly in the Southern part of Maputo Province, sharing the Tsetse fly spot area with South African Kwazulu Natal Province.

Another regional project was designed in 2007, which, besides Mozambique, it involves the Republic of Zimbabwe, the Republic of Zambia and the Republic of Malawi. The Mozambique component in this project comprises the Central part of the country including five provinces, namely Inhambane, Gaza, Sofala, Manica and Tete. For the implementation of this project we are looking for the financial support together with the PATTEC Coordination.

RAPPORT MOZAMBIQUE

Résumé

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Quatre espèces, notamment *Glossina morsitans morsitans*, *G. pallidipes*, *G. brevipalpis* et *G. austeni* sont présentes au Mozambique (Annexe 2), les *G. morsitans* et *G. pallidipes* étant les espèces les plus répandues. Actuellement, seules les *G. brevipalpis* et *G. austeni* sont présentes dans la partie australe du pays. L'infestation par la mouche tsé-tsé continue d'être l'une des principales causes qui influent sur la répartition du bétail sur l'ensemble du pays, concentrant davantage la production animale dans les zones exemptes de mouche tsé-tsé au sud de la rivière Save et dans certaines hautes terres de la région Nord et centrale du pays.

Les *Trypanosoma congolense*, *T. vivax* et *T. brucei* sont les espèces le plus souvent déclarées, la *T. congolense* étant l'espèce la plus courante chez le bétail et les petits ruminants.

Concernant la Trypanosomose humaine, la maladie est liée aux sous espèces de *T. brucei rhodesiense*. Elle survient dans les provinces de Tete, Niassa, Nampula et Cabo Delgado. On ne connaît pas la situation actuelle réelle, car aucune surveillance active n'a été effectuée depuis plus de 30 ans. Entretemps, quelques cas de maladie du sommeil ont été signalés dans les hôpitaux des provinces susmentionnées.

La population bovine réelle compte 1,6 millions de têtes environ dont 1/3 serait exposé. Les moutons et les chèvres sont au nombre de 4.639.433 environ, les porcs sont 1.349.502.

I. La lutte contre la mouche tsé-tsé et la trypanosomiase

1. Lutte contre la mouche tsé-tsé

Beaucoup de travaux ont été effectués sur la répartition des glossines avant l'indépendance (1975). Très peu a été fait sur la mouche tsé-tsé depuis lors. Toutefois des enquêtes en utilisant des pièges appâtés avec de l'acétone et un cocktail de phénols sont menées dans les zones où se déroulent les programmes de repeuplement et de la traction animale. La réduction de la densité de mouches a été notée dans certaines zones connues comme infestées en raison de la chasse et la dévastation des forêts pour des fins agricoles, de bois de chauffage, d'industrie de matériaux de bâtiment, etc. La mouche tsé-tsé est largement distribuée dans les régions centre et nord du pays bien qu'il n'y ait aucune région pouvant être considérée comme totalement exempte de mouche tsé-tsé.

2. Lutte contre la trypanosomiase

La trypanosomiase transmise par la mouche tsé-tsé se manifeste dans toutes les zones infestées et toute l'attention est donnée à son contrôle. Le programme de lutte contre la trypanosomiase repose sur d'administration de l'acéturate de diminazène et du chlorure d'isométymidium qui sont respectivement des médicaments curatifs et prophylactiques. Cependant, il y a une réduction significative du nombre d'animaux traités chaque année parce que depuis quelques années, le gouvernement a changé sa politique avec l'introduction du programme de recouvrement des coûts. Les agriculteurs sont prudents en achetant les trypanocides, le plus souvent en raison d'un manque de capacité financière.

Tous les animaux se déplaçant aux fins de reproduction sont testés pour la trypanosomiase avant leur mouvement.

3. La résistance aux médicaments trypanocides

La résistance aux médicaments a été suspectée et confirmée dans la province de Zambesia (annexe 3). Parce qu'il n'y a pas d'autres zones testées pour la chimiorésistance, nous croyons que le problème n'est pas limité à la province du Zambèze seulement et il y a un plan pour étendre les travaux aux provinces voisines de la Sofala.

II. La formation

Formation sur la surveillance des mouches tsé-tsé et de la trypanosomiase ainsi que sur le contrôle de la trypanosomiase a été

effectuée avec une attention particulière sur la formation sur le tas des agents sur leur lieu de travail.

Les services vétérinaires provinciaux ont la capacité minimale pour gérer les problèmes de la trypanosomiase. Toutefois, la faiblesse principale est le taux de renouvellement élevé du personnel formé déplacés à d'autres postes ou à des activités non liées au domaine de la tsé-tsé.

III. La Campagne Panafricaine d'Eradication de la Tsé-tsé et la Trypanosomiase (PATTEC)

Le programme a été lancé au cours de la 26^{ème} réunion du CSIRLT qui s'est tenue à Ouagadougou - Burkina Faso en 2001. Cependant le programme reste encore au stade embryonnaire au Mozambique. Le point focal national de la PATTEC n'a été nommé et l'équipe technique nationale désignée qu'au cours du second semestre de 2006. Des réunions bilatérales avec la République d'Afrique du Sud et la République du Zimbabwe, deux des cinq pays voisins qui sont infestés de glossines (annexe 4), ont été planifiées pour discuter de la mise en œuvre de l'initiative de la PATTEC.

Avec la République d'Afrique du Sud, nous avons conçu un projet binational avec l'appui technique de l'Agence internationale de l'énergie atomique qui a vu sa mise en œuvre commencée au cours de cette année (2009) et ce projet va durer 3 ans. L'objectif principal du projet est la collecte de données de référence afin de tester la méthodologie qui sera utilisée plus tard, sur l'éradication de la mouche tsé-tsé dans la partie sud de la province de Maputo, partageant la zone infestée de mouche tsé-tsé avec la province du Kwazulu Natal de l'Afrique du Sud.

Un autre projet régional a été conçu en 2007, qui, outre le Mozambique implique la République du Zimbabwe, la République de Zambie et la République du Malawi. La composante du Mozambique dans ce projet comprend la partie centrale du pays, dont cinq provinces, à savoir Inhambane, Gaza, Sofala, Manica et Tete. Pour la mise en œuvre de ce projet, nous sommes à la recherche du soutien financier ainsi que la coordination de la PATTEC.

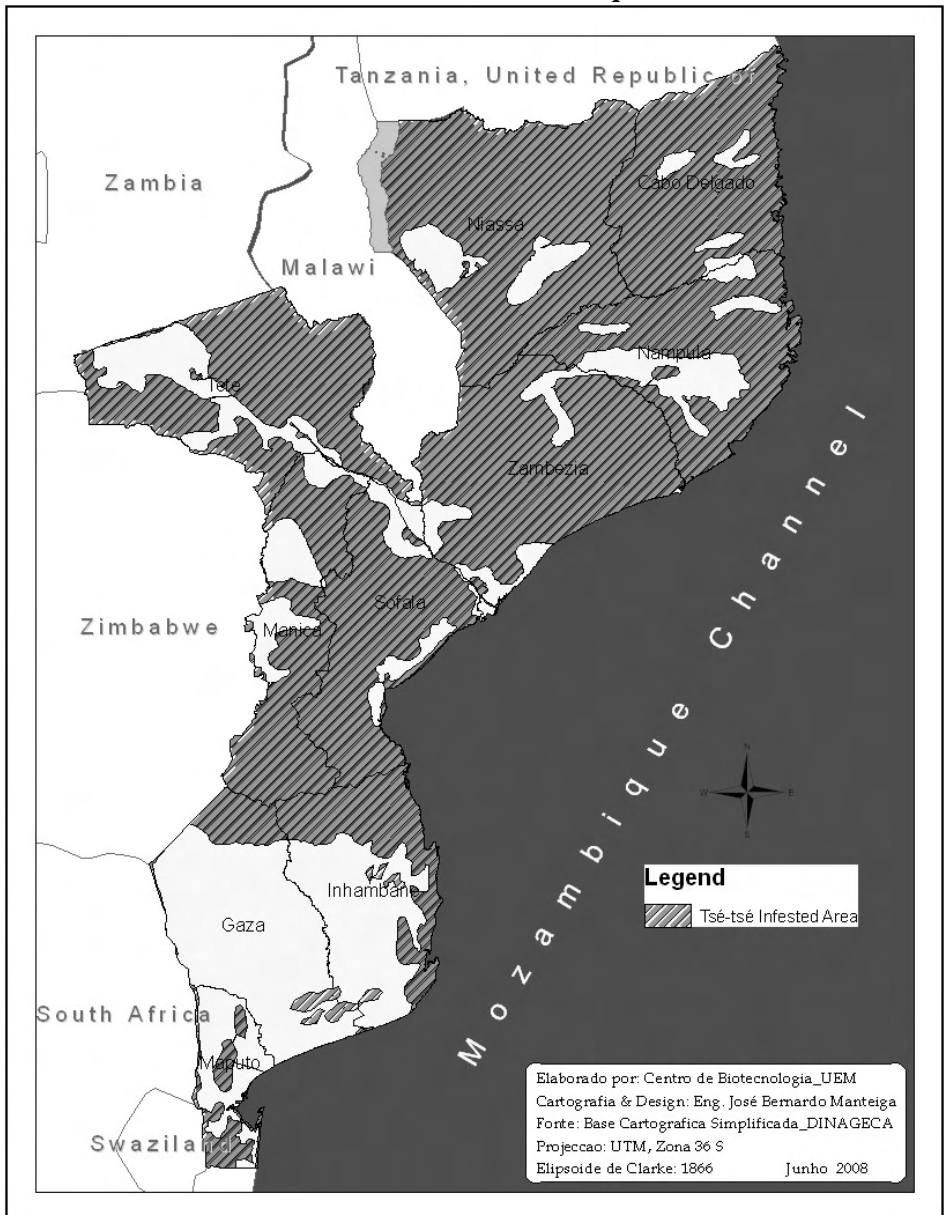
Annex 1

Mozambique situation



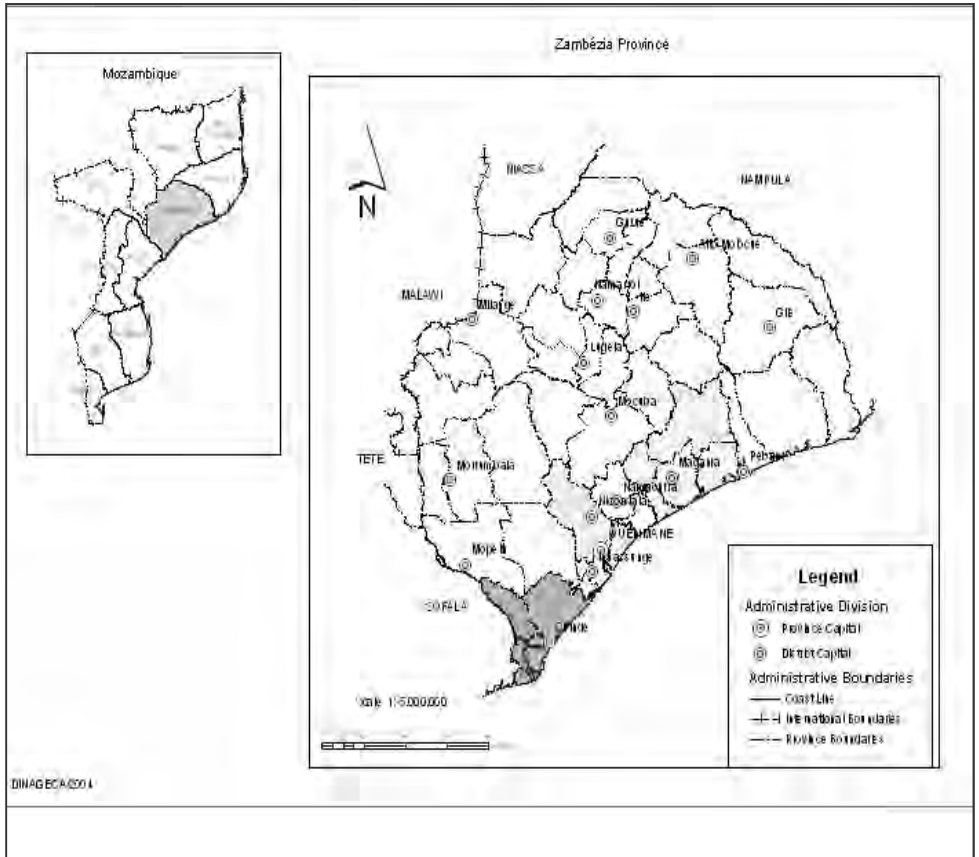
Annex 2

Tsetse distribution in Mozambique



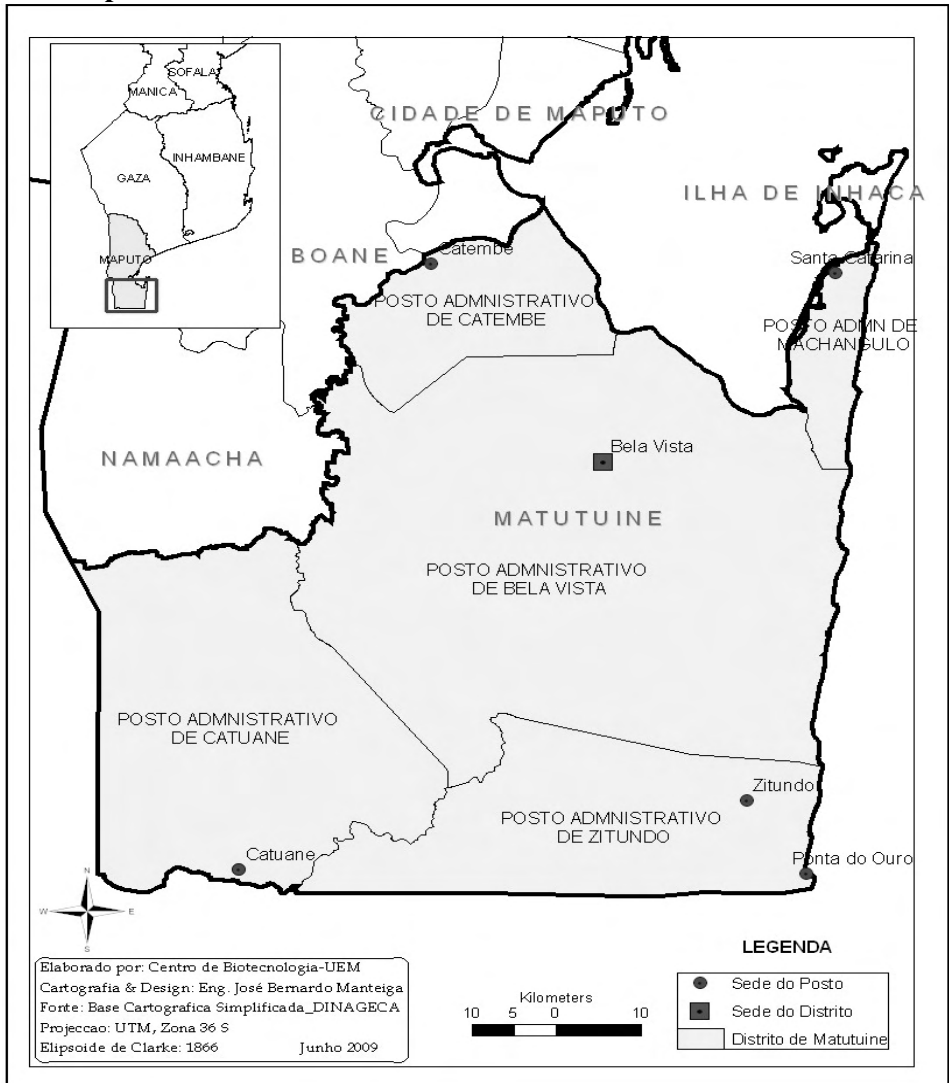
Annex 3

Zambezia Province: Trypanocidal drug resistance tested areas



Annex 4

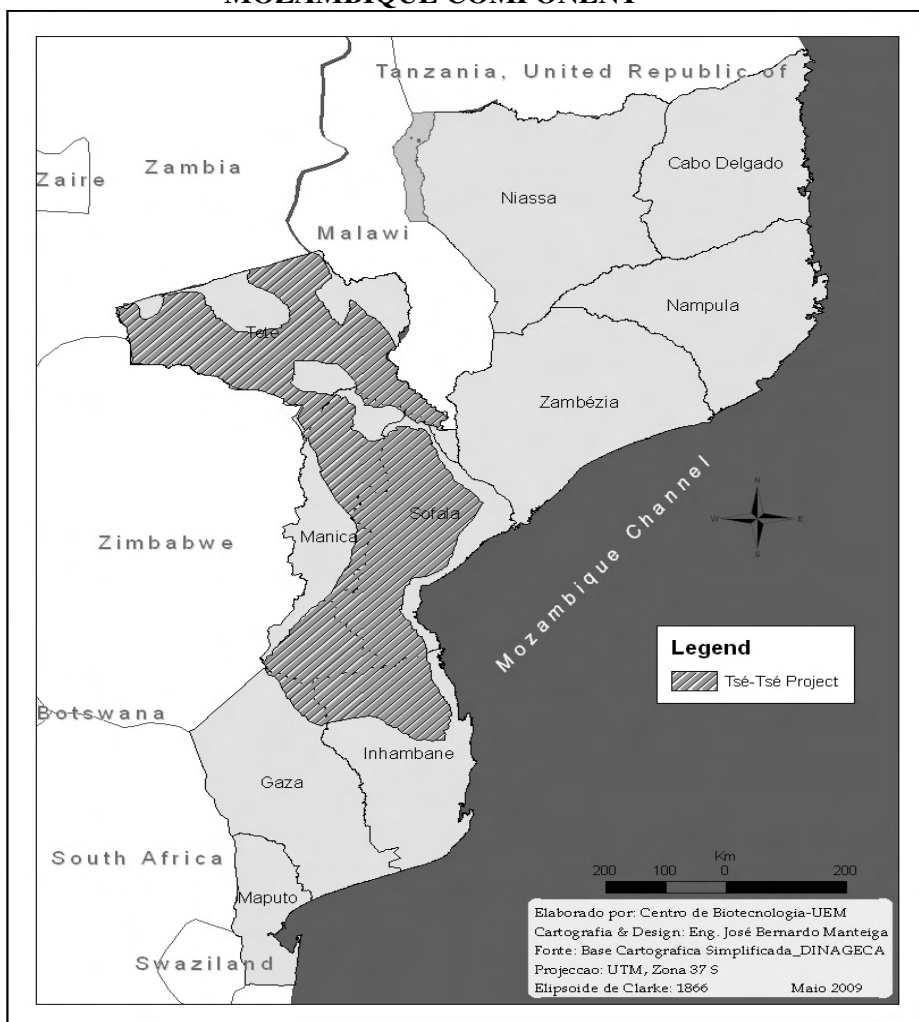
Maputo Province with the indication of Matutuine district



Annex 5

MULTINATIONAL PROJECT

MOZAMBIQUE COMPONENT



SOUTHERN SUDAN REPORT

Tereka T., A. Kwai and J. Korok

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Introduction

- The infested areas with Tsetse need to be resurveyed and mapped in order to obtain practical field results for intervention.
- Human and Animal Trypanosomiasis is a health and economic problem in Sudan.
- There has never been any standardized Tsetse control except some NGOS tried to trap Tsetse flies to confirm their presence, but records are not available at hand.
- Human and Animal Trypanosomiasis relied for many years on chemotherapy.

Visit of PATTEC Mission from Addis Ababa to Juba between 25th April to 5th May 2009

- The mission was led by Dr. John Kabayo, whose aim was to develop a comprehensive work plan for the initiation of activities for the implementation of PATTEC Sudan.
- In Juba the mission met H.E Vice President Dr. Riek Machar, HE Minister of Agriculture Dr. Samson Kwaje and HE Minister of Animal Resources and Fisheries Dr. Festo Kumba and Government dignitaries.
- It was agreed that a coordination office be established under Goss to help organize activities for Tsetse and Trypanosomiasis eradication.
 - HE the Vice President agreed to support the implementation of PATTEC in Sudan.

Tsetse and Trypanosomiasis stakeholders' workshop held in Juba on 12th -14th November 2008

Objectives:

- To refresh and update the participants on Tsetse and other biting flies, sleeping sickness and animal Trypanosomiasis.

- To formulate strategy for Tsetse and Trypanosomiasis control in Sudan.
- To forward practical recommendations to the stakeholders.

Recommendations

Recognizing that human sleeping sickness and animal Trypanosomiasis in Southern Sudan pose challenges in human and animal health that impedes the socio-economic growth of the South in Particular

The workshop arrived at the following general recommendations:

- Mobilization of resources;
- To identify and describe the role of all stakeholders and get them involved actively;
- To start working on policy frame work;
- To create active awareness programmes;
- Need for integrated model (Government, Universities and Research Institutions);
- Advocacy awareness at all levels, Government, Communities and NGOs;
- To develop pilot project

RAPPORT DU SUD-SOUDAN

**Tereka T., A. Kwai, J. Korok Courrier e-mail,
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Introduction

- Les zones infestées par la mouche tsé-tsé ont besoin d'être étudiées et cartographiées afin d'obtenir des résultats pratiques de terrain pour les interventions.
- Les trypanosomiasés humaine et animale sont des problèmes de santé et d'économie au Soudan.
- Il n'y a jamais eu de lutte normalisée contre les glossines, exception faite de quelques ONGs qui ont essayé de faire le piégeage des mouches tsé-tsé afin de confirmer leur présence, mais les résultats ne sont pas disponibles à portée de main.
- Le traitement des trypanosomiasés humaine et animale a été depuis de nombreuses années basé sur la chimiothérapie.

Mission de visite de la PATTEC d'Addis Abeba à Juba entre le 25 avril et 5 mai 2009

- La mission dont le but était d'élaborer un plan de travail détaillé pour le lancement des activités de mise en œuvre de la PATTEC au Soudan était dirigée par le Dr John Kabayo.
À Juba, la mission a rencontré SE le Vice-président M. Riek Machar, SE le Ministre de l'Agriculture Dr Samson Kwaje et SE le Ministre des Ressources animales et de la Pêche Dr Festo Kumba ainsi que des dignitaires du gouvernement.
- Il a été convenu que le bureau de coordination soit établi sous Goss pour aider à organiser les activités d'éradication de la mouche tsé-tsé et la trypanosomiasé.
- SE le Vice-président a accepté de soutenir la mise en œuvre de la PATTEC au Soudan.

Atelier des acteurs concernés par la mouche tsé-tsé et la trypanosomiasé tenu à Juba du 12 au 14 novembre 2008

Objectives:

- Actualiser et mettre à jour la connaissance des participants sur la mouche tsé-tsé et d'autres mouches piqueuses ainsi que la maladie du sommeil et de la trypanosomiasé animale.

- Formuler la stratégie de lutte contre la mouche tsé-tsé et la trypanosomiase au Soudan.
- Formuler des recommandations pratiques à l'attention des intervenants.

Recommandations

Reconnaissant que la maladie du sommeil humaine et la trypanosomiase animale posent des problèmes chez les personnes et animaux sains dans le Sud du Soudan, ralentissant ainsi la croissance socio économique du Sud en particulier,

L'atelier est parvenu aux recommandations générales suivantes,

- Mobiliser des ressources ;
- Identifier et décrire le rôle de tous les intervenants et les faire participer activement ;
- Commencer à travailler sur le document - cadre de politique ;
- Créer des programmes actifs de sensibilisation ;
- Développer un modèle intégré (gouvernement, universités et instituts de recherche).
- Plaider et sensibiliser à tous les niveaux, gouvernement, communautés et ONGs;
- Développer un projet pilote.

TANZANIA REPORT

TSETSE AND TRYPANOSOMOSIS CONTROL ACTIVITIES IN TANZANIA 2007-2009

ACTIVITES DE LUTTE CONTRE LA MOUCHE TSETSE ET LA TRYPANOSOMIASE EN TANZANIE, 2007 - 2009

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TANZANIA

Résumé

L'infestation de la mouche tsé-tsé et la Trypanosomiase demeurent des contraintes majeures pour la santé humaine et animale. Environ 60% de la Tanzanie, soit une superficie de 945 000 km², est infestée par sept espèces de Glossine. La trypanosomiase est la deuxième cause de mortalité du bétail, après la Fiebre de la vallée du Rift. La maladie est débilitante et réduit la capacité humaine et la puissance de traction de la production agricole dans la plupart des régions. La trypanosomiase transmise par la mouche tsé-tsé pose un risqué élevé pour 4 millions de personnes et 7 millions de têtes de bétail environ.

La trypanosomiase humaine africaine (THA) est endémique dans 11 districts de 6 régions. En 2007/08, seuls 5 districts ont signalé 158 cas de THA. Les cas de Trypanosome animale africain (TAA) signalés varient chaque année, en fonction du nombre de districts faisant rapport, à partir de 21 régions infestées par la mouche tsé-tsé. Il y avait 26 districts répartis dans 13 régions qui ont signalé 2872 cas et 93 morts en 2007. En 2008, les districts ayant fait rapport étaient au nombre de 22 sur 12 régions, avec 1613 cas et 64 morts ; alors que 10 districts de 7 régions ont signalé 279 cas 6 morts en 2009. La fourniture en cours de solution acaricide (pyrethroïde) dans l'ensemble des régions a eu un impact sur le problème de la mouche tsé-tsé. Cependant, la tendance montre une sous-déclaration de la TAA, du fait de ressources humaines et financières insuffisantes.

Au cours de la période 2007 à 2009, la Tanzanie a exécuté diverses activités dont la préparation de la mise en oeuvre de la Campagne panafricaine d'eradication de la mouche tsé-tsé et de la Trypanosomiase

(PATTEC). Parmi les autres activités on compte la formation et la recherche, les études.

Summary

Tsetse infestation and Trypanosomosis remain major constraints to human and animal health in Tanzania. About 60% of the country (or appr. 945,000 sq. km) is infested by seven species of *Glossina*. Trypanosomosis is second to East Coast Fever in causing cattle mortalities. The disease is debilitating and reduces human and draught power for agricultural production in most regions. Tsetse transmitted trypanosomosis pose a high risk to about 4 million people and 7 million cattle. Human African Trypanosomosis (HAT) is endemic in 11 districts in 6 regions. In 2007/08, only 5 districts reported 158 HAT cases. Animal African Trypanosomosis (AAT) reported cases vary in each year depending on the number of reporting districts from 21 tsetse infested regions. There were 13 regions with 26 districts reporting 2872 cases and 93 deaths in 2007. In 2008 reporting districts were 22 from 12 regions with 1613 cases and 64 deaths; whereas, 10 districts from 7 regions reported 279 cases and 6 deaths in 2009. The ongoing provision of dipping acaricide (pyrethroids) to all regions has an impact on tsetse challenge. However, the trend show under-reporting of AAT, due to inadequate human and finance resources. During the period of 2007 to 2009, Tanzania has been implementing various activities; among them are preparations to implement Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC). Other activities were training and research, tsetse surveys, mapping and community initiatives which are promising to control tsetse flies, HAT and AAT for improving food security.

1.0 INTRODUCTION

Tsetse infestation and Trypanosomosis remain major constraints to human and animal health. About 60% of Tanzania which covers an area of 945,000 sq. km is infested by seven species of *Glossina*. African Trypanosomosis (AT) is second to East Coast Fever in causing cattle mortalities. The disease is debilitating and reduces human and draught power for agricultural production in most regions. Tsetse transmitted trypanosomosis pose a high risk to about 4 million people and 7 million cattle.

Tsetse transmitted Africa Trypanosomosis is endemic in Tanzania. About 6762 cases of Nagana have been reported from 51 districts, and 165 deaths. There are 187 cases of sleeping sickness reported from 7 districts out of 10 HAT reporting districts in 6 regions from 2007 to June 2009.

Tanzania recognizes the severity of tsetse transmitted trypanosomosis and has continued to allocate a total of Tshs. 10 billion (USD 1.million) subsidies for the support to purchase synthetic pyrethroids for dipping for the control of both tsetse and ticks this is in line with Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC). The purpose of this report is to highlight tsetse and trypanosomosis control activities from 2007 to date in Tanzania.

2.0 ACTIVITIES UNDERTAKEN FROM 2007 TO 2009

During the period of 2007 to 2009, Tanzania has been implementing various activities such as research, training, tsetse surveys, mapping and control, publication and dissemination of booklets on HAT and AAT control for livestock keepers for improved food security. Furthermore, undertake preparatory activities to implement Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) on the Rwanda–Tanzania and Kenya–Tanzania borders.

2.1 TSETSE CONTROL

2.1.1 Community participation

In 2009 a total of 1500 T&Tc leaflets and 1500 booklets were produced and disseminated to different tsetse infested regions. Furthermore, a total of 229 farmers in Lindi region (168 Lindi and Kilwa 61 districts), Service providers 21 (Lindi 16, Kilwa 5 districts) and 7 Ward executive staff from 22 villages were trained on the use of insecticide treated live and artificial baits (cattle and screens) to control T&T in their grazing areas.

2.1.2 National Parks participation

Tsetse flies are nuisance and infest almost all the National Parks, Game and forest reserves in Tanzania. They transmit both Human and animal African trypanosomosis. Since the outbreak of sleeping sickness that

occurred in 2000, the National Park authorities has embarked on deploying targets (**Table 1**) to control tsetse flies in selected areas such as Tourist circuits, camps, lodges, ranger posts, walking paths, recreational areas and spraying vehicles of rangers. For example in Serengeti National Park (SENAPA) cars were sprayed at Seronera filling station, Naabi and Ndabaka entrance gates.

Table1: Tsetse Control in the National Parks in Tanzania

	No of Targets deployed		Insecticide cars	Sprayed
FINANCIAL YEAR	2007/08	2008/09	2007/08	2008/09
Serengeti	1769	1918	8915	11,403
Katavi	1380	1300	-*	-*
Mikumi			-*	-*
Ruaha	1550	1670	-*	-*

* No report

3.0 AFRICAN TRYPANOSOMOSIS (AT)

3.1 Human African Trypanosomosis (HAT)

HAT or Sleeping sickness threatens millions of people in 36 countries of sub-Saharan Africa. In the United Republic of Tanzania (URT) the disease is endemic in 3 western regions and 1 region in the northern east (Table 2), although tsetse flies are found in all 26 regions. However, it is not all seven *Glossina* species found in the country that can transmit the disease. Sleeping sickness generally occurs in remote rural areas where health systems are weak or non-existent and less than 1% of population at risk is under surveillance. Most of people are infected when performing their domestic (water and firewood collection) and economic occupational activities (farming, mining, hunting, and honey collection, livestock grazing, lumbering and fishing) in tsetse infested areas (map 1). The wide range of conservation areas is a source of *T. b. rhodesiense* infection from the wild animals. HAT cases are detected through few active surveillance but majority are from passive surveillance. Moreover, the Ministry of health undertake Sentinel surveillance in selected health centres (Kaliua & Magugu) any occurrence of disease is reported. There has been a delay in case detection and treatment of HAT cases due to low awareness and high prevalence of malaria in the same foci which have similar clinical signs to that of HAT. Delays is also caused by lack of diagnostic facilities

Table 2: Trend of HAT in 6 regions from 2005 -2009

REGION	DISTRIC T	2005	2006	2007	2008	2009
KIGOMA	<i>Kibondo</i>	15	12	5	12	-
	<i>Kasulu</i>	18	7	14	6	-
ARUSHA	<i>Babati</i>	-	1		1	-
	<i>Monduli</i>	-	-		-	-
	<i>Hanang</i>	-	-		-	-
TABORA	<i>Urambo</i>	85	56	89	33	-
RUKWA	<i>Nkansi</i>	18	9	-	1	-
	<i>Mpanda</i>	47	44	15	2	-
MBEYA	<i>Chunya</i>		1	-		
MARA	<i>Serengeti</i>		1	2	1	4
	TOTAL	183	131	128	55	4*

*Source: National Institute for Medical Research (NIMR) – Tabora. * By June 2009*

Currently there are few cases of HAT from Mara region in the North-Eastern Tanzania in the Serengeti ecosystem. The period of June to July is a dry season in Tanzania and has high influx of tourist in the Serengeti National Park (SENAPA). It is for this reason that there is a high chance of getting a bite from a tsetse fly. Due to recent reports on HAT cases from Serengeti, WHO – Collaborating Centre for Research and Training on HAT diagnostics - (Institute of Tropical Medicine - Antwerp in collaboration with National Institute of Medical Research (NIMR) Tabora Tanzania conducted a capacity building session on HAT diagnosis and treatment for 14 health facilities in Mugumu hospital to cover Serengeti National Park and the neighborhood areas.

3.2 AFRICAN ANIMAL TRYPANOSOMOSIS (AAT)

Animal Trypanosomosis has been reported country wide from different regions. It is obvious that the disease in domestic animals and particularly cattle (Table 1) is a major obstacle to the economic development of the livestock Industry in Tanzania.

Table 1: Animal trypanosomosis cases reported from 2007 to 2009

REGION	DISTRICTS		2007		2008		2009	
	Total	Reporting in 3 years	Case s	Deat h	Case	Deat h	Case	Deat h
Arusha	6	5	5	-	27	2	5	-
Dodoma	5	2	215	3	-	-	-	-
DSM	3	1	183	-	-	-	-	-
Iringa	6	4	180	7	3	1	-	-
Kagera	7	2	2	-	-	-	6	-
Kigoma	3	1	-	-	19	-	-	-
Kilimanjaro	6	4	80	4	3	5	-	-
Lindi	5	0	-	-	-	-	-	-
Mara	4	4	36	5	76	1	26	1
Mbeya	7	4	7	-	134	1	-	-
Morogoro	5	4	184	1	12	2	1	-
Mwanza	8	1	-	-	94	32	-	-
Mtwara	5	0	-	-	-	-	-	-
Pwani	6	5	225	45	43	3	82	4
Ruvuma	4	0	-	-	-	-	-	-
Singida	4	3	1	-	2	-	29	1
Shinyanga	7	1	3	-	22	10	12	4
Tabora	5	1	1	-	-	-	-	-
Tanga	8	9	1933	30	1180	7	234	1
Total	104		4988	92	1613	62	**161	**11

Source: Epidemiology Unit Ministry of Livestock development & Fisheries

Key: ** by July 2009

AAT is also a problem in the Livestock and wildlife interface areas. Screening of 518 cattle, 220 wild animals of different species and 115 domestic dogs undertaken in Western Serengeti using PCR; indicated that the overall *T. brucei brucei* prevalence was 5.4% (n=518) in cattle, 1.25% (n=155) in domestic dogs and 5.5% (n=220) in wild animals The overall prevalence of Human infective trypanosome

T. b. rhodesiense was 1.2% in cattle and 1.8% in wild animals. Certainly, there are unrecorded numbers of deaths among the wild animals.

4.0 RESEARCH

Tsetse and Trypanosomosis Research Institute has continued to undertake various research activities with the following titles:

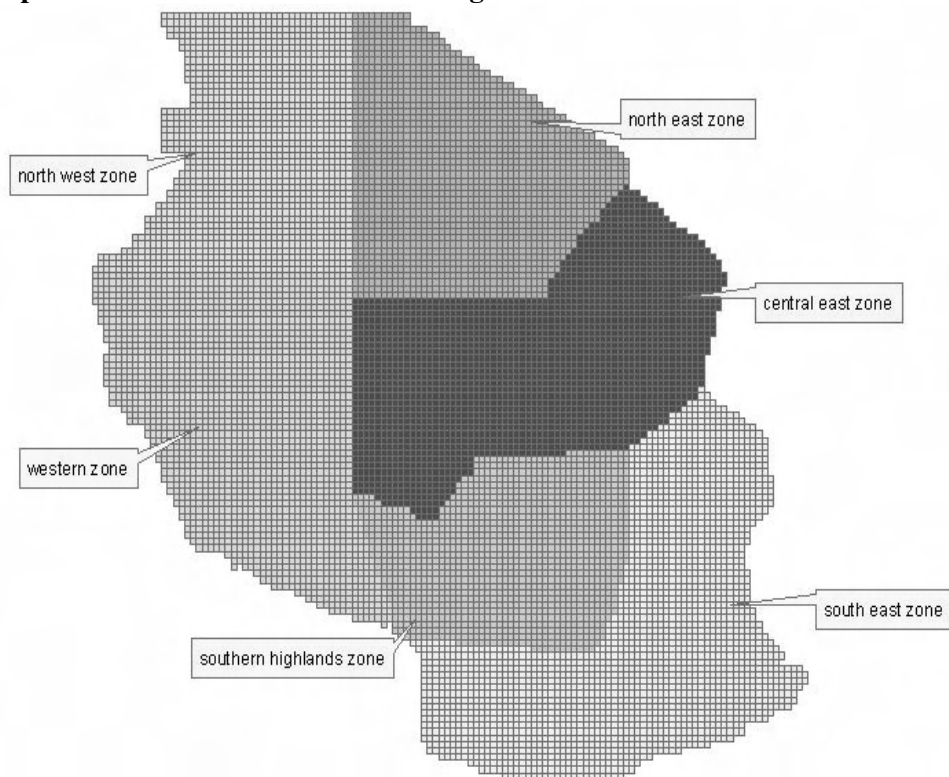
- Seasonal effects of blood diet on tsetse (*Glossina austeni*) performance mass reared for the Sterile Insect Techniques (SIT)
- Study of the ecology and population dynamics of *Glossina swynnertoni* at the game/livestock/people interface of the Tarangire National Park, Tanzania
- Improving SIT for tsetse flies through research on their symbionts and pathogens
- Tsetse density and prevalence of trypanosomiasis in Kisarawe and Rufiji districts
- Integrated tsetse fly ecology and genetics for improved HAT control
- Decision supports tools in HAT control
- Standardization and optimization of tsetse fly trapping/target devices for area wide control
- The seasonal studies of tsetse flies and epidemiology of animal Trypanosomiasis at the Ngorongoro Conservation Area.
- Assistance to a feasibility study for the use of the Sterile Insect Technique in northern Tanzania
- Seasonal studies of the tsetse flies in Mafia Island
- Use of molecular diagnostic techniques to determine epidemiological status for animal trypanosomiasis

5.0 TRAINING

There is a problem of few field staff to effectively implement the control Programmes. In order to overcome this problem, human resource

development and recruitment by both the public and private Sector is necessary. In response to overcome this problem the Capacity of the lead ministries has been strengthened through training and recruitment of trained personnel from the Livestock Training Institute (LITI) Morogoro. As from 1990 to date LITI has trained 136 Technicians on Range Management and Tsetse Control at Certificate and Diploma levels. In addition to above training the IAEA consultant conducted a Grid sampling course in May 2008 to staff from the Ministry of Livestock development and Fisheries, Tanzania National Parks (TANAPA) and Ngorongoro National Park Conservation Authority. Tanzania was divided into 6 zones for future tsetse and trypanosomosis interventions (Map 1).

Map 1: Tanzania zones for T &T management



IAEA conducted a regional training on tsetse field data collection and data base management whereby two participants attended in March 2008

held in Dakar Senegal. Moreover, AU-PATTEC in partnership with Arab Bank for Africa Development (BADEA) supported a PATTEC managers / coordinators training in March 2009 in the Kingdom of Swaziland.

In support of the PATTEC program, the Foundation of Innovative New Diagnostics (FIND), in September 2009 held a Workshop in Tanzania on Capacity Strengthening in African Trypanosomosis and data Management. The objective was to develop data management, mapping and GIS capacity within countries to aid the management of HAT and AAT. Furthermore, to develop tools to guide surveillance and eradication interventions.

The Way forward

In collaboration with other stakeholders the government will strengthen:

- The on going activities such as tsetse and trypanosomosis research, survey and mapping, control, diagnosis and treatment
- Capacity building for tsetse and range management staff.
- Advocacy on HAT and AAT control techniques in collaboration with other stakeholders
- Preparation for the implementation of PATTEC in Rwanda–Tanzania Kagera basin area and along Kenya-Tanzania border

**HUMAN AFRICAN TRYPANOSOMIASIS/
TRYPANOSOMIASE HUMAINE AFRICAINE**

POPULATION GENETICS OF *TRYPANOSOMA BRUCEI* *GAMBIENSE*: A CLONE IN THE DARK

GENETIQUE DES POPULATIONS DE *TRYPANOSOMA* *BRUCEI GAMBIENSE*, UN CLONE DANS LE NOIR

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Résumé

La Trypanosomose Humaine Africaine (THA) (ou maladie du sommeil) due à *Trypanosoma brucei gambiense* groupe 1) affecte l'Afrique de l'Ouest et du Centre. *T. brucei* s.l. a une grande faculté d'adaptation à différents hôtes et de nombreuses questions sont débattues quant à son régime principal de reproduction, ses capacités de dispersion et l'importance de sa population réelle. Nous avons analysé le polymorphisme de *T. b. gambiense* au niveau de huit loci microsatellites à partir de souches isolées de patients atteints de THA en Côte d'Ivoire et en Guinée en vue de découvrir comment l'information génétique est répartie au sein des isolats, entre isolats, entre foyers et entre échantillons espacés dans le temps.

Les analyses montrent (1) une absence d'échange à l'échelle de l'Afrique de l'Ouest et que même en Guinée, les deux foyers étudiés échangent peu de gènes; (2) que la taille réelle (génétique) des populations de trypanosomes en termes d'hôtes infectés dépasse probablement le nombre estimé à partir des surveillances épidémiologiques ; et (3) que *T. b. gambiense* groupe 1 se reproduit probablement exclusivement par voie asexuée.

Summary

Human African trypanosomiasis (HAT) (or sleeping sickness) due to *Trypanosoma brucei gambiense* occurs in Western and Central Africa. *T. brucei* s.l. displays a huge diversity of adaptations and host specificities, and questions about its reproductive mode, dispersal abilities, and effective size remain under debate. We have investigated genetic variation at eight microsatellite loci of *T. b. gambiense* strains isolated from HAT patients in Côte d'Ivoire and Guinea, with the aim of knowing how genetic information was partitioned within and between individuals, between foci and between temporally spaced samples. The results indicate that (i) migration of *T. b. gambiense* group 1 strains does not occur at the scale of West Africa, and that even at a finer scale (e.g. within Guinea) migration is restricted; (ii) effective population sizes of trypanosomes as reflected by infected hosts are probably higher than what the epidemiological surveys suggest; and (iii) *T. b. gambiense* group 1 is most likely a strictly clonally reproducing organism.

Key words: *Trypanosoma brucei gambiense*; genetic diversity; clonality; Western Africa; microsatellite markers; genetic differentiation; effective population size

Introduction

The causative agent of Human African trypanosomiasis (HAT) or sleeping sickness, *Trypanosoma brucei* is subdivided into three subspecies (Hoare, 1972): *T. brucei gambiense* (*T. b. gambiense*) is responsible for the chronic form of HAT in Western and Central Africa, *T. b. rhodesiense* is the agent of the acute form of HAT in East Africa, and *T. b. brucei* does not infect humans but causes animal trypanosomiasis (nagana) in cattle. During the last decades, molecular methods have been developed for typing *T. brucei* s.l. stocks in order to study its population structure and taxonomy. Only one group could be

clearly identified as a distinct genetic entity: *T. b. gambiense* group 1, which is considered to be the main causative agent of HAT in Western and Central Africa (Gibson, 1995; Gibson, 2007).

Trypanosoma brucei s.l. displays a huge diversity of adaptations and host specificities and questions about its reproductive mode, dispersal abilities, and effective population size remain under debate. Like most protozoan parasites, *T. brucei* s.l. has been assumed to be clonal (Tibayrenc, 1995; Tibayrenc, 1998; Tibayrenc *et al.*, 1990), although some authors have reported the occurrence of sexual reproduction (Tait, 1980; Jenni *et al.*, 1986; Gibson, 1989; Gibson, 1995; MacLeod *et al.*, 2005a; MacLeod *et al.*, 2005b). The presence or absence of a sexual process will crucially determine the genetics at both, individual and population levels. Estimates of how genetic diversity is portioned within individuals (reproductive system), within and among subpopulations (population structure) may indicate how species track continuously varying environments and adapt to local conditions in the face of gene flow among diverse populations (Criscione *et al.*, 2005). Thus, a better understanding of the reproductive system of such organisms might be crucial for optimizing field control strategies (Tibayrenc *et al.*, 1991; Milgroom, 1996; Taylor *et al.*, 1999; Tibayrenc, 2005).

Recently, microsatellite markers were shown to be polymorphic enough to highlight the existence of genetic diversity within *T. b. gambiense* group 1 (Koffi *et al.*, 2007; Koffi *et al.*, 2009). In the present paper, we revisit the results presented in (Koffi *et al.*, 2009) with particular attention to reproductive modes of *T. brucei gambiense* 1 and other *T. brucei* species and on population size. We conclude on optimal sampling strategies that should further be undertaken to fully understand the population biology, hence epidemiology, of these important pathogens.

Material and Methods

Trypanosome isolates and genotyping

Trypanosome isolates (one and more rarely two per patient) were taken from three geographical zones and four sampling dates: in Guinea, Boffa 2002, Dubreka 1998, and Dubreka 2002; in Côte d'Ivoire, Bonon 2000, Bonon 2002, and Bonon 2004 (Koffi *et al.*, 2009) (Figure 1). In Bonon the isolates were 17 in 2000, 14 in 2002 and 17 in 2004. In Guinea, the isolates were 15 in Dubreka 1998, seven in Dubreka 2002, and 20 in Boffa 2002. The study area in Bonon concerns 30,000 inhabitants distributed in 400 km², with an approximate mean prevalence

of 0.004 (Kaba *et al.*, 2006), leading to an estimate of about 120 infected persons (Table 1). In Boffa and Dubreka, these values were extrapolated from medical survey results (Camara *et al.*, 2005) taking into account evaluated population at risk (Table 1), and lead to estimates of 187 and 295 infected persons, in Dubreka and Boffa respectively.

We studied seven microsatellite loci: *M6c8*, *Mt3033* (Biteau *et al.*, 2000), *Micbg1*, *Micbg5*, *Micbg6*, *Misatg4* and *Misatg9* (Koffi *et al.*, 2007). Complete genotypes and multilocus genotypes (MLG) are given in (Koffi *et al.*, 2009). Because *Micbg6* did not vary across all samples (all individuals displayed the same genotype), this locus was removed from the data set in further analyses, except when specified.

Data analysis

The most widely used parameters to infer population structure are the *F*-statistics (Wright, 1951; e.g., (Nagylaki, 1998)). Typically, these parameters are defined for three hierarchical levels. F_{IS} measures the identity (or homozygosity) of alleles within individuals within sub-populations relative to that measured between individuals; it is thus a measure of deviation from local panmixia (random union of gametes producing zygotes). It varies between -1 (single class of heterozygote) as expected in a very small and isolated clonal population (De Meeûs, Balloux, 2005) and +1 (all individuals are homozygous for different alleles), as expected in fully selfing species; and it equals 0 in panmictic populations. F_{ST} measures the identity between individuals within sub-populations, as compared to individuals from other sub-populations within the total population, or the total relative homozygosity due to the Wahlund effect (Wahlund, 1928). It is thus a convenient measure of differentiation between sub-populations that varies between 0 (no structure) and 1 (all populations fixed for one or other allele). These *F*-statistics were estimated by Weir and Cockerham's unbiased estimators (Weir, Cockerham, 1984), with FSTAT version 2.9.3.2 ((Goudet, 2001), updated from (Goudet, 1995)), and their significant deviation from 0 was tested by randomizing alleles between individuals within subsamples and randomizing individuals among sub-samples. Randomizations were set to 10,000 and implemented by FSTAT 2.9.3.2.

To get an encompassing picture of genotypic distribution across space, time, and subspecies, a NJTREE was computed by the MEGA 3.1 software (Kumar *et al.* 2005, updated from (Kumar *et al.*, 2004)). As recommended (e.g., (Takezaki, Nei, 1996; De Meeûs *et al.*, 2007)), the tree was built according to a Cavalli-Sforza and Edwards chord distance

matrix (Cavalli-Sforza, Edwards, 1967) computed with Genetix 4.05 (Belkhir *et al.*, 2004).

In purely clonal populations, a negative F_{IS} , homogeneous across loci, is expected, while a substantial variance of F_{IS} across loci can be the sign of rare sexual events (De Meeûs *et al.*, 2006). Nevertheless, in clonal organisms F_{IS} is also dependent on mutation rate (the higher mutation rate the higher F_{IS}) (De Meeûs *et al.*, 2006). Consequently, if mutation rate varies from one locus to the other, so will the F_{IS} . Mutation rate of trypanosome microsatellites is unknown but a direct positive relationship is expected between mutation rate and genetic diversity, hence between F_{IS} and, for instance, Nei's unbiased estimate of genetic diversity H_s (Nei, Chesser, 1983). We tested this with a linear regression under R software (R-Development-core-team, 2008).

Inferring clonal sub-population size and migration rate

We used the model developed by Balloux *et al.* (Balloux *et al.*, 2003). Consider a subdivided monoecious population of diploid individuals, like *T. brucei gambiense* are (Koffi *et al.*, 2009), with non-overlapping generations. Individuals reproduce clonally with probability c and sexually with probability $(1-c)$. Self-fertilization occurs at a rate s . There are n sub-populations, or demes, each composed of N individuals. Migration between the subpopulations follows an island model (Wright, 1951), with a migration rate m . The mutation rate is u for all alleles and therefore the probability that two alleles, identical by descent before mutation, are still identical after mutation is $\gamma=(1-u)^2$. We further assume stable census sizes and no selection and a two-population framework, which we assume being the case in both Côte d'Ivoire and Guinea, with total clonality.

Three probabilities of identity by descent can be defined: Q_i , the probability that two alleles drawn at random from a single individual are identical by descent; Q_s , the probability that two randomly sampled alleles from two different individuals within a subpopulation are identical by descent; and Q_T , the probability that two randomly sampled alleles from two individuals in different subpopulations are identical by descent.

The recurrence equations between generations t and $t+1$ for the different identities by descent are:

$$\begin{cases} Q_{i(t+1)} = \gamma \left\{ cQ_{i(t)} + (1-c) \left[s \left(\frac{1+Q_{i(t)}}{2} \right) + (1-s)Q_{s(t)} \right] \right\} \\ Q_{s(t+1)} = \gamma \left\{ q_s \left[\frac{1}{N} \left(\frac{1+Q_{i(t)}}{2} \right) + \left(1 - \frac{1}{N} \right) Q_{s(t)} \right] + (1-q_s)Q_{T(t)} \right\} \\ Q_{T(t+1)} = \gamma \left\{ q_d \left[\frac{1}{N} \left(\frac{1+Q_{i(t)}}{2} \right) + \left(1 - \frac{1}{N} \right) Q_{s(t)} \right] + (1-q_d)Q_{T(t)} \right\} \end{cases}$$

with

$$\begin{cases} q_s \cong (1-m)^2 + \frac{m^2}{n-1} \\ q_d = \frac{1-q_s}{n-1} \end{cases}$$

and where q_s is the probability that two individuals taken at random within the same sub-population after migration were born in the same subpopulation and q_d the probability that two individuals sampled after migration in different sub-populations originated from the same subpopulation (Wang, 1997).

Wright's F -statistics, can be defined following Cockerham (Cockerham, 1969; Cockerham, 1973) as:

$$\begin{cases} F_{IS} = \frac{Q_i - Q_s}{1 - Q_s} \\ F_{ST} = \frac{Q_s - Q_T}{1 - Q_T} \\ F_{IT} = \frac{Q_i - Q_T}{1 - Q_T} \end{cases}$$

Assuming no selfing (i.e. $s=1/N$), the systems of equations (1), (2) and (3) lead to:

$$\left\{ \begin{array}{l} F_{IS} = \frac{\gamma\{q_s - c[\gamma(q_s - q_d) - 1] - 1\}}{2N(1 - c\gamma)[\gamma(q_s - q_d) - 1] - \gamma\{q_s - c[\gamma(q_s - q_d) - 1] - 1\}} \\ F_{ST} = \frac{\gamma(1 - c\gamma)(q_s - q_d)}{[2N(1 - c\gamma) - \gamma(1 - c)][1 - \gamma(q_s - q_d)] + \gamma\{q_d[\gamma(c + 1) - 1] - q_s[\gamma(c + 1) - 2]\}} \end{array} \right.$$

In Côte d'Ivoire there are two foci, and thus two putative subpopulations, Bonon and Sinfra, as is the case for Guinea (Dubreka and Boffa) (Koffi *et al.*, 2009). In a two sub-populations framework with total clonality ($n=2$, $c=1$), as it is probably the case in the two areas investigated in the present study, we get:

$$\left\{ \begin{array}{l} q_s = (1 - m)^2 + m^2 = 1 - 2m(1 - m) \\ q_d = 1 - (1 - m)^2 - m^2 = 2m(1 - m) = 1 - q_s \end{array} \right.$$

and combining equations (4) and (5):

$$\left\{ \begin{array}{l} F_{IS} = \frac{\gamma\{q_s - \gamma(2q_s - 1)\}}{2N(1 - \gamma)[\gamma(2q_s - 1) - 1] - \gamma\{q_s - \gamma(2q_s - 1)\}} \\ F_{ST} = \frac{\gamma(1 - \gamma)(2q_s - 1)}{[2N(1 - \gamma)][1 - \gamma(2q_s - 1)] + \gamma\{(1 - q_s)[2\gamma - 1] + 2q_s[1 - \gamma]\}} \end{array} \right.$$

After neglecting terms in u^2 and u ($\ll 1$ or q_s) and simplifications, these equations can be rearranged into:

$$\left\{ \begin{array}{l} F_{IS} = -\frac{1 - q_s}{(1 - q_s)(1 + 8Nu)} \\ F_{ST} = \frac{2u(2q_s - 1)}{(1 - q_s)(8Nu + 1)} \end{array} \right.$$

From equation (7) it is easy to see that when $q_s \neq 1$ (i.e., m is in]0,1[) F_{IS} becomes independent from migration and can provide an estimate for N in the simple form:

$$N = -\frac{1 + F_{IS}}{8uF_{IS}}$$

If we combine (7) and (8) we can also obtain an estimate for q_s :

$$q_s = \frac{1 + F_{ST} \frac{8Nu + 1}{2u}}{2 + F_{ST} \frac{8Nu + 1}{2u}}$$

Because we are in a two populations case, the genetic effect of migration is symmetric around 0.5 ($m=0.49$ is equivalent to $m=0.51$). We can thus focus on values below 0.5 for m . From there it is easy to see from (5) that:

$$m = \frac{1}{2} \left[1 - \sqrt{2q_s - 1} \right]$$

and thus combining (9) and (10) gives us access to m as:

$$m = \frac{1}{2} \left[1 - \sqrt{\frac{F_{ST} \frac{8Nu + 1}{2u}}{2 + F_{ST} \frac{8Nu + 1}{2u}}} \right]$$

that can be finally combined with (8) to obtain:

$$m = \frac{1}{2} \left[1 - \sqrt{\frac{F_{ST}}{F_{ST} - 4uF_{IS}}} \right]$$

Inferring clonal effective population size

Temporal samples offer the opportunity to estimate effective population sizes (N_e , the size of panmictic adults required to drift at the same rate as the observed population) with the method developed by Waples (Waples, 1989) and implemented in NeEstimator v 1.3 (Peel *et al.*, 2004). For this purpose, we only used the multilocus genotypes data (MLG), which we rendered diploid by duplication of the allele of the single artificial locus obtained. MLG's were chosen because in clonal organisms all loci are linked and heterozygosity excess affects differentiation estimates (De Meeûs *et al.*, 2006). In order to estimate the number of trypanosome generations passed within the time windows (two and four years), we used two drastically different methods. The first method assumes that populations are mainly defined as the infra-populations of cells contained in each individual host. In that case, generation time must be close to the time between two cell divisions. T .

brucei cells divide every 5.7 hours (Salmon *et al.*, 2005), which yields 4.2 generations per day and thus 1537 per year. The second method assumes that each host is colonized by a limited number of strains (~1) and that the generation time corresponds more to the time it takes for a human individual newly infected by a trypanosome after a tsetse bite to become infectious for a new tsetse, and for this second tsetse to become infectious to a human individual again. Incubation in human hosts lasts on average 25 days (Gouteux, Artzrouni, 2000), while between 12 and 24 days are required for a newly infected tsetse fly to become infectious for a vertebrate host (Dale *et al.*, 1995; Van den Abbeele *et al.*, 1999). This gives a generation time window of 37 to 49 days for trypanosomes, leading to 7-10 generations per year.

Results

Heterozygosity within sub-samples

Nearly all stocks were heterozygous at each microsatellite locus. Mean $F_{IS} = -0.62$, the relative heterozygosity measured within subsamples, is strongly negative (strong heterozygote excess as compared to Hardy-Weinberg expectations) with small variance across loci that is almost entirely explained by the genetic diversity maintained at each locus and hence by mutation rates (Figure 2), meaning that individuals are extremely heterozygous at all loci (genome wide heterozygous state) and populations are totally clonal.

In Table 1 it can be seen that no positive relationship exists regarding the surface of the investigated geographical areas, prevalence of infection, or number of infected persons. Moreover, GPS data for Bonon 2000 and 2002 can be used to build groups of trypanosomes from infected patients from different sub-areas in each zone. The F_{IS} computed for the six loci is extremely close (and indeed higher) to the one computed without GPS coordinates so that Wahlund effects can safely be disregarded (Koffi *et al.*, 2009).

Genetic differentiation

In Figure 3 it can be seen that trypanosome strains first differentiate between countries, then between sites (in Guinea), between temporal samples (apparently more pronounced in Dubreka, Guinea, than in Bonon, Côte d'Ivoire), and that the sampling method does not have any impact.

Population sizes.

From the F_{IS} analysis, according to De Meeûs *et al.* criteria (De Meeûs *et al.*, 2006), full clonality can be assumed for *T. brucei gambiense* group 1 for the studied populations. According to (Hellegren, 2000), microsatellite mutation rates mostly range between 10^{-3} and 10^{-4} . We use these two values for estimating clonal effective population sizes with equation (8) of Material and Methods. The results are presented in Figure 4.

If we assume that generation time corresponds to cell divisions, Waples moment-based method (Waples, 1989) gives huge estimates ($N_e \approx 12,000$ -30,000 cells). During the surveys, it was observed that the expected number of trypanosome cells would approximately range from 7,000,000 in Guinea to 400,000,000 in Bonon. With such values a glance at equation (7) leads to an expected $F_{IS} \sim 0$, which is far from being the case. Thus the cell is not the individual unit and the individual host does not correspond to a demographic unit (population) for trypanosomes. Figure 4 also presents the results obtained with the trypanosome life-cycle-based method for generation time (37 to 49 days, see Material and Methods). For temporal MLG-based estimates, the values obtained are probably much smaller than the "real" N_e , as indicated by the extremely high upper bounds of the 95% confidence intervals, so that the F_{IS} -based method is probably more accurate, as suggested in general from theoretical analyses of fully clonal populations (De Meeûs, Balloux, 2005).

From Figure 4, the estimated numbers of infected patients seem to almost perfectly match all N_e estimates. This is unexpected if infected patients are to reflect *T. brucei gambiense* group 1 census sizes, which should be at least slightly over N_e .

With $u=10^{-4}$, F_{IS} -based N_e reaches 297, 760 and 1479 for Boffa, Bonon and Dubréka respectively. These values match well other estimates in Boffa, but clearly surpass the observed number of infected patients in Bonon and Dubreka, for which a reasonable match is reached with $u=10^{-3}$.

Discussion

According to the De Meeûs *et al.* criterion (De Meeûs *et al.*, 2006), if some sex occurred, even very rarely, a higher F_{IS} with a much stronger variance of F_{IS} across loci would have been observed. We must conclude that the populations studied never sexually recombined in a reasonable length of time. Our results also indicate that within each country, *T. b. gambiense* group 1 populations are relatively small and do

not exchange many migrants. For instance, in Guinea, where two sites were sampled in 2002, with equations (8) and (12) from our model (Material and Methods) and a reasonable mutation rate of $u=10^{-4}$, the effective clonal population size and migration rate respectively are $N_{ec}=297$ and $m=0.001$ in Boffa and $N_{ec}=1479$ and $m=0.0008$ in Dubreka. Obviously, migration is weak.

At the scale of West Africa (between Côte d'Ivoire and Guinea), our results show that any strain transfer between the two countries is too rare to leave any signature in the investigated microsatellite polymorphism (no MLG in common).

The effective population size results support a complete parasitic-cycle-based generation time and reject a cell-based generation time. Population regulation thus occurs at the scale of a focus. This means that even if multiple infection of the same patient is not rare, at the scale of the focus, it has no demographic influence; probably because the vector will itself always harbors a single genotype (trypanosome individual). This is in line with the absence of recombination found in this study and suggests that tsetse flies represent the main regulatory factor of *T. brucei gambiense* 1. Interestingly enough, the effective population size and migration rate computed for *Glossina palpalis gambiensis* in Dubreka (Solano *et al.*, 2009) was around $N_e \sim 1000$ and $m \sim 0.005$ (recomputed here) and are very close to what is found for the parasite assuming a mutation rate of 10^{-4} . As discussed in (Koffi *et al.*, 2009), the clonal population sizes found here strongly support the existence of hidden hosts (animal reservoirs or asymptomatic infected humans) (Jamonneau *et al.*, 2004). These hidden hosts may represent a potential parasite reservoir that could be responsible for the persistence of transmission and re-emergence of sleeping sickness. It would be of interest to sample both healthy humans and animals living next to the HAT cases and identify with microsatellite loci the trypanosomes they may harbor.

Control of the disease at a country scale would probably be efficient in the long term before new strains reinvade the area. Nevertheless, our data also reveal a high degree of local genetic polymorphism, either because of larger population sizes than epidemiological surveys can account for, or because of high mutation rates, which suggests that *T. b. gambiense* may quickly respond to new selective pressures, such as the one imposed by chemical treatment with a new drug.

Finally, the strong heterogeneity found in other sub-species raises a doubt on the unity of each of these taxa. This is particularly

interesting for *T. brucei brucei* that is currently assumed to experience frequent recombination, hidden by an epidemic population structure (MacLeod *et al.*, 2000). But mixing different taxa could also lead to much more confusion (Prugnolle, De Meeûs, 2009) so this issue will require further investigations like differentiation tests as a function of host species.

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Table 1: Data from epidemiological surveys of the investigated areas and estimated F_{IS} obtained with the six most reliable loci in the different *T. brucei gambiense* sub-samples. F_{IS} is a standardized measure of heterozygosity deviation, expected null if reproduction is sexual and random, its value is influenced by reproductive mode and/or undetected subdivision within subsamples. Prevalence is the ratio between the number of infected persons (Infected) to the number of persons examined.

Sub-sample	Surface of study (in km²)	Human population	Prevalence	Infected	F_{IS}
Bonon 2000	400	30000	0.004	120	-0.671
Bonon 2002	400	30000	0.004	120	-0.645
Bonon 2004	400	30000	0.004	120	-0.555
Dubreka 1998	1600	25000	0.0075	187	-0.440
Dubreka 2002	1600	25000	0.0075	187	-0.505
Boffa 2002	2400	25000	0.0118	295	-0.808

Figure Legends

Figure 1: Localization of sampling areas (stars). (Drawing by Fabrice Courtin)

Figure 2: Regrassion of F_{IS} per locus against their genetic diversity H_s . For this analysis only the values obtained for locus *Micbg6* ($F_{IS}=-1$, $H_s=0.5$) were used.

Figure 3: Rooted NJTREE of the different isolates combined with reference strains available from the Supplementary Table 2 of (Koffi *et al.*, 2006a) article where the complete information and origins, year and publication references can be found in their Table 3. The tree is based on Cavali-Sforza and Edwards' chord distances computed on the eight loci described in Koffi *et al* ige. *T. brucei gambiense* 1 reference strains are in purple and all included in one cluster within the sub-tree comprising all strains studied in the present paper (in black). *T. brucei gambiense* 2 reference strains are in red, *T. brucei rhodesiense* in blue and *T. brucei brucei* in green. This particular tree was rooted with strain Feo of *T. brucei brucei* but any other strain of this sub-species or

of *T. brucei rhodesiense* equally illustrates the monophyly of *T. brucei gambiense* 1 and polyphyly of all other *Trypanosoma brucei* types. Bootstrap values of principal nodes (above 750 %) are given (obtained with Phylip 3.68, J. Felsenstein 2008)

Figure 4: Effective population size (N_e) obtained with the F_{IS} -based method (see Material and Methods Equation 1) (“model”), with $u=10^{-3}$ and $u=10^{-4}$, and with Waples' method from temporally spaced samples (with MLG as a single locus), using trypanosome's life cycle as the generation time with the shortest (sgt=37 days) or largest (lgt=49 days) generation times (see text). Black squares are the means with 95% confidence intervals (CI) (small lines) (averaged over 2000-2002, 2000-2004 and 2002-2004 for Bonon). The dotted line corresponds to the estimated number of infected persons in the different areas according to epidemiological surveys. For Waples' method, CI come from a chi-square distribution with a degrees of freedom (a is the number of alleles, here of different MLG's) (Waples, 1989). For the F_{IS} -based method, confidence intervals correspond to those of F_{IS} obtained by bootstrap over loci.

Figure 1



Figure 2

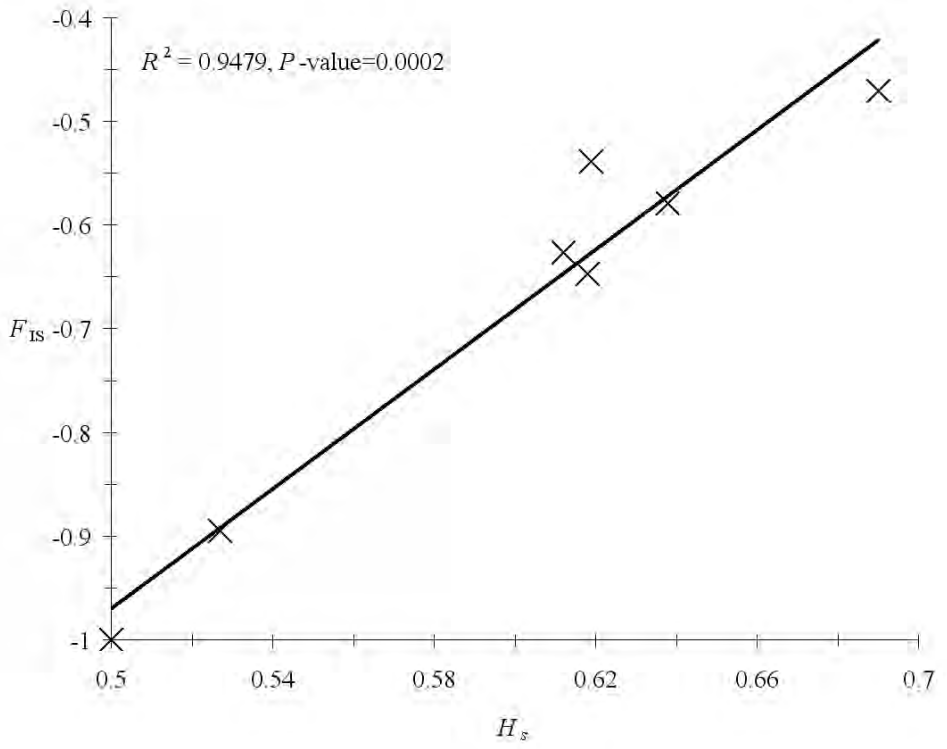


Figure 3

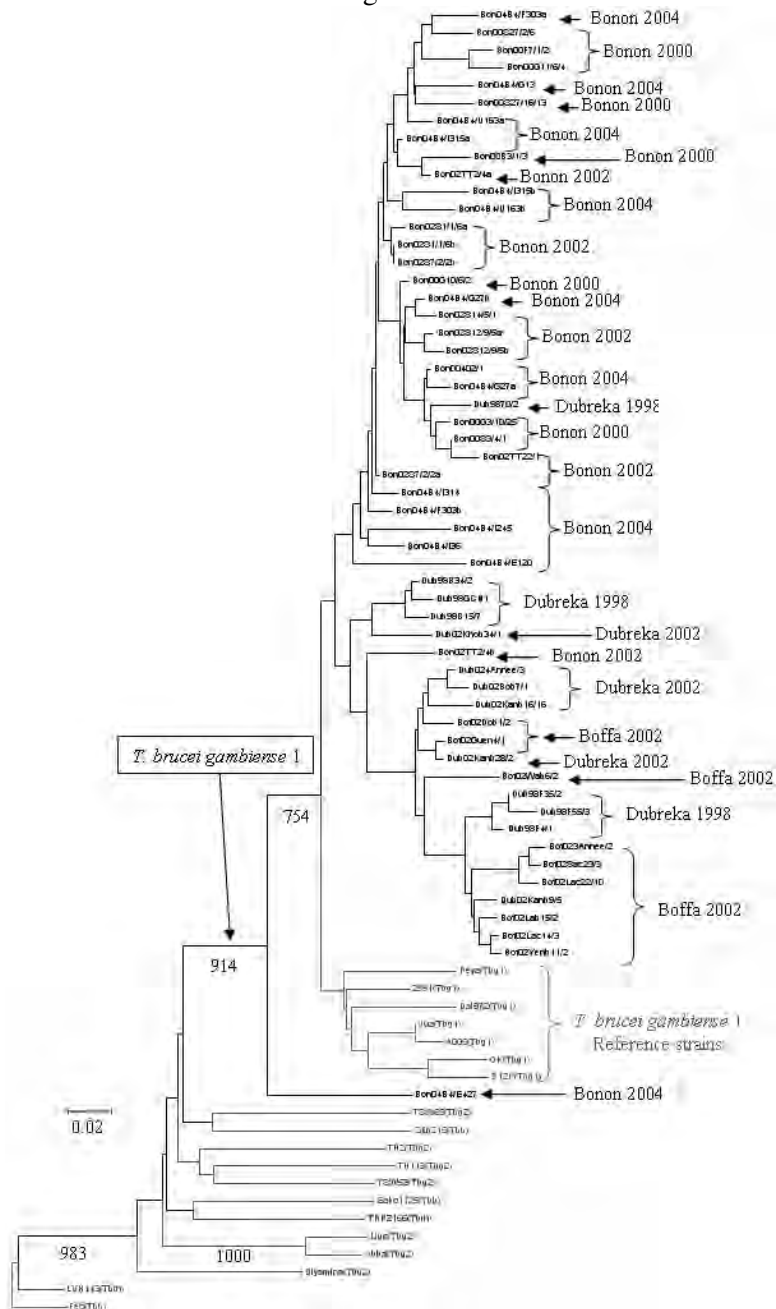
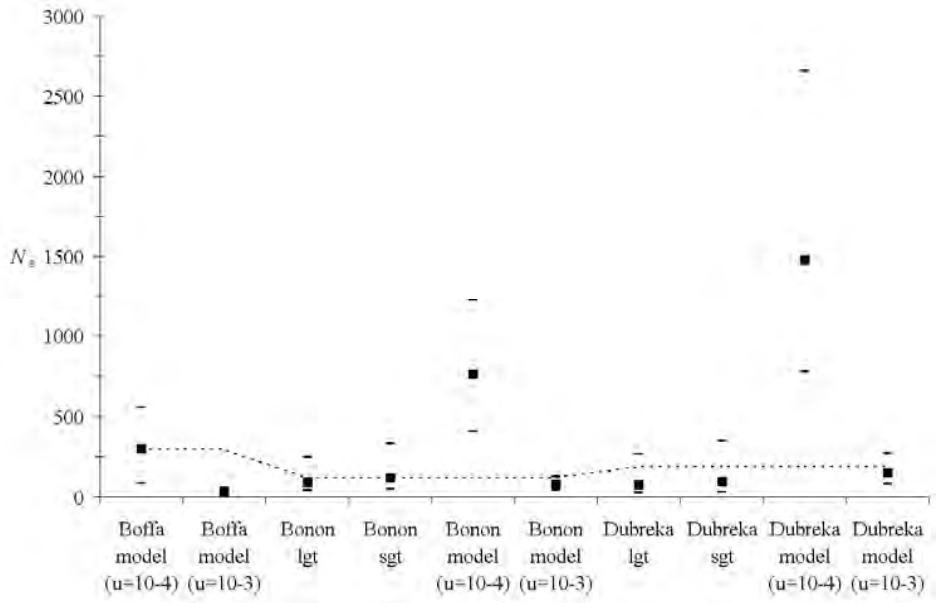


Figure 4



HOW TO SHORTEN PATIENT FOLLOW-UP AFTER TREATMENT FOR T.B. GAMBIENSE SLEEPING SICKNESS?/

COMMENT REDUIRE LA PERIODE DE SUIVI APRES TRAITEMENT DES PATIENTS SOIGNES POUR LA TRYPANOSOMIASE HUMAINE AFRICAINE A T.B. GAMBIENSE?

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Résumé

Un problème majeur dans la prise en charge des patients souffrant de la Trypanosomiase Humaine Africaine (THA) à *T.b. gambiense* est celui de

la nécessité d'une période de suivi après traitement de 2 ans, avec les examens de contrôle à 3,6,12,18 et 24 mois. Cette étude a été menée dans le but de raccourcir la durée du suivi des patients trypanosés en proposant de nouveaux critères applicables dans les conditions de terrain. Une étude longitudinale a été conduite pour évaluer plusieurs marqueurs biologiques du liquide céphalorachidien (la cytorrachie, le taux d'IgM par LATEX/ IgM, les anticorps spécifiques anti-trypanosomes) nécessaires pour le suivi des patients. Une cohorte des patients a été recrutée, traitée et suivie pendant 36 mois. Le taux de rechute a été de 37 %. La cytorrachie a été le marqueur biologique le plus précoce et précis pour distinguer les patients guéris de patients rechutés. Par conséquent, nous avons combiné les critères de guérison et de rechute basés sur 2 marqueurs: la présence des trypanosomes et la cytorrachie. Quatre algorithmes ont été évalués pour raccourcir la durée du suivi. L'algorithme procédant au test de guérison à 12 mois semblait être la mieux appropriée pour les programmes de lutte contre la THA et les études cliniques.

Summary

A major problem in clinical management of *Trypanosoma brucei.gambiense* sleeping sickness is the need for a patient follow up period of two years with follow up (FU) visits at 3, 6, 12, 18 and 24 months post-treatment. This study was undertaken to investigate if and how FU of HAT patients could be shortened by applying improved treatment outcome criteria suitable for use under field conditions. A longitudinal study was carried out to evaluate several biological markers in the cerebrospinal fluid (WBC count, Latex/IgM end titer and trypanosome specific antibodies) for monitoring clinical outcomes in HAT treatment. A cohort of 360 *gambiense* Human African Trypanosomiasis (HAT) patients was enrolled, treated and followed for up to 36 months. The overall relapse rate was 37 %. The CSF WBC count was the earliest and most accurate marker that allowed distinguishing cures from relapses. We therefore combined criteria for relapse and cure based on 2 markers: presence of trypanosome and CSF WBC count. Four algorithms were then evaluated for shortening the duration of FU. The algorithm using a test of cure (ToC) at 12 months seemed the most appropriate for HAT control and clinical trials.

Introduction

Human African Trypanosomiasis (HAT) is endemic in sub-Saharan Africa. In 2006, 11,382 cases were reported, including 8,023 from the D.R. Congo (DRC). *Trypanosoma brucei gambiense* causes chronic HAT in West and Central Africa. Treatment is stage-dependent and untreated HAT is fatal.

Clinical management of sleeping sickness requires a two-year patient follow-up (FU) with visits at 3, 6, 12, 18 and 24 months post-treatment. At each FU visit, lymph, blood and cerebrospinal fluid (CSF) is examined for trypanosomes and CSF white blood cells (WBC) counted. However, compliance of HAT patients with scheduled FU drops with time after treatment.

This study investigates if and how FU of HAT patients can be shortened by applying novel biological criteria under field conditions. We describe treatment outcomes in a prospectively recruited cohort of patients, evaluate risk factors and biomarkers for treatment failure and validate criteria for shorter follow-up periods.

Methods

Study design

Longitudinal study was conducted to evaluate biomarkers for monitoring clinical outcomes in HAT treatment.

Study site The study was conducted in Dipumba hospital, Mbuji Mayi, East Kasai Province, DRC.

Patients

Patients were recruited between May 2005 and May 2008.

Inclusion criteria were:

- Trypanosomes in lymph, blood or CSF, regardless of disease stage;
- Age 12 years old and above;
- Living within a 100 km perimeter around Mbuji-Mayi.

Exclusion criteria were:

- Pregnancy;
- No guarantee for follow-up;

- Moribund;
- Haemorrhagic CSF;
- Concurrent serious illness (tuberculosis, bacterial or cryptococcal meningitis).

Patients never treated before for HAT (i.e. treatment naïve) were classified as "primary cases" and those who presented with a relapse at enrolment as "retreatment cases". Informed consent of patients or their guardian was asked prior to enrolment.

Diagnosis

Each patient underwent a clinical examination. CATT positive individuals and those showing clinical signs suggesting HAT were examined for trypanosomes in lymph node aspirate or in blood via capillary tube centrifugation or mini-anion exchange centrifugation technique. In CSF, WBC count was done using disposable counting chambers (Uriglass, Menarini) and trypanosomes were searched for by modified single centrifugation (MSC). Retreatment cases were enrolled on the basis of trypanosome positive CSF. Trypanosome specific antibodies and total IgM in CSF were measured with LATEX/*Tb gambiense* and LATEX/IgM. All tests were repeated 24 h after the last drug administration and at each FU assessment. HIV status was determined with the Vironostika HIV Uni-Form II Ag/Ab (bioMérieux), followed by INNO-LIA HIV I/II Score (Innogenetics) if positive. Indeterminate specimens were tested with INNOTEST HIV Antigen mAb (Innogenetics). HIV PCR on peripheral blood mononuclear cells was performed to confirm HIV infection.

Treatment

Patients got free treatment and food during hospitalisation. Pretreatment consisted of mebendazole (100 mg, twice a day, for 3 days) and a single dose of combination sulfadoxine 500 mg - pyrimethamine 25 mg (1 tablet/20 kg body weight). HAT treatment was according to PNLTHA guidelines.

Patient monitoring after treatment

Treatment outcome was assessed 1 day (end of treatment [EoT]) and 3, 6, 12, 18 and 24 months post-treatment. During FU, patients were

classified according to WHO recommendations as death, relapse, probable relapse, favourable evolution, uncertain evolution with the following adaptations. Probable relapse: no trypanosomes but CSF WBC count increased with >30 WBC/ μ l compared to the lowest previous count or neurological signs not due to another condition than HAT and requiring rescue treatment in the opinion of the physician in charge. Favourable evolution: for stage 1: ≤ 5 WBC/ μ l and no trypanosomes; for stage 2: no trypanosomes and ≤ 20 WBC/ μ l or no trypanosomes and WBC not increased with >10 WBC/ μ l compared to the lowest previous value and without clinical deterioration. Uncertain evolution: no trypanosomes and not falling in any other category. Test of cure (ToC) assessment was made 24 months post-treatment or later.

Data analysis

Tolerance windows of 2-4, 5-9, 10-16, 17-21 and ≥ 22 months were defined around the scheduled FU at 3, 6, 12, 18 and 24 months. Patients reaching a final treatment outcome (non responder, relapse, probable relapse, death) prior to the 24 months ToC were excluded from data analysis at subsequent time points.

Several algorithms for detecting relapse with shorter FU period were constructed and evaluated on the cohort data, as well as on another dataset obtained in a clinical trial conducted between 1998 and 2001 in Bwamanda, DRC.

Results

Study population

Between May 2005 and February 2006, the Dipumba hospital admitted 384 eligible HAT patients and 360 patients were enrolled.

Treatment outcomes

The patients were treated as follows: 41 received pentamidine; 207 started melarsoprol monotherapy but 8 developing intolerance to melarsoprol before day 8 were switched to eflornithine; 63 received melarsoprol-nifurtimox combination but 6 were switched to eflornithine as nifurtimox ran out; 1 received melarsoprol-eflornithine combination; 1 received nifurtimox-eflornithine combination. Altogether, 61 patients received eflornithine monotherapy.

Compliance with follow-up was respectively 94.4% (323/342) at 3 months, 91.9% (262/285) at 6 months, 83.6% (194/232) at 12 months, 71.6% (151/211) at 18 months and 84.2% (171/203) at 24 months and was not significantly different between treatment groups.

Overall cure rate was 48.6 % (175/360) and case fatality rate was 10.3% (37/360).

The proportion of treatment failures during FU was 37% (133/360): 95 relapses, 32 probable relapses, 6 deaths due to HAT. The highest failure rate, 59% (117/199), was observed in the melarsoprol group and the lowest with eflornithine containing regimens (0–5%). There were 1 relapse and 3 probable relapses in the pentamidine group, (10% (4/41)). Thirty eight percent (51/133) of all recurrences were detected within 3 months, 78% (104/133) within 6 months, 92% (122/133) within 12 months and 97% (129/133) within 18 months.

Baseline characteristics as risk factors for relapse

Adjusting odd ratios for drug regimen, baseline CSF WBC count ≥ 100 WBC/ μ l ($p=0.008$), presence of trypanosomes in CSF ($p=0.005$) at baseline and baseline LATEX/IgM titer $\geq 1:16$ ($p<0.001$) were significantly associated with higher relapse rates. Gender, age, trypanosome specific antibodies (LATEX/ *T.b.gambiense*), early treatment termination and HIV status were not. In the final logistic model, only drug regimen and latex IgM were independent predictors of relapse.

Post-treatment evolution of CSF parameters

In cured patients, median CSF WBC count dropped from 213 WBC/ μ l to 36 WBC/ μ l at EoT and steadily decreased during FU, while in relapsed patients it dropped from 259 WBC/ μ l to 59 WBC/ μ l at EoT, rose to 101 WBC/ μ l at 6 months and decreased subsequently to 37 WBC/ μ l at 24 months. Median WBC counts were always higher than in cured patients. In cured patients median LATEX/IgM titers were 1:64 before treatment and dropped to 1:2 at 18 and 24 months, while in relapses they decreased until 3 months, stabilized at 1:16 between 3 and 12 months and decreased further thereafter. The proportion of patients with positive LATEX/*T.b.gambiense* decreased from 82% pre-treatment to 14% at 24 months in cured patients, in relapses it dropped from 80% pre-treatment to 52% at 3 months and rose to 56-68% between 6 and 18 months.

Accuracy of criteria for relapse detection

For CSF WBC count the AUC was >0.80 from 3 to 24 months. The Youden index was highest at 12 and 18 months with 87-89% sensitivity and 100% specificity. A cut-off of >20 WBC/ μ l had similar high sensitivities and specificities than cut-offs of 23 and 29 WBC/ μ l at 12 and 18 months respectively. A cut-off of >5 WBC/ μ l was 89-96 % sensitive and a cut-off of ≥ 50 WBC/ μ l was 95-100% specific.

For CSF LATEX/IgM titer the AUC was >0.80 at 12 months only (0.84, CI 0.72-0.96); with a cut-off of $\geq 1:16$, sensitivity was 69% and specificity 97%.

For trypanosome specific CSF antibodies the maximum Youden index was 0.43 at 12 months with 65% sensitivity and 78% specificity.

Evaluation of algorithms for shortening FU duration

Based on the above, we constructed several algorithms with variable duration of FU time and using a composite definition to discriminate between cure and relapse at each FU visit. Under this definition, patients with ≤ 5 WBC/ μ l at any FU visit are considered 'cured' and do not need further FU, while those ≥ 50 WBC/ μ l are considered 'relapsed' and should receive rescue treatment. Trypanosome negative patients with 6-49 WBC/ μ l are considered as "uncertain evolution" and should continue FU. At the final ToC assessment, those with presence of trypanosomes or > 20 WBC/ μ l are considered as 'relapse'. Combining these FU and ToC criteria, 4 algorithms were formulated and their accuracy was checked against our study data (Figure 1).

In algorithm A the FU lasts for 24 months. Of second stage patients with known treatment outcome, 85 are considered cured at 6 months, 40 at 12 months, 7 at 18 months and 10 at 24 months (142, including 4 wrongly); 56 patients are considered relapsed at 6 months, 10 at 12 months and 6 at 18 months (72, including 2 wrongly). Specificity over 24 months is 98.6% (138/140, CI 95-100%) and sensitivity 94.6% (70/74, CI 87-99).

In algorithm B the FU lasts for 18 months. At 6 months 85 patients are considered cured, at 12 months 40 and at 18 months 9 (134, including 4 wrongly); 56 patients are considered relapsed at 6 months, 10 at 12 months and 6 at 18 months (72, including 2 wrongly). Specificity is 98.5% (130/132, CI 95-100%) and sensitivity 94.6% (70/74, CI 87-99).

In algorithm C the FU lasts for 12 months; 85 patients are considered cured at 6 months and 50 at 12 months (135, including 4 wrongly); 56 patients are considered relapsed at 6 months and 15 at 12 months (71, including 3 wrongly). Specificity over 12 months is 97.8% (131/134, CI 94-100%) and sensitivity 94.4% (68/72, CI 86-98).

In algorithm D the ToC criterion lasts for 6 months; 136 patients are considered cured (including 13 wrongly); 77 patients are considered relapsed (including 13 wrongly). Specificity is 90.4% (123/136, CI 84-95) and sensitivity 83% (64/77, CI 73-91).

Applying these algorithms on a dataset of second and intermediate stage patients from another clinical trial, the specificities were identical whereas sensitivities were lower than those of this study for algorithms B [specificity 98.9% (173/175, CI: 96-100%), sensitivity 84.2 (32/38, CI: 69-94%)] and C [specificity 98.8% (167/169, CI: 96-100%), sensitivity 79.4% (27/34, CI: 62-91%)]. For algorithm A, sensitivity was only 85.4% (35/41, CI: 71-94%). It appears there is a risk to miss relapses with algorithms B and C but waiting for 24 months is not better. We recommend that patients classified as cured should be instructed to come back if they feel sick.

Discussion

This study was conducted to assess how the WHO-recommended 24 months post-treatment FU of HAT patients could be shortened using field applicable criteria. We investigated for the first time, the usefulness for FU of a combination of criteria for relapse and cure based on 2 markers, trypanosome presence and CSF WBC count, distinguishing 3 groups. Group I: patients with ≤ 5 WBC/ μ l from 6 months onwards and without trypanosomes are at low risk for relapse. Group II: patients with ≥ 50 WBC/ μ l and/or trypanosomes at any FU assessment are considered relapses. Group III: patients with 6-49 WBC/ μ l and without trypanosomes have “indeterminate status” and should attend the next scheduled FU assessment. For ToC, a cut-off of >20 WBC/ μ l was used, as in other studies. The usefulness of 4 different FU algorithms (combining one criterion for FU and one criterion for cure) with ToC at 6, 12, 18 or 24 months was checked against our study data.

Algorithm analysis was restricted to second stage patients. Applying algorithm B or C would result in a considerable reduction of the number of patients needing further FU (respectively beyond 18 and 12 months) and shorter FU duration. As their sensitivity was identical and the difference in specificity was not statistically significant ($p=1$), algorithm C with ToC at 12 months seems the most appropriate for HAT control and clinical trials.

Our study had the following limitations. Although several investigated markers had cut-offs based on studies in other foci and many of our observations were similar to those of other authors, our conclusions are

based on a cohort in Mbuji Mayi, where the relapse rate after melarsoprol monotherapy was unusually high. Nonetheless, when we validated our algorithms on a second cohort, quite similar results were obtained. We were constrained to restrict our algorithm analysis to second stage patients, as the number of first stage patients was low. Our analysis comprised only field applicable tests, while more sophisticated techniques (like PCR or IL-10 measurement) might allow reducing FU duration even further. The majority of our patients were treated with melarsoprol, which is increasingly being replaced by eflornithine or nifuritmox-eflornithine combination therapy. In conclusion, by using trypanosome detection and CSF WBC count as FU criterion at 6 months and by introducing "no trypanosomes and ≤ 20 WBC/ μ l CSF" as criterion for cure at 12 months, post-treatment FU for second stage *gambiense* HAT patients can be reduced from 24 months to 12 months.

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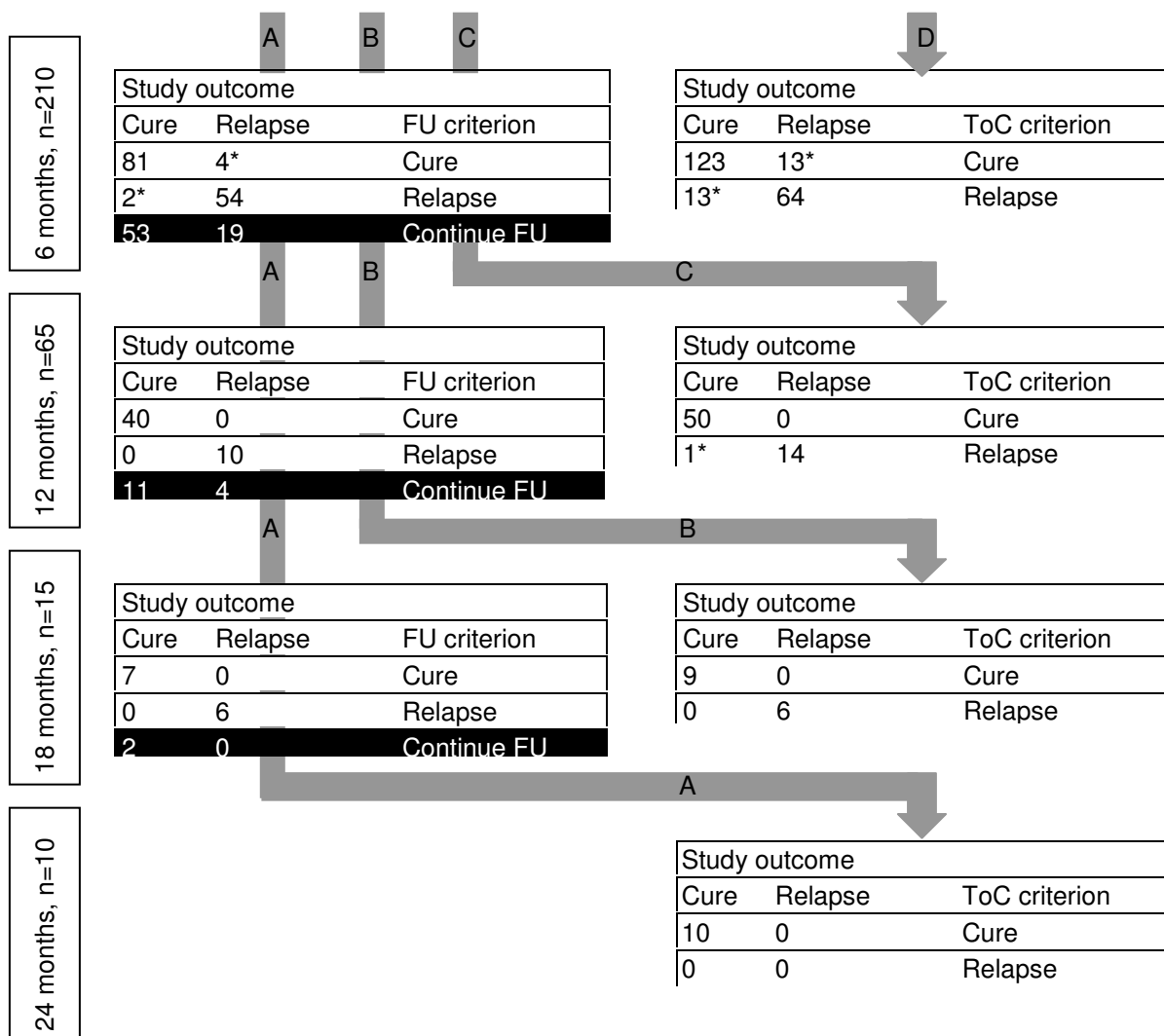


Figure 1: Impact on FU duration in enrolled second stage HAT patients of 4 algorithms (A, B, C, D) based on a combination of 2 different criteria for relapse and cure.

FU criterion: Cure = ≤ 5 WBC/ μ l CSF and no trypanosomes, relapse = ≥ 50 WBC/ μ l or trypanosomes, continue FU = 6-49 WBC/ μ l and no trypanosomes.

ToC criterion: Cure = ≤ 20 WBC/ μ l and no trypanosomes, relapse = > 20 WBC/ μ l or trypanosomes

* Wrongly classified (not corresponding with real treatment outcome).

FU: follow-up. ToC: test of cure.

CONTINENT-WIDE MAPPING OF HUMAN AFRICAN TRYPANOSOMIASIS/

CARTOGRAPHIE CONTINENTALE DE LA TRYPANOSOMIASE HUMAINE AFRICAINE

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Résumé

L'Atlas de la Trypanosomose Humaine Africaine (THA) est une initiative de l'Organisation Mondiale de la Santé (OMS), exécutée en collaboration avec la FAO dans le cadre du Programme de Lutte contre la Trypanosomose Africaine (PLTA). Les données épidémiologiques sont fournies par les programmes nationaux de lutte contre la trypanosomose (PNLTHA), les organisations non gouvernementales et les instituts de recherche. Les cas de THA sont localisés au niveau du village au moyen de GPS, de bases de données des endroits peuplés et de cartes papier et numériques. Les connaissances des techniciens qui travaillent sur le terrain contribuent aussi au processus de géoréférencement. L'utilisation de Systèmes d'Information Géographique (SIG) permet de cartographier la répartition de la maladie et l'intensité de transmission avec une résolution spatiale sans précédent. Des résultats préliminaires sont disponibles pour 17 pays endémiques, où, jusqu'à présent, environ trois quarts des cas reportés dans la période 2000-2008 ont été cartographiés. Les PNLTHA contribuent et participent

au développement des cartes, qui devraient devenir des outils importantes pour le support des activités de contrôle et de surveillance. Les résultats finaux et les données utilisées pour l'Atlas seront finalement rendus disponibles à travers les sites Internet de l'OMS et de la FAO/PLTA, et bénéficieront aux services sanitaires nationaux, aux scientifiques, aux communautés concernées et aux décideurs et donateurs. Les capacités des PNLTHA pour gérer les données seront renforcées pour permettre la mise à jour de l'Atlas au niveau national.

Summary

The Atlas of Human African Trypanosomiasis (HAT) is a WHO-led initiative executed in collaboration with FAO within the Programme against African Trypanosomiasis (PAAT). The Atlas aims at geo-referencing all cases of HAT reported in sub-Saharan Africa. Epidemiological data are provided by national sleeping sickness control programmes (NSSCP), non-governmental organizations and research institutes. HAT cases are geo-referenced at the village level by means of global positioning systems (GPS), databases of named locations, paper and digital maps. The experience of health workers in affected countries also contributes to the geo-referencing process. The use of Geographic Information Systems allows the depiction of the disease distribution and transmission intensity with unprecedented spatial accuracy. Preliminary outputs have been generated for 17 disease-endemic countries, where approximately three quarters of cases reported in the period 2000-2008 have been mapped to date. NSSCPs provide input and participate in the development of the maps, which are expected to become important tools to support control activities and disease monitoring and surveillance. The final outcomes as well as the input data used for the Atlas will eventually be made available in the public domain through WHO and FAO/PAAT websites for the benefit of national health services, scientists, concerned communities, policy makers and donors. The capacity of NSSCPs for data management will be strengthened to enable regular updates of the Atlas at the national level.

Introduction

The latest map of the distribution and extent of Human African Trypanosomiasis (HAT) transmission areas comprised over 250 active and historical foci. It was assembled in the 1990s for the World Health Organization (WHO), and it was generated from formal and grey

literature, as well as from expert opinion (WHO, 1998). Since then, the rapid diffusion of Geographic Information Systems (GIS) and Global Positioning Systems (GPS) has provided new tools for a novel and more accurate approach to sleeping sickness mapping. GPS enables rapid and accurate measurement of the geographic coordinates of endemic and screened villages, whilst GIS provides a framework for storing, analyzing and presenting georeferenced data, as well as for regular updating. As GIS software and GPS instruments became increasingly available, the need for more precise and standardized delineation of HAT endemic areas was recognized, and in 2005 the mapping of disease distribution was set by WHO as a priority in its regional strategy for the control of sleeping sickness (WHO, 2005). Following up on this resolution, in 2007 WHO launched the HAT Atlas initiative, jointly implemented with the Food and Agriculture Organization of the United Nations (FAO), in the framework of the Programme against African Trypanosomiasis (PAAT) (Cecchi *et al.*, 2009a). The Atlas aims at mapping all sleeping sickness cases reported from sub-Saharan Africa by using the vast amount of epidemiological data collated by WHO in recent years. The epidemiological maps that are being generated are expected to become important tools to support control activities and disease monitoring and surveillance. This paper summarizes the methodology for developing the Atlas and presents the progress status and future prospects for the WHO/FAO initiative.

Materials and methods

National Sleeping Sickness Control Programmes (NSSCPs), Non-Governmental Organizations (NGOs) and Research Institutes collect the epidemiological data upon which the Atlas of HAT is based. As shown in Figure 1, input data are provided to WHO in different formats, including epidemiological reports, spreadsheets and databases. Data from the field increasingly include geographic coordinates of the locations of epidemiological interest (i.e. towns, villages or other settlements where active screening is carried out, as well as the places of residence of passive cases detected by health care facilities). A central repository hosted by WHO and FAO/PAAT is used to store information provided from the field. Data processing follows, whereby selected epidemiological and geographical information is imported into a single database (DB). The epidemiological data imported in the DB include (i) the number of new HAT cases detected per year and per geographic location, (ii) parasite sub-species (either *Trypanosoma brucei gambiense*

or *T.b. rhodesiense*), and (iii) surveillance type (either active or passive). Disease stage (either haemolympathic or meningoencephalitic), number of people screened and number of people living in the screened areas are also included when available. The patients' village of residence and the relevant coordinates are imported into the DB to map the geographic distribution of HAT cases. GIS software allows to directly generate maps based on the data stored in the HAT DB. Importantly, screening activities are included in the DB and in the maps even when no cases were detected. The loop is closed when maps are shared with data providers for refinement and verification. Upon completion and consolidation, all inputs and results of the HAT Atlas initiative will be widely disseminated, both in hard copy and in digital format.

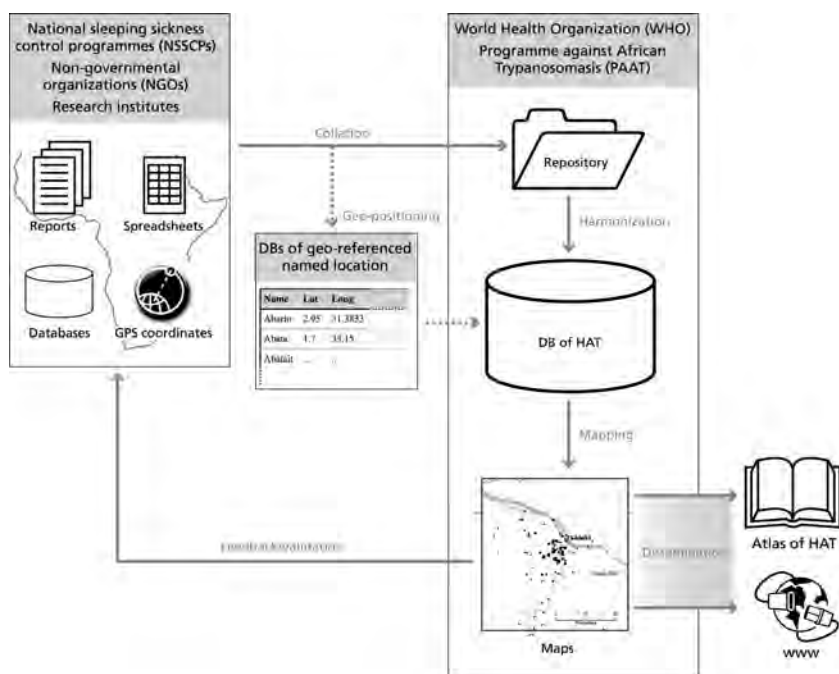


Figure 1: The information system for the Atlas of HAT: data providers, data collation and processing, archiving, outputs, verification and dissemination channels (Cecchi *et al.*, 2009a).

From the mapping standpoint, the chief challenge for the HAT Atlas initiative is to measure or estimate the geographic coordinates of all locations of epidemiological interest. The ideal situation occurs when

mobile teams involved in HAT active case-finding missions acquire geographic coordinates by GPS, whose measurements are characterized by a high level of accuracy. If GPS coordinates are not available in the epidemiological reports, the second option is to geo-position villages by matching their names with those available in gazetteers, which are dictionaries of geographical information that list names and coordinates of geographical entities. In addition to reported GPS coordinates and gazetteers, many other sources are used to geo-reference HAT cases, including paper and digital maps and hand-drawn sketches of the endemic areas. Crucially important are also the indications provided by field workers having direct knowledge of the often remote areas where sleeping sickness occurs. Descriptions such as “village x is located at n km from village y , along the road to village z ”, allow to geo-locate numerous hamlets and small settlements in a relatively approximate but highly efficient manner. As the HAT Atlas initiative progresses and the capacity of NSSCPs is strengthened, approximate coordinates are replaced with more precise measurements, resulting in a geographical accuracy that continuously improves.

The initial goal of the HAT Atlas initiative is to map all reported cases and active screening activities for the period 2000-2009, and the present paper concentrates on the study period 2000-2008. Close collaboration with and capacity building of NSSCPs will pave the way for future regular updates.

Results

At the time of writing, preliminary mapping has been completed for 21 countries (study period 2000 – 2008), comprising eight countries in West Africa (Benin, Burkina Faso, Côte d’Ivoire, Ghana, Guinea, Mali, Nigeria and Togo), seven countries in Central Africa (Cameroon, Central African Republic, Chad, Congo, Equatorial Guinea, Gabon and Sudan) and six countries in Eastern and Southern Africa (Kenya, Malawi, Mozambique, Zambia, Zimbabwe and United Republic of Tanzania). Mapping of a given country is considered completed when all available data sources have been fully processed.

Work is in progress to map HAT in the other affected countries for which data are available (Angola, Democratic Republic of the Congo and Uganda). Because of the lack of recent reports, the epidemiological situation in eight countries (i.e. Burundi, Ethiopia, Gambia, Guinea-Bissau, Liberia, Niger, Rwanda, Senegal and Sierra Leone) still needs to be clarified (Simarro *et al.*, 2008). The list of countries considered

endemic for HAT is completed by Botswana, Namibia and Swaziland. In the two former countries transmission of sleeping sickness is likely to have been interrupted by successful Tsetse elimination campaigns (Allsopp and Pillemon-Motsu, 2002; Kgori *et al.*, 2006), whilst in the latter recent entomological surveys showed that *Glossina austeni*, a species considered of very limited or *nihil* importance in HAT transmission, is currently the only species of Tsetse fly present in the country (Saini and Simarro, 2008). The progress status of the HAT Atlas is summarized in Figure 2.

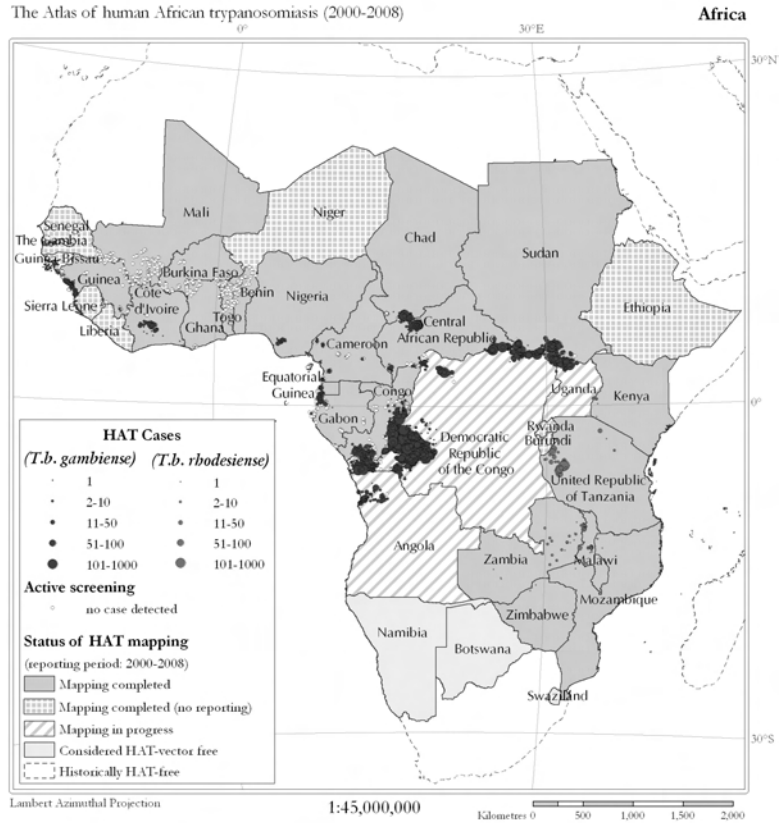


Figure 2: The Atlas of Human African Trypanosomiasis: progress status. Dark grey dots represent cases of Gambian sleeping sickness and light grey dots represent cases of Rhodesian sleeping sickness. Active screening events where no cases were detected are represented as white dots. Mapping is considered completed (countries in solid grey) if all available data sources for the period 2000 – 2008 have been analysed

In West Africa significant disease incidence only continues to be reported from coastal Guinea, in the mangrove biotope, and from central Côte d'Ivoire, most notably in cocoa and coffee plantations (Cecchi *et al.*, 2009b). By contrast, extensive active screening has not detected any evidence of local transmission in vast areas of the Sudanian savannah ecoregion, which includes a number of sleeping sickness foci that had been active in the 20th century. This evolution appears to have been driven by demographic and climatic dynamics that would have shrunk the distribution of the Tsetse fly in the region and reduced the possibility of contact between people and the vector of HAT (Courtin *et al.*, 2008). The picture in Central Africa is more complex, with several areas in Sudan, Central African Republic and Congo still reporting relatively high incidence rates. Lastly, in the mapped countries where *T.b. rhodesiense* is endemic, cases concentrate in the proximity of national parks, game reserves and other protected areas, where people enter into contact with parasites circulating in the wild animal reservoir.

The HAT DB presently contains approximately 100,000 cases. However, it must be stressed that even in some countries where mapping has been completed not all cases officially reported could be geo-referenced at the village level. Around 83% of the cases reported from completed countries have been mapped so far. Most of the unmapped cases originate from reports of passive surveillance, in which HAT cases are sometimes only reported to WHO as focus-level aggregates, without specific reference to the patient's village of residence.

The geographic database of HAT will enable different outputs to be generated, including disease maps at different geographic scales. In Figure 3 an example of country-level map is given, which shows the distribution of sleeping sickness cases in Guinea.

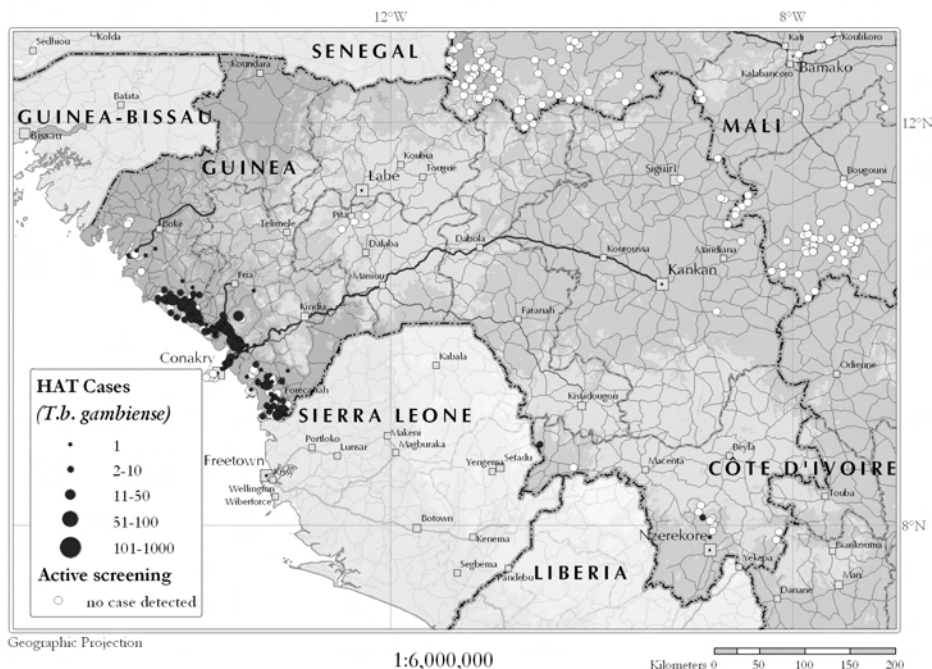


Figure 3: Cases of human African trypanosomiasis (*T.b. gambiense*) reported from Guinea in the period 2000-2008. Data sources: National Sleeping Sickness Control Programme and Research Institutes. Guinea-Bissau, Liberia, Senegal and Sierra Leone are masked out as no recent report on the status of HAT in these countries is available

This type of small scale map provides at a glance the state of knowledge on the HAT distribution in a country, including the known areas of transmission, the areas where no cases were detected by active case-finding surveys and, importantly, the areas where the lack of adequate information may call for new field investigations.

In addition to the continental and country-level maps, the high level of spatial accuracy of the HAT DB - preliminarily estimated in the order of 1 km - also allows larger scale, focus-level maps to be produced. An example is given in Figure 4 for the focus of Forécariah in Guinea. If country-level maps may be more relevant for strategic planning of HAT surveillance and control activities, higher resolution maps represent a

valuable tool for field workers to plan and carry out active screening activities.

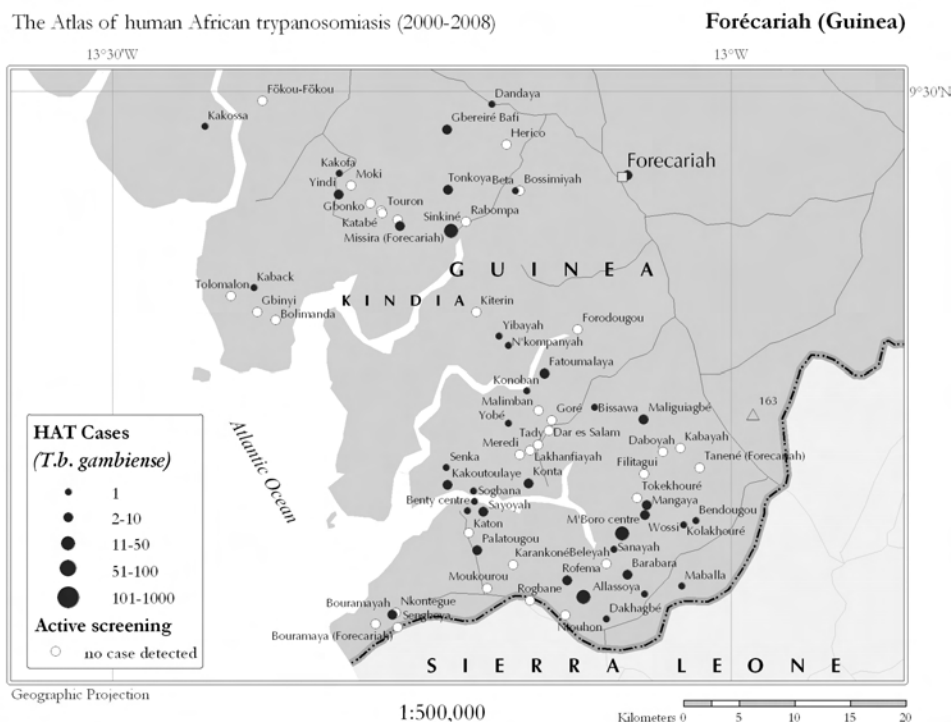


Figure 4: Cases of human African trypanosomiasis (*T.b. gambiense*) reported from the Forécariah focus, in Guinea (period reported: 2000-2008). Data sources: National Sleeping Sickness Control Programme and Research Institutes.

Conclusions

The ambitious, however achievable goal of mapping the continental distribution of sleeping sickness could only be targeted by building on the contributions and commitment of a broad range of stakeholders. NSSCPs, NGOs and the Research Institutes provide inputs to the Atlas by collecting epidemiological information in the challenging conditions often encountered in HAT endemic areas. The United Nations, with its specialized agencies WHO and FAO, provide the technical expertise, coordination and institutional umbrella for a continent-wide initiative of this scope to be carried out.

NSSCPs of affected countries play a central role in building the knowledge base needed for the Atlas, a role that will be further strengthened as the initiative progresses. Training in data management and GIS, as well as provision of software and hardware, will help build the capacity at national level, with a view towards the optimal utilization and regular updating of HAT epidemiological maps. Two regional workshops with key representative from NSSCPs have already been organized for (i) sharing the methodology and preliminary results of the HAT Atlas initiative, and (ii) refining and consolidating preliminary maps.

The Atlas substantially improves the knowledge of HAT geographic distribution, but the room for further enhancement is not exhausted. From the reporting standpoint, a more widespread inclusion of GPS measurements in the epidemiological reports would contribute to increasing the geographical accuracy of the maps. Furthermore, efforts are still needed to reduce the numbers of undetected and unreported cases, despite the intensification of active surveillance achieved in the last decade (Simarro *et al.*, 2008).

Upon completion, a range of dissemination media and outreach activities are envisaged for the Atlas of HAT. A proper Atlas will provide disease maps at different scales as well as an index of all geo-referenced endemic locations. Eventually, the Atlas and all input data used to generate it will be made available to the public domain through WHO and FAO/PAAT website.

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EVALUATION OF THE ANTIBODY RESPONSE DIRECTED AGAINST TSETSE SALIVA ANTIGENS IN HUMANS/

EVALUATION DE LA REPONSE ANTICORPS DIRIGEE CONTRE LES ANTIGENES SALIVAIRES DE TSETSE CHEZ L'HOMME

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Résumé:

La salive des arthropodes hématophages est composée de nombreux composés actifs dont la fonction première est d'interférer avec la réponse hémostatique de leur hôte. Des études ont montré que certaines de ces molécules étaient immunogènes et capables d'induire une réponse anticorps, suggérant que ces anticorps peuvent servir de marqueur d'exposition aux vecteurs hématophages. L'objectif de cette étude était d'évaluer si la réponse IgG dirigée contre les antigènes salivaires de glossines est bien représentative du contact homme-tsé-tsé. Pour cela la salive de *Glossina palpalis gambiensis* a été collectée afin d'évaluer la réactivité immunologique de plasmas humains par un test ELISA indirect. L'échantillon était composé de 301 plasmas collectés dans 2 foyers actifs de THA en Guinée (Forécariah et Dubreka), 2 foyers

historiques du sud-ouest Burkina Faso (Batié et Loropéni) et à Bobo-Dioulasso (zone indemne). Les plus fortes réponses anti-salive ont été observées dans les foyers Guinéens alors que les réponses étaient significativement plus faibles à Bobo-Dioulasso et chez les sujets provenant de la zone de Loropéni. Des réponses élevées ont également été observée à Batié, indiquant la persistance d'un fort contact homme-glossine dans cette zone où les risques de réémergence de la maladie apparaissent importants dans le contexte de mouvements de population saisonniers vers les zones endémiques de Côte d'Ivoire. Ces résultats suggèrent que l'évaluation de la réponse humorale anti-salive pourrait être une bonne alternative aux méthodes d'évaluation entomologique classiques et pourrait permettre de cibler les populations les plus fortement exposées aux glossines et pour évaluer l'efficacité de la lutte anti-vectorielle.

Summary

Saliva from blood sucking arthropods contains a rich array of pharmacologically active compounds whose primary function is to prevent the hemostatic mechanisms of the host. Furthermore, recent studies have shown that many of these saliva molecules are immunonogenic and elicit an antibody response. Such antibodies directed against saliva antigens may thus serve as marker of exposure to the bite by hematophagous vectors. The objective of this work was to assess if the IgG response directed against *Glossina* saliva was representative of the human-tsetse contact. For this purpose saliva from *Glossina palpalis gambiensis* was collected by an experimental procedure, and reactivity of human plasma was evaluated by indirect ELISA. The study sample was composed of 301 plasma, from two active HAT foci in Guinea (Forécariah and Dubreka), two historical HAT foci in South-West Burkina Faso (Batié and Loropéni) and from volunteers living in Bobo-Dioulasso (a tsetse free area). The highest anti-saliva responses were observed in the HAT foci of Guinea, whereas responses were significantly lower in subjects living in Bobo-Dioulasso and in the Loropeni area. High responses were also observed in Batié indicating that this population is still highly exposed to tsetse bites and that the risk of re-emergence of HAT is important notably with numerous subjects travelling to endemic areas of Côte d'Ivoire for seasonal activities. These results suggest that the anti-saliva response evaluation may be a good alternative or complementary approach to classical entomological

methods to target most exposed populations and to evaluate efficiency of tsetse elimination programs.

Introduction

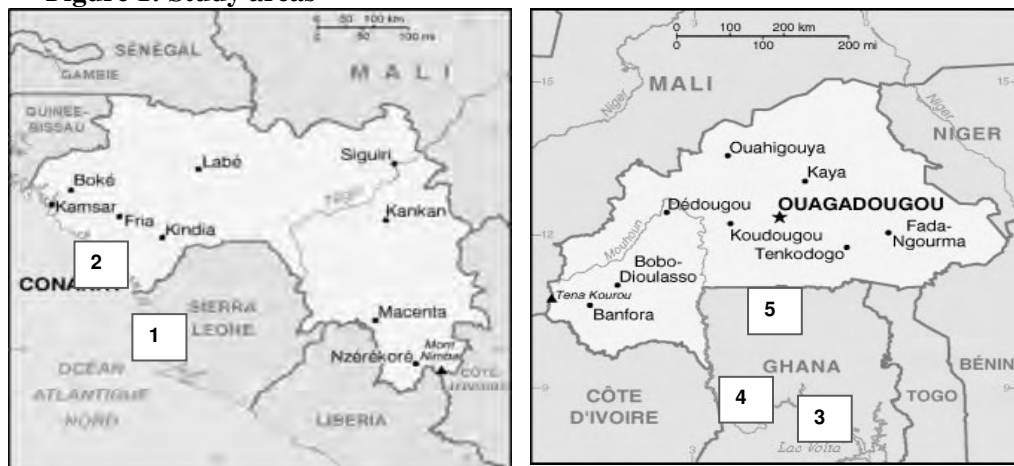
Human African Trypanosomiasis (HAT) caused by protozoan of the *Trypanosoma brucei* group is endemic in 36 countries of sub-Saharan Africa, where over 60 million people are exposed (WHO, 2001). Previous studies have shown that, as most hematophagous arthropod vectors, *Glossina* species inject micro-quantities of saliva to their hosts during their blood meals. This saliva contains anti-platelet, anti-clotting and vasodilatory molecules which play an important role in the blood meal success and transmission of parasites (Ribeiro and Francischetti, 2003). In addition, it was also shown that the presence of specific antibodies directed against specific salivary compounds could be used as a marker of exposure to the bite by several hematophagous arthropods such as ticks, bugs, mosquitoes (Remoue et al., 2005) and sand flies (Barral et al., 2000). Such studies both in mice (Caljon et al., 2006a) and humans (Poinssignon et al., 2008) also suggest that the analysis of the IgG response against *Glossina* salivary antigens may be efficient in assessing exposure to tsetse bites thus providing a tool for assessing the risk of HAT transmission. The objective of this study, conducted at the International Centre for Research-Development in Subhumid zone (CIRDES) was to determine the anti-saliva response from several populations living in different epidemiological context in order to assess the value of this new entomological tool to evaluate human-tsetse fly contacts.

Materiel and methods

Study areas and plasma samples

The sample was composed of plasmas collected from 256 individuals from two active foci of THA in Guinea Conakry (Forécariah, Dubreka-Boffa) and from two historic foci of HAT of Burkina Faso (Loropéni, Batié) (Figure 1). Among these individuals there were 115 healthy subjects, 64 patients (including the various stages of the disease) and 35 seropositive individuals (serological suspects without parasitological confirmation). The patients were treated and monitored during one year. The negative control group comprised 18 individuals from Bobo-Dioulasso which is assumed to be free of Tsetse flies.

Figure 1: Study areas



Guinea (1: Forecariah
2: Dubreka-Boffa)

Burkina Faso (3: Batié
4: Loropéi 5: Bobo-Dioulasso)

Quantification of anti-saliva antibodies

The anti-saliva response was measured by an indirect ELISA test using *Glossina palpalis gambiensis* salivary total antigens obtained by a Glossina saliva collection technique. The concentration of salivary proteins was measured by the BCA Kit and ELISA plates were coated with 100µl/well of a 2mg/ml solution.

Data analysis

Statistical analysis was carried with the JMP 5.1 software. The significance level was set at 0.05. Mean between groups were compared by the ANOVA and T-test and the matched pairs test was used to compare means between paired data (serological follow-up).

Results and discussion

The anti-saliva response correlates with exposure to Tsetse flies:

As expected the anti-saliva response was the lowest in the population of Bobo-Dioulasso where the inhabitants experience no or few contact with Tsetse flies (Figure 2). On the contrary the highest responses were

observed in the two populations of Guinea endemic areas in agreement with the fact that these subjects are highly exposed during daily activities in the mangrove areas infested by Tsetse flies (>60% of the population is above the positive threshold). Interestingly contrasting responses were observed in the two historical foci of Burkina Faso. The population of Loropéni is not significantly different from that of Bobo whereas in Batié, anti-saliva responses were similar to those observed in the active HAT foci of Guinea. This correlates well with the observation of a high level of anthropisation in Loropéni that led to the degradation/disappearance of the *Glossina* biotope in this area.

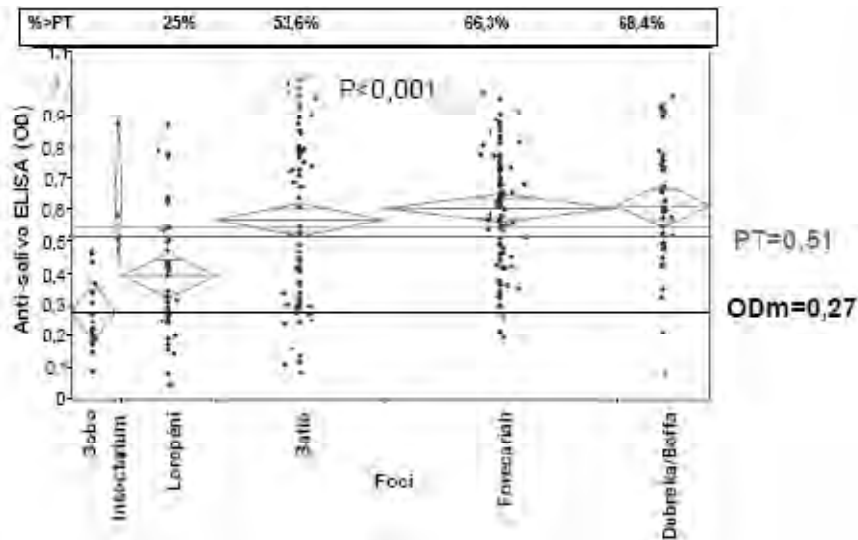


Figure 2: Anti-saliva response level in the different fociODm: mean optical density in subjects from Bobo-DioulassoPT: The Positive Threshold was define as the ODm + 2 SD

In addition, in the Batié area, we were able to analyse the anti-saliva response on a geographic basis (Fig 3) and along with potential Tsetse exposure factors (Fig 4). The three villages with the highest anti-saliva responses were those located near the most important hydrographic networks with preserved vegetation (Yapouoteon and Poni villages) or characterized by the absence of an hydraulic planning (Titinateon village). Concerning human activities, daily attendance of the river for water supply (associated with the absence of a water pump or well near the house) was significantly associated with high anti-saliva responses (P=0.005).

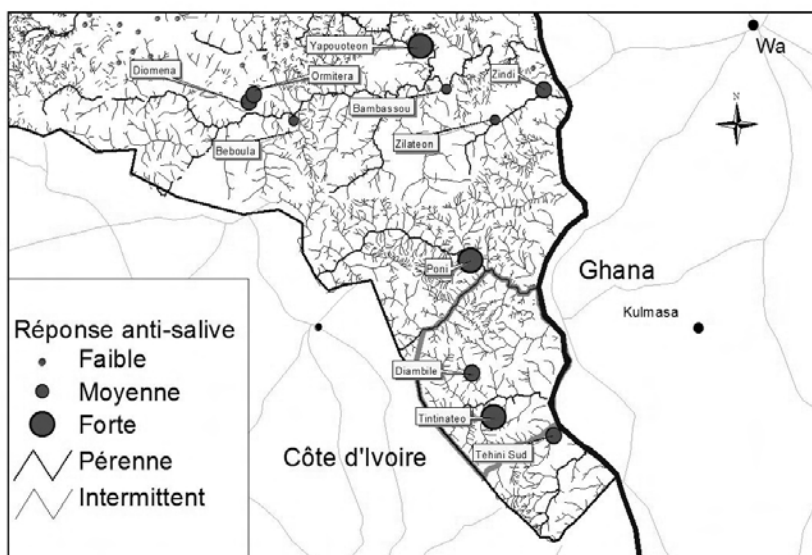


Figure 3: Anti-saliva response in the prospected villages in the Batié area

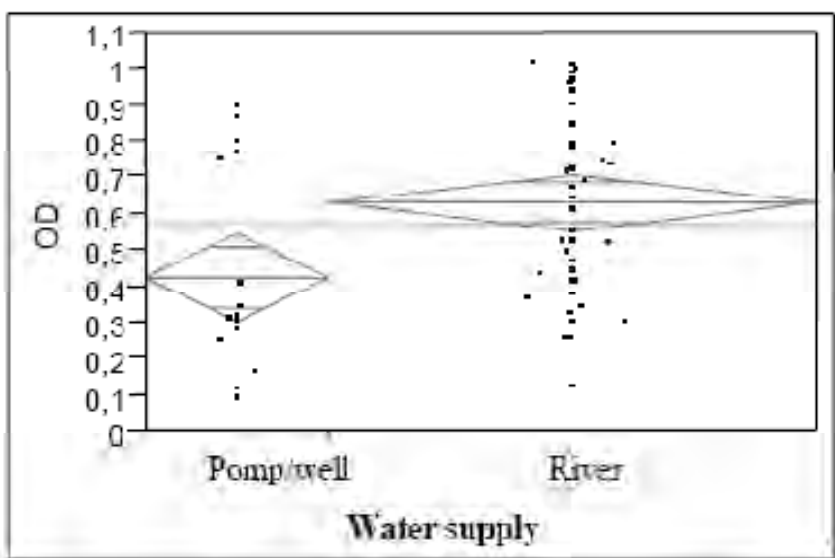


Figure 4, Anti-saliva response according to the water supply

The kinetics of the anti-saliva response appears to be a dynamic process:

In Guinea, a significant decrease ($p=0,007$) of the anti-saliva response was observed in patients between their time of diagnosis and their 6 month follow up visit whereas the anti-saliva response was similar on both visit for HAT serological suspects (Fig 5). This difference may be explained by the fact that HAT patients had to leave their home and activities for at least a month period to go to the treatment centre whereas serological suspect remained in the area.

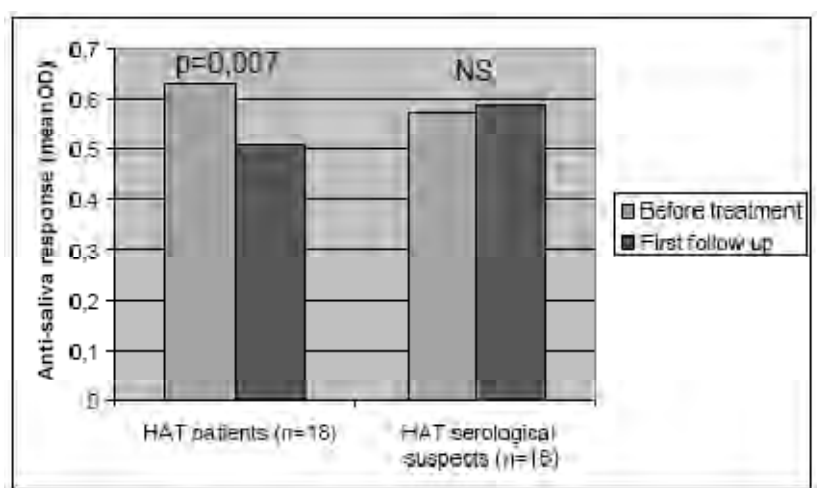


Figure 5: Follow-up of the anti-saliva response in patient Before and after treatment and in serological suspects

Conclusion

This preliminary study suggests that the analysis of the humoral response directed against total *Glossina* salivary antigens can be a good serologic marker of human exposure to Tsetse bites. We believe that this new tool can constitute a good alternative or complementary approach to classical entomological evaluation techniques that are both expensive and human labour demanding. It could be used to rapidly screen, on large geographic scales, populations that are the most exposed to Tsetse, thus at risk of epidemics or re-emergence of the disease (such as in the

Batié area) to which vector control measures should be directed to. Furthermore the apparent dynamic kinetics of anti-saliva IgG makes it an interesting and easy way of assessing vector control campaigns by analysing the evolution of the anti-saliva response in the Tsetse hosts.

The next step is now to identify *Glossina* salivary specific antigens in order to define synthetic peptides that would enable an easy and reproducible test to be developed with higher sensitivity and specificity.

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ADAPTATION D'UNE STRATEGIE DIAGNOSTIQUE DE LA TRYPANOSOMIASIS HUMAINE AFRICAINE AU FOYER DE MANDOUL, TCHAD/

ADAPTATION OF A HUMAN AFRICAN TRYPANOSOMIASIS DIAGNOSTIC STRATEGY IN THE FOCAL AREA OF MANDOUL, CHAD

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Summary

A consensus was reached in the early 2000s on a methodological approach to the diagnosis of Human African Trypanosomiasis during mass surveys conducted in the field. Practically, the detection chain is divided into three main parts i) front office, sampling and CATT serological test ii) laboratory (palpation/aspiration of lymph node, CATT titration, CTC and mAECT) and iii) diagnostic phase (lumbar puncture and reading of the cytorachia level). This simple and logical approach enables the examination of 500 to 600 people per day. However, in some Human African Trypanosomiasis foci in densely populated areas, the use of this methodology requires the presence on the ground of a mobile medical team for about 5 weeks, which is too long, or one of two teams for three weeks, which is too expensive. An adaptation of this methodology is to have a team set up consisting of two front office, sampling and CATT serological testing posts, for one laboratory and diagnostic phase post. This adapted method enabled the whole focus area to be surveyed in 14 days at half the cost: 1,000 persons were examined per day, without excessive work overloading and virtually no additional equipment, and the cost of the survey was only 397 FCFA per person examined against 572 FCFA with the standard methodology. This result is important given the logistical difficulties and staff shortages faced by most national Human African Trypanosomiasis control programs.

Key-words: Sleeping sickness ▪ Diagnosis ▪ Chad

Résumé:

Un consensus a été établi au début des années 2000 autour d'une approche méthodologique du diagnostic de la Trypanosomiase Humaine Africaine lors des prospections de masse sur le terrain. En pratique, la chaîne de dépistage est subdivisée en trois grandes parties i) secrétariat, prélèvement et sérologie CATT, ii) laboratoire (palpation/ponction ganglionnaire, CATT titration, CTC et mAECT) et iii) diagnostic de phase (ponction lombaire et lecture de la cytorachie). Cette approche, simple et logique, permet d'examiner 500 à 600 personnes par jour. Cependant, dans certains foyers de Trypanosomiase Humaine Africaine densément peuplés, l'application de cette méthodologie nécessite la présence sur le terrain d'une équipe mobile pendant environ 5 semaines, ce qui est trop long, ou celle de deux équipes pendant trois semaines, ce qui est trop onéreux. Une adaptation de cette approche méthodologique en mettant en place une équipe constituée de deux postes de secrétariat, prélèvement et sérologie CATT pour un poste de laboratoire et un poste de diagnostic de phase, a permis de prospecter l'ensemble du foyer en 14 jours et de réduire de moitié le coût de l'activité, 1 000 personnes ont été examinées par jour, sans surcharge excessive de travail et quasiment sans matériel supplémentaire, et le coût de la prospection n'a été que de 397 FCFA par personne examinée contre 572 FCFA avec la méthodologie classique. Ce résultat est important à prendre en compte au regard des difficultés logistiques et de la pénurie en personnel que connaissent la plupart des programmes nationaux de lutte contre la Trypanosomiase Humaine Africaine.

Mots-clés, Maladie du sommeil ▪ Diagnostic ▪ Tchad

Introduction

L'arbre décisionnel de dépistage et de diagnostic de la Trypanosomiase Humaine Africaine (THA) proposé par l'Organisation Mondiale de la Santé (OMS) et utilisé dans le dépistage actif sur le terrain, est une séquence de 8 actes, enregistrement, prise de sang au bout du doigt dans un tube capillaire, test sérologique CATT (*Card Agglutination Trypanosomiasis Test*) sur sang total, palpation et ponction ganglionnaire, titration CATT (test sérologique CATT sur des dilutions de plasma de raison 2, centrifugation en tubes capillaires (CTC), minicolonne échangeuse d'anions (mAECT), ponction lombaire avec lecture de la

cytorachie [1]. La position de chaque acte dans la chaîne diagnostique a été précisée et fixée à la suite d'études sur le terrain, en Angola, en République Démocratique du Congo, au Soudan, en Guinée Equatoriale et en Ouganda [2]. L'approche méthodologique a été testée et validée en 2005 en Guinée lors d'une prospection formative sur le terrain qui a regroupé les techniciens de six pays d'Afrique de l'Ouest, Guinée, Côte d'Ivoire, Bénin, Burkina Faso, Mali et Togo (Diarra, communication personnelle). Au terme de ces études, la chaîne diagnostique adoptée est apparue comme celle ayant le meilleur rendement au moindre coût, ce qui n'est pas négligeable pour des programmes nationaux de lutte contre la THA (PNLTHA) confrontés à des difficultés budgétaires et des insuffisances permanentes en personnels et en moyens logistiques. Elle est aujourd'hui acceptée et utilisée par la quasi-totalité des PNLTHA.

LA STRATEGIE DIAGNOSTIQUE SUR LE TERRAIN

Dans la pratique, on peut subdiviser la chaîne diagnostique en trois parties distinctes (fig. 1),

- le secrétariat et les prélèvements (SP), cette phase comprend l'enregistrement, la prise de sang capillaire au bout du doigt et le test sérologique CATT sur sang total. Cinq personnels sont nécessaires à son bon fonctionnement, 2 au secrétariat, dont 1 secrétaire lettré recruté dans le village, 2 à la prise de sang capillaire et 1 au CATT sur sang total (CATT_{WB}). Ce poste utilise très peu de matériel, ne nécessite pas de personnel particulièrement qualifié et fonctionne aisément avec une seule batterie de 12 volts comme source d'énergie.
- le laboratoire (LAB), y sont pratiqués palpation et ponction ganglionnaire (PPG), test sérologique CATT sur des dilutions de plasma de raison 2 (titration CATT) et techniques parasitologiques, centrifugation en tubes capillaires (CTC) et minicolonne échangeuse d'anions (mAECT). Trois techniciens qualifiés et très expérimentés suffisent à la tâche. Le matériel nécessaire pour cette étape comprend, microscopes, centrifugeuses à tubes et à microhématocrite, micropipettes) et une source électrique fournie par un groupe électrogène de moyenne puissance.
- la ponction lombaire (PL) et la lecture de la cytorachie, Un technicien très expérimenté est nécessaire pour ce poste. Pour la lecture de la cytorachie, il utilise un des microscopes du laboratoire. Il n'a donc besoin que du matériel nécessaire à une PL pratiquée avec des règles d'asepsie les plus rigoureuses. Il est assisté par un personnel local qui sert d'interprète et met en confiance le malade à ponctionner.

Au total, cette approche nécessite 10 techniciens et permet l'examen de 500 à 600 personnes par jour, les limites étant constituées par la population du village prospectée et la capacité de prélèvement du poste SP.

APPLICATION DE CETTE APPROCHE METHODOLOGIQUE AU FOYER DE TRYPANOSOMIASE HUMAINE AFRICAINE DU MANDOUL, TCHAD

Années 2002 et 2003, délimitation du foyer du Mandoul.

Avec l'appui de l'OMS, l'arbre décisionnel de dépistage et de diagnostic classique a été appliqué par le PNLTHA du 14 Novembre au 16 Novembre 2002 puis du 7 au 17 Avril 2003, soit au cours de la même saison sèche (Novembre-Mai), pour déterminer les villages endémiques afin de délimiter le foyer de THA du Mandoul, au sud du Tchad.

Au cours de ces deux prospections, 19 389 personnes ont été examinées et 443 malades diagnostiqués, soit une prévalence de 2,28 % (Fig. 2).

Pour atteindre ce résultat, il a fallu déboursier 5 208 000 FCFA et employer 10 techniciens pendant 42 jours. Le poste SP a prélevé 462 personnes par jour en moyenne. Le poste LAB a réalisé 1 872 examens (1 177 CATT titrations, 353 PPG, 255 CTC et 87 mAECT), soit une moyenne de 44,6 examens par jour. Pour le diagnostic de phase, le poste PL a pratiqué 441 ponctions lombaires, soit une moyenne de 10,5 ponctions lombaires par jour.

Année 2004, nouvelle application de la stratégie diagnostique.

Pour raccourcir la durée de la prospection, l'OMS et l'OCEAC (Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale) ont apporté leur appui au PNLTHA du 21 Septembre au 5 Octobre 2004.

Deux équipes constituées chacune de 10 personnes ont prospecté la totalité du foyer en 15 jours effectifs de travail, 18 406 personnes ont été examinées et 284 malades diagnostiqués, soit une prévalence de 1,54 % (Fig. 2).

Cette prospection a coûté 7 920 000 FCFA. Les postes SP ont prélevé 1 227 personnes par jour en moyenne, soit 613,5 par jour et par poste. Les postes LAB ont réalisé 1 455 examens (738 CATT titrations, 320 PPG, 257 CTC et 140 mAECT), soit une moyenne de 97 examens par jour et de 48,5 examens par jour et par poste. Pour le diagnostic de phase, les postes

PL ont pratiqué 284 ponctions lombaires, soit une moyenne de 19 ponctions lombaires par jour et de 9,5 par jour et par poste.

Le calcul du coût de cette prospection n'a pas pris en compte tous les équipements (véhicules, microscopes, centrifugeuses, rotateurs, etc.) qu'il a fallu doubler, le coût que constitue la prise en charge (déplacement avion, indemnités de déplacement) des techniciens de l'OCEAC et des PNLTHA voisins venus en appui (Cameroun, Congo, Gabon, Guinée Equatoriale, République Centrafricaine, République Démocratique du Congo). Il est très vite apparu que cette expérience ne pouvait se renouveler au cours des prospections ultérieures.

Année 2006, Adaptation de l'approche méthodologique de diagnostic.

Pour des raisons budgétaires et logistiques évidentes, la nécessité de repenser l'approche à mener pour prospecter l'ensemble des villages endémiques du foyer du Mandoul s'est imposée. Le problème posé était, comment examiner tout le foyer dans un délai considéré comme normal (trois semaines) et avec un personnel limité,

Sur le constat qu'au cours des prospections de 2002, 2003 et 2004 le poste SP était saturé et que les postes LAB et PL étaient relativement sous employés, il a été décidé de faire l'essai d'un doublement du poste SP, avec un seul poste LAB et un seul poste PL (Fig. 2). Les deux postes SP étaient placés dans des villages voisins si leur population était limitée ou à deux extrémités d'un même village de population importante.

Par précaution, la durée de la prospection a été maintenue à trois semaines et le programme de prospection, village par village, établi en ce sens. Avec l'appui de l'OMS et de l'OCEAC, la prospection s'est déroulée du 23 Novembre au 12 Décembre 2006, toujours en saison sèche, afin de permettre une plus grande participation des populations qui ne sont pas très occupées par les travaux champêtres.

L'équipe « bicéphale » qui a été constituée (deux postes SP pour 1 poste LAB et 1 poste PL) comprenait 14 techniciens et a coûté 7 524 000 FCFA. En 19 jours effectifs de travail, 10 970 personnes ont été examinées et 78 malades diagnostiqués, soit une prévalence de 0,71 %.

Les postes SP ont prélevé 577 personnes par jour en moyenne, soit 288,5 par jour et par poste. Le poste LAB a réalisé 359 examens (200 CATT titrations, 88 PPG, 44 CTC et 27 mAECT), soit une moyenne de 18,9 examens par jour. Pour le diagnostic de phase, le poste PL a pratiqué 75 ponctions lombaires, soit une moyenne de 3,9 ponctions lombaires par jour.

Au terme de cette prospection, il est apparu que parce que la population examinée avait nettement diminué et que la prospection avait duré 19

jours, les postes SP avaient été nettement sous-employés, avec une moyenne de 288,5 personnes prélevées par jour, les postes avaient cessé toute activité vers 12 ou 13 heures. Et parce que la prévalence observée, 0,78 %, était exceptionnellement basse, les postes LAB et PL avaient également été très peu employés. Il a alors été décidé de maintenir cette stratégie « bicéphale », mais sur une prospection d'une durée ramenée à deux semaines.

Année 2009, une prospection ramenée à deux semaines.

La prospection de la THA dans le foyer du Mandoul s'est déroulée du 2 au 15 Mars 2009, soit sur une durée de 14 jours effectifs. Comme pour celle de 2006, elle a bénéficié de l'appui de l'OMS et de l'OCEAC, a coûté 5 544 000 FCFA et a nécessité 14 techniciens (Fig. 2).

A l'issue de ces 14 journées de travail, 13 989 personnes ont été examinées et 144 malades diagnostiqués, soit une prévalence de 1,03 %.

Les postes SP ont prélevé 999 personnes par jour en moyenne, soit 499,5 par jour et par poste. Le poste LAB a réalisé 541 examens (240 CATT titrations, 150 PPG, 89 CTC et 62 mAECT), soit une moyenne de 38.6 examens par jour. Pour le diagnostic de phase, le poste PL a pratiqué 130 ponctions lombaires, soit une moyenne de 9,3 ponctions lombaires par jour.

Discussion

Les résultats obtenus en 2009 sont comparables à ceux de 2002-2003 et de 2004 pour les postes SP, LAB et PL. Il n'y a donc pas eu surcharge de travail en 2008, malgré les 1 000 personnes examinées par jour.

Pour le calcul du prix de revient d'une prospection, nous n'avons pris en compte que les variables indemnités de déplacement et dépenses en carburant dans le foyer. Les autres paramètres n'ont pas été pris en compte car invariants quelle que soit la méthodologie (achats de consommables, réactifs, médicaments, etc.) ou parce qu'ils ne s'appliquaient pas à toutes les méthodologies (déplacements par avion des experts, indemnités de déplacement des superviseurs, etc.).

Selon ce barème, la prospection la moins onéreuse (5 208 000 FCFA) a été celle des années 2002 et 2003 où la chaîne de dépistage classique a été utilisée. Cette prospection a été également la plus longue (42 jours) et la moins efficace en termes de rendement (moins de 500 personnes examinées par jour). En revanche, tout en étant la plus efficace (999 personnes examinées par jour), la méthodologie diagnostique de 2008 a

été à peine plus onéreuse (5 544 000 FCFA), sans surcharge excessive de travail.

L'analyse en terme de rapport coût/efficacité (coût/population examinée) montre que la prospection de 2006 est revenue à 685 FCFA/personne examinée, celle de 2002-2003 à 269 FCFA, celle de 2004 à 430 FCFA (mais il faudrait ici ajouter le coût du doublement du matériel et des véhicules) et celle de 2009 à 397 FCFA par personne examinée.

Conclusion

Ainsi, une petite modification de la chaîne de dépistage classique (deux postes SP au lieu d'un seul) a permis de doubler le rendement de la prospection et de réduire de manière significative sa durée, quasiment sans matériel supplémentaire, sans surcharge de travail et sans augmentation significative de son coût.

Ce résultat est important à prendre en compte au regard des difficultés logistiques et de la pénurie en personnel que connaissent la plupart des PNLTHA. Le modèle demande cependant à être éprouvé dans d'autres foyers THA, parfaitement adapté au Mandoul (villages très proches les uns des autres, bonne participation de la population, etc.), il ne le serait peut-être pas dans d'autres foyers de THA, où les villages sont plus éloignés les uns des autres et où la population adhérerait moins aux campagnes de dépistage.

Remerciements

Les auteurs remercient tous les techniciens du PNLTHA du Tchad, sans qui ces études n'auraient pas été possibles, l'OMS pour son soutien constant, financier et logistique, et pour son expertise, l'OCEAC pour son appui technique et son expertise, et MM. Vincent Ebo'o Eyenga (PNLTHA Cameroun), Jean-Charles Kounda Gboumbi (PNLTHA République Centrafricaine), Frédéric Louya (PNLTHA Congo), André Luslatomi et Norbert Mwandeke (PNLTHA RD Congo), Eustaquio Nguema Ndong (PNLTHA Guinée Equatoriale) et Jean-Luc Obiang (PNLTHA Gabon) pour leur expertise.

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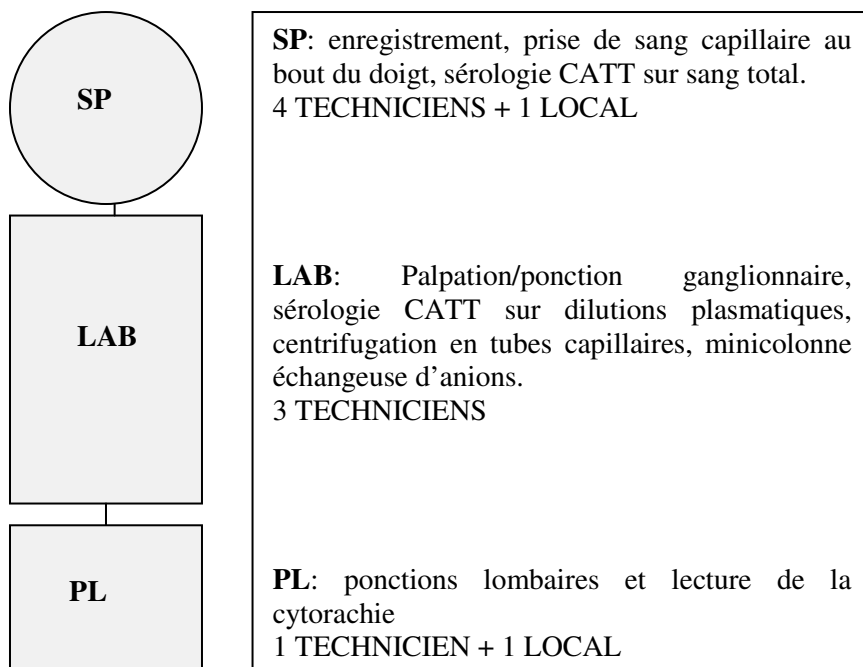


Figure 1 – Organisation du dépistage actif sur le terrain, selon le schéma usuel en vigueur depuis 2000-2001

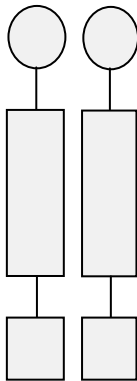
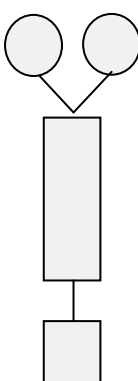
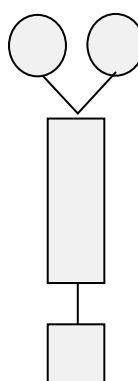
Année	2002-2003	2004	2006	2009
Stratégie diagnostique				
Personnel Utilisé	8 + 2 locaux	16 + 4 locaux	11 + 3 locaux	11 + 3 locaux
Population examinée	19 389	18 406	10 970	13 959
Prévalence	2.28 %	1.54 %	0.78 %	1.03 %
Nombre de jours	42	15	19	14
Population examinée par jour par « SP »	462	1 227	577	999
Moyenne par poste	462	613.5	288.5	499.5
Analyses effectuées par jour par « LAB »	44.6	97	18.9	38.6
Moyenne par poste	44.6	48.5	18.9	38.6
Ponctions lombaires effectuées par jour par « PL »	10.5	19	3.9	9.3
Moyenne par poste	10.5	9.5	3.9	9.3

Tableau I – Prix de revient des prospections

Années	2002 et 2003	2004	2006	2009
Approche méthodologique	1 chaîne de dépistage	2 chaînes parallèles de dépistage	1 Chaîne « bicéphale »	1 chaîne « bicéphale »
Population examinée	19 389 personnes	18 406 personnes	10 970 personnes	13 959 personnes
Durée de la prospection	42 jours	15 jours	19 jours	14 jours
Nombre de personnes examinées par jour	462	1 227	577	999
Coût de la prospection (FCFA)	5 208 000	7 920 000	7 524 000	5 544 000

**PREVALENCE OF HUMAN INFECTIVE
TRYPANOSOMES IN CATTLE IN KABERAMAIDO AND
DOKOLO DISTRICTS, NORTH EASTERN UGANDA
USING PCR TECHNIC/**

**DETECTION DE TRYPANOSOMES POTENTIELLEMENT
INFECTIEUX POUR L'HOMME AU NIVEAU DES BOVINS
DU DISTRICT DE KABERAMAIDO, AU NORD-EST DE
L'UGANDA, GRACE A L'UTILISATION DE LA
TECHNIQUE DE REACTION EN CHAINE PAR
POLYMERASE**

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Résumé

La Trypanosomiase Animale Africaine (THA) causée par les trypanosomes et transmise par la mouche tsé-tsé (*Glossine* spp.), demeure un obstacle majeur à la sécurité alimentaire dans les zones endémiques de la trypanosomiase de l'Afrique. La mouche tsé-tsé transmet les parasites responsables de la maladie du sommeil chez les humains. Une enquête sur le terrain sur la trypanosomiase a été entreprise dans les sous comtés de Bululu, Alwa et de Dokolo du district de Kaberamaido, au cours de laquelle un total de 658 bovins ont été examinés grâce à la technique de centrifugation de l'hématocrite (HCT) et la/les techniques(s) du frottis sanguins. Tout le bétail présenté aux sites de dépistage a été examiné. 13 (5,46%) sur 238 bovins examinés grâce aux techniques parasitologiques dans le sous comté de Bululu ont été testées positives. Cinq (4,1%) des bovins à Alwa sur les 120 examinés étaient positifs et dans le sous-comté de Dokolo, un total de 300 bovins ont été examinés et 9 (3%) ont été testées positifs. L'ADN Trypanosomal a été extrait de tous les échantillons testés positifs et analysés grâce aux techniques TBR-PCR, ITS-PCR et SRA-PCR et les

résultats étaient les suivants, 21 (77,7%) des 27 ADN trypanosomal isolés du bétail étaient testés positifs grâce à la technique TBR-PCR, indiquant qu'ils étaient des sous groupes de *Trypanozoon* des trypanosomes, alors que 6 (22,2%) étaient testés négatifs, suggérant qu'ils pourraient être des *T. vivax* ou *T. congolense*. Cependant, lorsque l'ITS-PCR a été effectué sur tous les 27 échantillons testés parasitologiquement positifs à l'aide d'amorce ITS à plusieurs espèces, il s'est avéré que 6 (22,2%) étaient des espèces *T. vivax*, 16 (59,3%) des *T.brucei* et les 5 (18,5%) restants portaient une infection avec les deux *T.vivax* et *T.brucei*, alors que le *T. congolense* n'a pas été détecté. Pour établir la présence de trypanosomes humains infectieux pour l'homme chez le bétail échantillonné, tous les 21 échantillons testés positifs ont été soumis au PCR-SRA qui est spécifique au *T.b.rhodesiense*, 4 (19%) des échantillons testés positifs confirmant que le bétail était un réservoir potentiel de trypanosomes *T.b.rhodesiense* infectieux pour l'homme.

Summary

Tsetse transmitted trypanosomiasis is a great hinderance to livestock development and human health in large areas of sub-Saharan Africa. This study aimed at improving livestock production and human health through unequivocal diagnosis of trypanosome species and it covered sub counties of Bululu, Alwa and Dokolo. A total of 658 cattle were examined of which 27 tested positive using Haematocrit Centrifugation and thin blood smear techniques. All the twenty seven samples were subjected to molecular PCR technique using TBR-PCR, ITS-PCR and SRA-PCR. It was revealed that using TBR-PCR, 21(77.7%) were positive suggesting the presence of *T. brucei* subgroup. However, when all the samples were analysed using ITS-PCR, 6 (22.2%) were *T. vivax*. Using PCR-SRA, it was revealed that 4(19%) of the 21 *T. brucei* positive samples were positive confirming the presence of *T. b. rhodesiense* in the areas surveyed.

Introduction

Tsetse transmitted Trypanosomiasis in Africa is a serious constraint to both livestock production and human health. About 10 million square km of sub-Saharan Africa are infested by Tsetse covering about 38 countries (Finelle, 1980). About 30% of approximately 147 million cattle in the continent are said to be at risk of Trypanosomiasis (Murray and Gray, 1984). In Uganda, Tsetse and Trypanosomiasis are widely

distributed in about 2/3 of all the agro ecological zones. The control of Trypanosomiasis in both Animals and Humans is difficult because of the low sensitivity of the parasitological techniques usually used in the field detection of trypanosomes.

Objective

The objective was to improve livestock production and human health through unequivocal diagnosis of trypanosomes.

Materials and Methods

Study site

The study was conducted in two sub-counties of Bululu and Alwa in Kaberamaido district and neighbouring Dokolo Sub-county in Dokolo district, North Eastern Uganda (**Figure.1**).

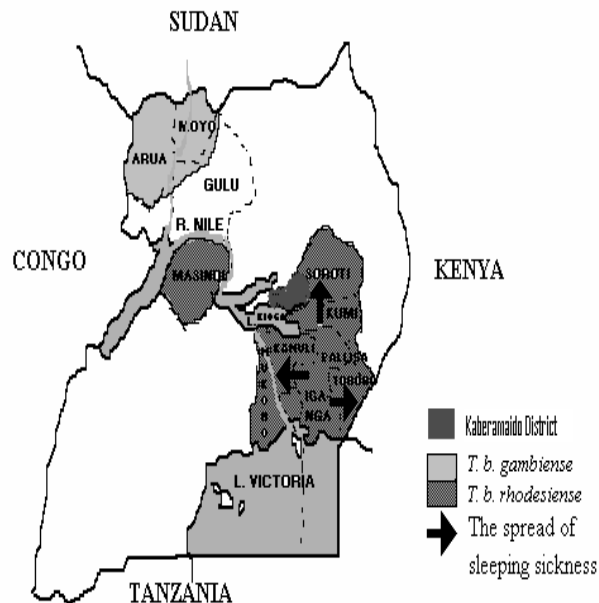


Figure 1: Map of Uganda showing the study areas (Kaberamaido and Dokolo districts)

Animal Trypanosomiasis Survey

Farmers in each selected parish were earlier mobilized through the local authorities to bring their animals to the designated screening sites. Each cattle was restrained in order to draw blood from the jugular vein. Five millilitres of blood were obtained by puncture of the jugular vein using a syringe. Blood was immediately transferred to a vial containing EDTA as an anti-coagulant. Two microliters of each blood sample were examined by a wet blood smear and Haematocrit Centrifugation techniques under a high power light microscope at a magnification of x 400. The blood with mobile trypanosomes in view was recorded as positive.

DNA extraction

DNA extraction from blood samples was done using Chelex® 100 (BioRad) protocol as described by Solano, P., *et al.*, (2002) with some modifications. Blood samples were removed from the deep freezer at -20°C and left at room temperature for at least 30 minutes prior to extraction of DNA to allow thawing. 250 µl of the blood was mixed with 250 µl of lysis buffer (0.32M sucrose, 0.01M Tris, 0.005M MgCl₂, 1% Triton X-100, pH 7.5). The mixture was left at room temperature for about 10 minutes followed by centrifugation at 1200 rpm for 2 minutes and the supernatant discarded. The pellet was then washed three times with 250µl of the lysis buffer and each time the mixture centrifuged and the supernatant discarded as before leaving the pellet behind. The pellet was then re-suspended in distilled water and 10 µl of proteinase k was added and the mixture vortexed for thorough mixing. The mixture was then incubated at 56°C for one hour, and thereafter heated at 95°C for 10 minutes. 250 µl of chelex (1 %) were added and the mixture incubated at room temperature for 15 minutes and thereafter centrifuged at 8000rpm for 5 minutes. The supernatant was pipetted into a new tube containing 1/10 of 50 µl 0.3M sodium acetate pH 5.2 and an equal volume (250µl) of 98-100% alcohol added and the mixture was centrifuged at 14000 rpm for 5 minutes and the supernatant discarded. The remaining pellet was washed with 200 µl of 70% ethanol, centrifuged at 14000 rpm for 3 minutes and the supernatant discarded. The pellet was then dried at 37°C and finally re-suspended into 60 ml of molecular biology grade water.

Molecular DNA analysis

Polymerase Chain Reaction using species specific primers for *T. brucei*

The PCR amplification was carried out as described by Moser *et al.*, (1989) in 25µl reaction mixture containing final concentrations of 10mM Tris-HCl, pH 8.3, 50mM KCl, 1.5mM MgCl₂, 0.1%(v/w) Triton X-100, 200 moles each of dATP, dCTP, dGTP and dTTP, 1.0µM each of the 5' and 3' primers, 50ng DNA template or 2µl crude DNA preparation and 1 unit of Red Taq. DNA polymerase. The amplification started with initial denaturation step at 94°C for 3 minutes and subjected to 35 cycles involving denaturation for 1 minute at 94°C, annealing at 56°C for 1 minute and extension at 72°C for 1 minute and final elongation of 5 minutes at 72°C. The absence of contaminants were routinely checked by inclusion of negative control samples in which the DNA samples were replaced with sterile water. Fifteen microlitres of each sample alongside a control and a marker DNA were electrophoresed in 1.5% (w/v) agarose gel containing 0.5µg/ml ethidium bromide stain. The amplified products were observed by UV transillumination.

Polymerase Chain Reaction using ITS-1 specific primers

Polymerase Chain Reaction (PCR) that amplifies DNA of all the pathogenic mammalian trypanosomes using ITS C-F and ITS B-R primers. PCR amplification was performed using 50ng of DNA extracted from purified *T. b. brucei*, *T. congolense* and *T. vivax* trypanosomes as control DNA, or 10µl of DNA extracted from domestic animal blood. The DNA amplifications was carried out in 25 µl reaction mixtures containing final concentrations of 20 µM Tris-HCl, pH 8.3, 100 mM KCl, 0.1% (v/v)Triton X-100, 3.0 mM MgCl₂, 200 µM each of dATP, dCTP, dGTP, and dTTP, 0.2 µM each primers and 0.75 units of Red Taq. DNA polymerase with the initial incubation for 5 minutes at 94°C, followed by 35 cycles involving denaturation for 1 minute at 94°C, annealing at 56 °C for 1minute, extension at 72°C for 1 minute and a final extension for 10 minutes at 72 °C. The absence of contaminants will be routinely checked by inclusion of negative control samples in which the DNA samples were replaced with sterile water. Twenty microlitres of each sample were electrophoresed in 1.5% agarose containing 1 µg/ml ethidium bromide. The amplified products were observed by UV transillumination.

Polymerase Chain Reaction using *T. b. rhodesiense* specific primers

Polymerase Chain Reaction using primers that recognize *T. b. rhodesiense* SRA gene were conducted to identify *T. b. rhodesiense* among trypanosomes isolated from domestic animals as described by Radwanska *et al.*, (2002). PCR amplification was performed using 50ng of DNA extracted from suspected cattle blood. The DNA amplifications were carried out in 25 µl reaction mixtures containing final concentrations of 20 mM Tris-HCl, pH 8.7, 100 mM KCl, 50 mM (NH₄)₂ SO₄, 1.5 mM MgCl₂, 200 µM each of dATP, pH 8.7, 100 mM KCl, 50 mM each primers and 2.5 units of HotStar Taq of dATP, dCTP, dGTP, and dTTP, 0.5 µM each primers and 2.5 units of HotStar Taq DNA polymerase. PCR will be performed using a GeneAmp PCR system 9700 from Applied Biosystems with the initial Incubation for 15 minutes at 95°C, followed by 45 cycles involving denaturation for 1 minute at 94°C, annealing at 68°C for 1 minute extension at 72°C for 1 minute and a final extension for 10 minutes at 72°C. The absence of contaminants was routinely checked by inclusion of negative control samples in which the DNA samples were replaced with sterile water. Twenty microlitres of each sample was electrophoresed in a 1.5 % agarose containing 1 µg/ml ethidium bromide. The amplified products were observed by UV transillumination.

Results

A total of 658 cattle were examined for the presence of trypanosomes in Bululu, Alwa and Dokolo sub counties. Fifteen samples were positive in Bululu, four in Alwa and eight in Dokolo (**Table 1**). All the 27 parasitological positive samples were analysed using PCR technique. It was revealed that using TBR-PCR, 21(77.7%) (**Table 3**) were positive suggesting the presence of *T. brucei* subgroup trypanosomes. However, when all the samples were subjected to ITS-PCR, 16 (59.3%) were *T. brucei*, 11(40.7%) were *T. vivax* and 5(11.5%) were mixed infection of *T. brucei* and *T. vivax* (**Table 2**). Using PCR-SRA, it was revealed that 4(19%) of the 21 *T. brucei* positive samples were positive confirming the presence of *T. b. rhodesiense* in the areas surveyed (**Table 3**).

Table 1: Showing the prevalence of trypanosomes in the three sub counties by Parasitological technique (Wet blood smear technique).

Location / Sub county	Total number of cattle examined	Number of Positives	Infection rate (%)
Bululu	238	15	6.3
Alwa	120	04	3.3
Dokolo	300	08	2.7
TOTAL	658	27	Av.IR 4.1

Table 2: Molecular differentiation of *Trypanosome spp* from infected cattle blood samples using ITS- PCR.

Animal Species	Origin (Sub county)	Number Positive by Microscopy	ITS 1 - PCR						
			+						
			Tb	Tv	Tc	Tb+ Tc+ Tv	Tb +T v	Tb+ Tc	Tv+ Tc
Cattle	Bululu	15	12	01	00	00	02	00	00
	Dokolo	08	04	03	00	00	01	00	00
	Alwa	04	00	02	00	00	02	00	00
TOTAL		27	16	11	00	00	05	00	00

Table 3: Molecular differentiation of *T. b. rhodesiense* trypanosomes from infected cattle blood samples.

Animal	Origin	Number positive-microscopy	TBR-PCR		SRA-PCR Positive
			+	-	
Cattle	Bululu	15	14	01	03
	Dokolo	08	05	03	01
	Alwa	04	01	03	00
	TOTAL	27	21	06	04

Discussion

To assess the risk of *T. b. rhodesiense* sleeping sickness, *T. b. rhodesiense* parasites must be unequivocally distinguished from *T. b. brucei* the non human infective form which jointly infects cattle. This is usually done by analysing all the *T.brucei* positive samples for the presence of the Serum Resistance Associated (SRA) gene which have been confirmed to be present in *T.b.rhodesiense* stocks (Enyaru *et al.*, 2006). On analysing the 27 samples using PCR technique, 21(77.7%) belonged to *T.brucei*, and 4(19%) were SRA positive confirming that there were *T.b.rhodesiense* which is linked to the trypanosomiasis in the region and this directly implicates cattle in the transmission of the disease in the area which further confirms the results as obtained by Welburn *et al.*, 2001. The presence of *T.b.rhodesiense* in the area puts at risk the 133,000 population (Fe'vere *et al.*, 2005) in Kaberamaido district of getting infected with the *T.b.rhodesiense* sleeping sickness.

Acknowledgement

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EPIDEMIOLOGY OF HUMAN AFRICAN TRYPANOSOMOSIS IN THE FORECARIAH MANGROVE FOCUS (GUINEA): CLINICAL AND DIAGNOSIS FEATURES/

EPIDEMIOLOGIE DE LA TRYPANOSOMIASE HUMAINE AFRICAINNE DANS LE FOYER DE MANGROVE DE FORECARIAH (GUINEE), ASPECTS CLINIQUES ET DIAGNOSTIQUES

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Résumé

La zone de mangrove en Guinée est probablement le foyer le plus touché par la THA au *Trypanosoma brucei gambiense* (Tbg) en Afrique de l'Ouest. Bien que la plupart des cas de ces dernières années aient été signalés à partir du foyer de Dubreka, au nord de Conakry, seules quelques informations étaient disponibles à partir de la zone de Forecariah, au sud du pays. Les résultats des enquêtes médicales menées entre Novembre 2007 et Octobre 2008 indiquent que, bien que la maladie semble moins épidémique que dans le foyer de Dubreka, la THA survient également à Forecariah, avec plusieurs zones géographiques où la transmission semble active. Une spécificité du diagnostic de la THA dans la zone de Forecariah comparée aux foyers de Dubreka et de Côte d'Ivoire, est que presque tous les patients ont des aspirats de ganglions lymphatiques parasitologiquement positifs, alors que presque tous sont

testés négatifs au mAECT. En plus des 28 patients atteints par la THA et diagnostiqués dans le cadre de cette étude, il a été identifié 32 suspects sérologiques avec des degrés CATT-plasma $\geq 1/8$ mais sans aucune confirmation parasitologique. Le suivi de ces sujets par PCR (Tbr1/Tbr2) et par un test sérologique de Trypanolysis (TL) (test spécifique au *Tbg*) a été effectué pour clarifier leur rôle dans l'épidémiologie de la THA. La positivité du PCR était plus élevée chez les suspects sérologiques TL+ que chez les suspects TL (50% contre 18% respectivement) et alors que les degrés CATT-plasma ont baissé chez les patients atteints de THA et traités et les suspects sérologiques TL-, les suspects positifs au TL ont maintenu des degrés sérologiques élevés durant toute la période de suivi. Ces résultats laissent supposer que ces sujets sont de porteurs asymptomatiques et qu'ils devraient donc être pris en compte dans le cadre des programmes de lutte ou d'éradication de la THA.

Summary

The mangrove area in Guinea is probably the most affected focus of *Trypanosoma brucei gambiense* (*Tbg*) HAT in West Africa. Although most cases these last years were reported from the Dubreka focus in the north of Conakry, few information were available from the Forecariah area, south of the country. The results of medical surveys performed between November 2007 and October 2008 indicate that although the disease appears less epidemic than in the Dubreka focus, HAT also occurs in Forecariah with several geographically defined areas where transmission seems to be active. A diagnosis specificity of HAT in the Forecariah area compared to the Dubreka and Côte d'Ivoire foci, is that almost all patients have parasitologically positive lymph node aspirates whereas almost all are negative to mAECT. In addition to the 28 HAT patients diagnosed in the frame of this study, 32 serological suspects with CATT plasma titers $\geq 1/8$ but no parasitological confirmation were identified. Follow up of these subjects by PCR (Tbr1/Tbr2) and by the Trypanolysis (TL) serological test (a *Tbg* specific test) was performed to clarify their role in the epidemiology of HAT. PCR positivity was higher in TL+ than in TL- serological suspects (50% vs. 18% respectively) and whereas CATT plasma titers decreased both in treated HAT patients and TL- serological suspects, TL positive suspects maintained high serological CATT titers throughout the follow up. These results suggest that these individuals are asymptomatic carriers and should thus be taken into account in the frame of HAT control or elimination programs.

Introduction

Human African Trypanosomiasis (HAT) caused by protozoan of the *Trypanosoma brucei* group is endemic in 36 countries of sub-Saharan Africa, where over 60 million people are exposed. The mangrove area in Guinea is probably the most affected focus of *T. b. gambiense* (*Tbg*) HAT in West Africa although the situation remains unknown in several countries such as Sierra Leone, Liberia or Guinea Bissau. Although most cases these last years were reported from the Dubreka focus north from Conakry, few information were available from the Forecariah area, south of the country at the border area with Sierra Leone. In endemic area control of HAT is highly depend on mass screening and treatment of patients in an attempt to reduce the parasite reservoir and thus lower or disrupt transmission to tsetse flies. However, an important fraction of the population positive to serological test (CATT) is negative to direct parasitological examination (seropositive subjects). A better characterization of these subjects is of crucial importance as previous work carried out in Côte d'Ivoire suggests that at least some of them could be asymptomatic carriers of parasites and thus could play a role in transmission. The objective of the present work was to characterize the epidemiology of HAT in the Forecariah mangrove area located at the border with Sierra Leone (Fig 1). Three medical surveys were carried out in the area between November 2007 and October 2008. Patients and seropositive subjects were followed up for 9 months, and blood and plasma samples collected on the different visits were analyzed by the Trypanolysis serological test (specific of *Tbg*) and by PCR TBR1/TBR2 (a highly sensitive PCR diagnosis technique).

Results and discussions

Geographic distribution of HAT cases

Results of medical surveys are given in Fig 2. The overall disease incidence was 0.22 %. Although less endemic than in the Dubreka focus, these results show that the disease is also present at the south of the country and strongly argue for extending surveillance activities in Sierra Leone. HAT cases were not evenly distributed in the area and were rather clustered in specific geographic. Since Tsetse flies are present almost every where this result suggests that transmission of parasite only occurs in some specific and restricted foci. Targeting vector control to

these areas could therefore have a noticeable impact in reducing HAT transmission in the Forecariah focus.

Diagnosis specificities in the Forecariah focus

A striking feature of HAT in Forecariah is that unlike what is observed in Côte d'Ivoire or in the Dubreka focus, almost all patients have parasite positive lymph node aspirates whereas their mAECT is negative (Fig 3). This suggests that blood parasitemia is very low in Forecariah, possibly due to parasite virulence differences in this area. This observation prompts us to test a new mAECT method where 350µl of buffy coat is used instead of blood. These newly developed test is simple and proved to be more sensitive and easier to read (collectors contained in average 3 times more parasites than in the classical blood mAECT).

Follow up of seropositive subjects suggests the presence of asymptomatic carriers of parasite

An important aspect of the work was the follow-up of study subjects in order to better characterize the status of serological suspects (Fig 4). In HAT patients we observed an overall general decrease of CATT titers after treatment and all patients displayed TBR1/TBR2 PCR negative results on their second follow-up. All but one seropositive subjects negative to the Trypanolysis test became serologically negative (CATT<1/8) during their follow up and positivity to TBR1/2 PCR was rare. These results argue for CATT false positive results in these subjects either due to *T. b. brucei* transient infections or to cross reactivity with other diseases. On the contrary Trypanolysis positive individuals remained with high CATT titers during their follow-up and parasite DNA was detected by PCR in half of them. All together, the presence of parasite DNA and the high and long lasting CATT reactivity observed in these subjects (in contrast to what is observed in treated patients) strongly suggests that these individuals are asymptomatic carriers of *T. b. gambiense* with low blood parasitemia.

Conclusions

This epidemiological survey has shown that although less endemic than in the Dubreka Foci, HAT is also present in the mangrove area of Forecariah south of Conakry. This result strongly suggests that this is

also the case on the other side of the border in Sierra Leone were the HAT situation remains unknown. In the Forecariah focus, the disease was not evenly distributed and rather clustered in some specific area. The most affected individuals were observed in the active population (20-50 years old) and very few cases were evidenced in children. Together these results suggest that transmission do not occur inside villages but is rather associated with human agricultural or fishing activities in some geographically limited areas. Thus identification of these micro transmission foci and targeted vector control program should be efficient in reducing transmission intensities and thus disease incidence in this area.

A diagnosis specificity of the Forecariah focus compared to the Dubreka foci is that blood parasite parasitemia seems to be very low: almost all patients were negative to blood mAECT and were diagnosed by parasitological examination of lymph node aspirates. This suggests the existence of parasite virulence differences between the two foci. Furthermore, this field observation prompted us to perform mAECT with buffy coats rather than blood to increase parasite concentration. This technique was indeed more sensitive (more patients were positive to mAECT on buffy coats and easier to read since mAECT collectors contained in average 3 times more trypanosomes). It is noteworthy that in Forecariah, mAECT on buffy coats enabled to diagnose 2 advanced stage II patients that were parasitologically negative to both blood mAECT and lymph node aspirates examination and that would otherwise have remained undiagnosed.

Finally an important aspect of the work presented here was to provide a better characterization of apparently aparasitemic seropositive individuals with high CATT titers that constitute an important fraction of subject found during medical surveys. Application of the trypanolysis test, a serological test specific of *Tbg*, proved that some of these individuals indeed experienced previous infection/contact with *Tbg* parasites whereas CATT positivity in TL- individuals is rather due to cross reaction due to other *Trypanosoma* species or other pathogens infections. Interestingly follow-up of these patients for a year period showed that these individuals maintained high serological responses in time and that parasite DNA could be evidenced by PCR in half of these subjects. In contrast in patients after treatment we observed a general decreased of CATT titers associated with a disappearance of parasite DNA detected by PCR. All together these observations suggest that in seropositive TL+ subjects, high serological responses are maintained by

the presence of *Tbg* infections at low parasitemia level undetectable by classical microscopic examination. This last observation is important for two reasons. First it adds some new evidences to the existence of trypanotolerance/resistance phenotypes in man as it as already been demonstrated in several cow breeds or in mice experimental models. Understanding why such individual are naturally able to resist infection and metabolic pathways involved could open the way to the identification of new therapeutic or prophylactic targets. Second, the fact that these individuals may be asymptomatic carriers of parasites raises important question about their epidemiological role in transmission. Indeed, they could act as reservoir of parasite facilitating disease persistence in endemic areas and should thus be taken into account by control programs.

Figure 1: Study area

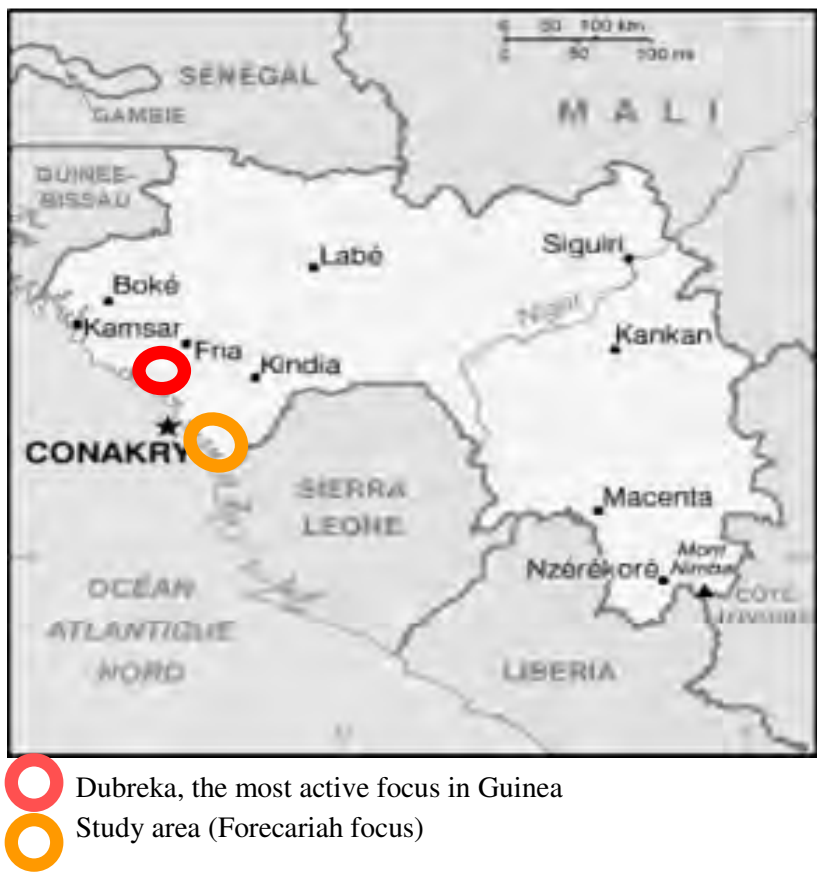


Figure 2: Geographic distribution of HAT and seropositive subjects in the Forecariah focus

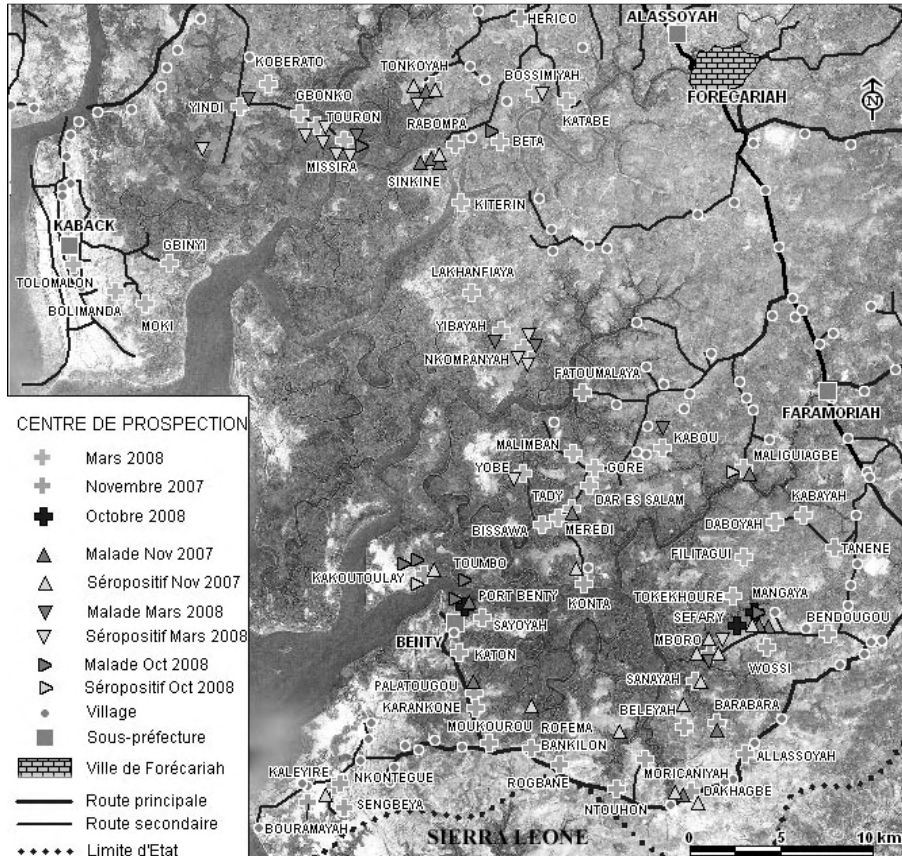


Figure 3: Diagnosis specificities in the Forecariah focus compared to Dubreka

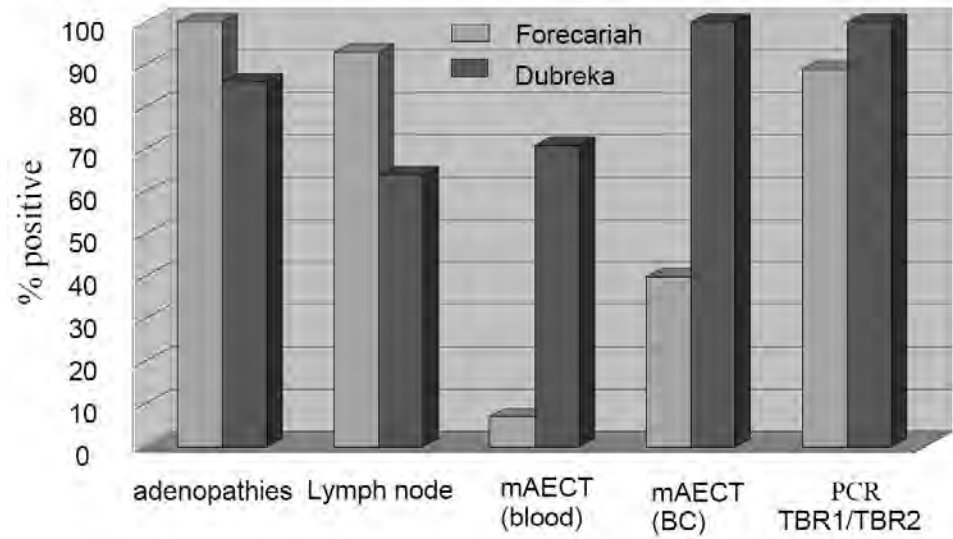
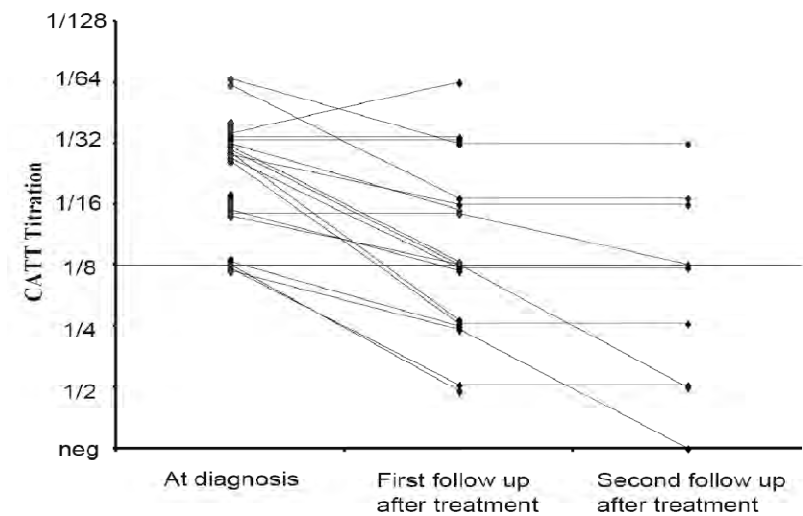
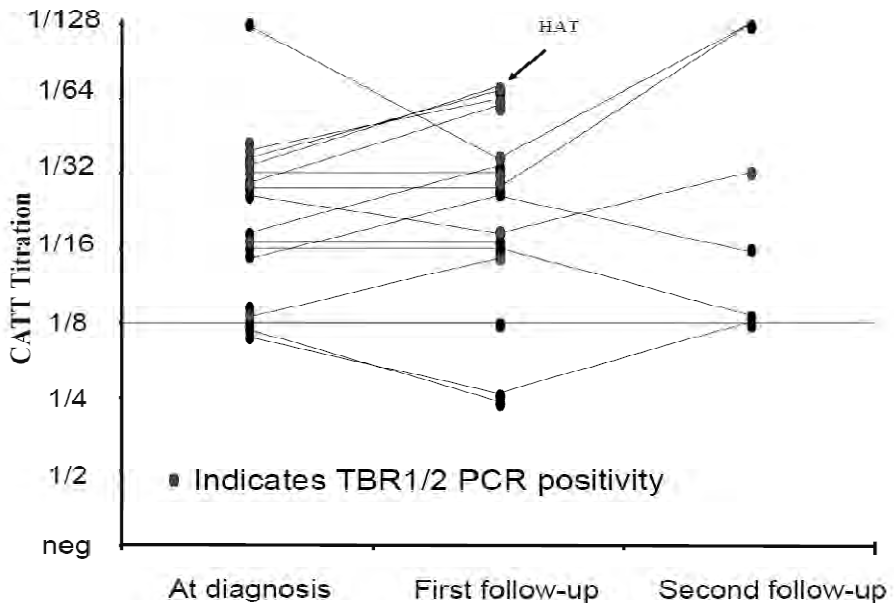


Figure 4: Follow-up of A- HAT treated patients, B- Trypanolysis (TL) positive serological suspects and C- Trypanolysis negative serological suspects

A- HAT patients



B- Seropositive subjects (TL +)



C- Seropositive subjects (TL -)

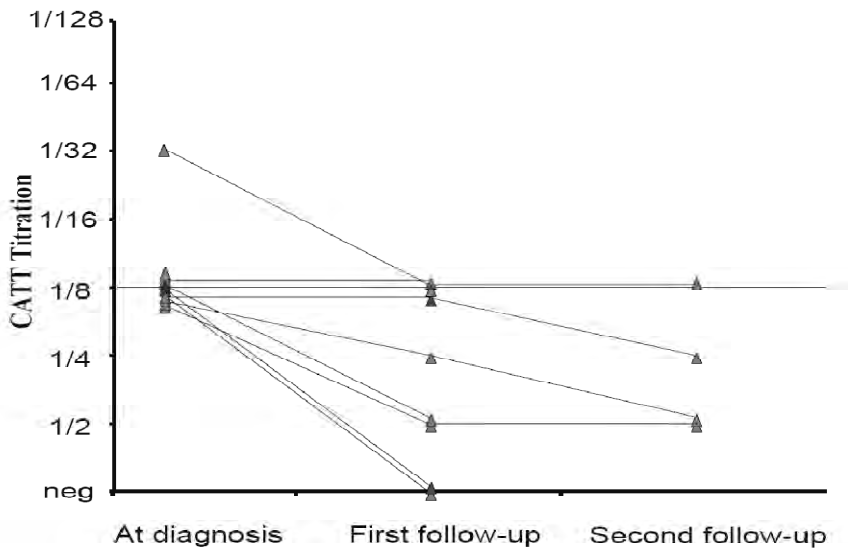


Figure 5:

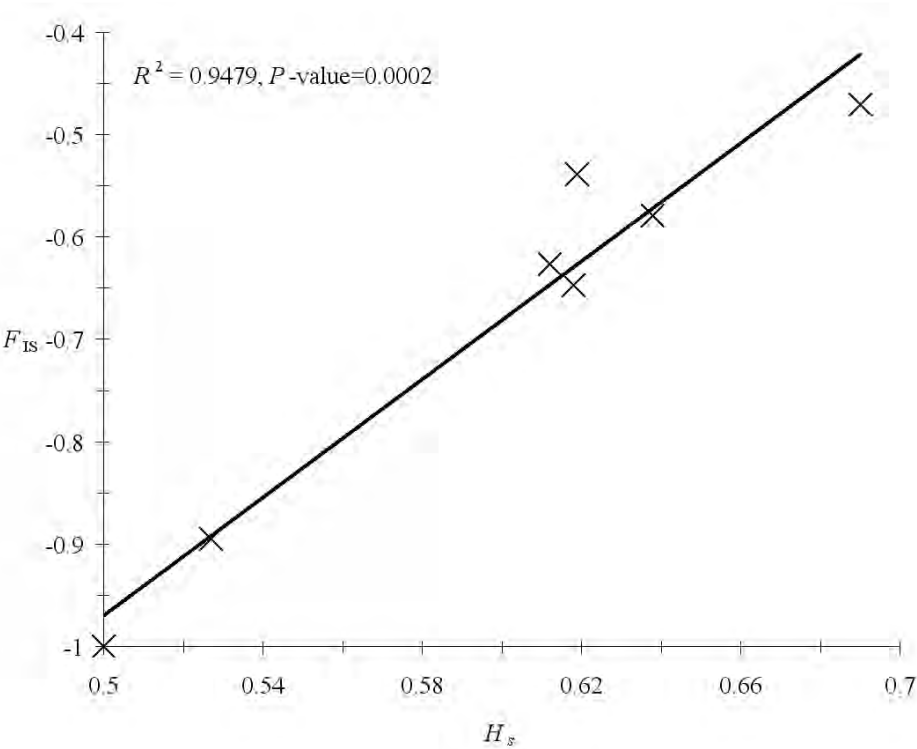


Figure 6:

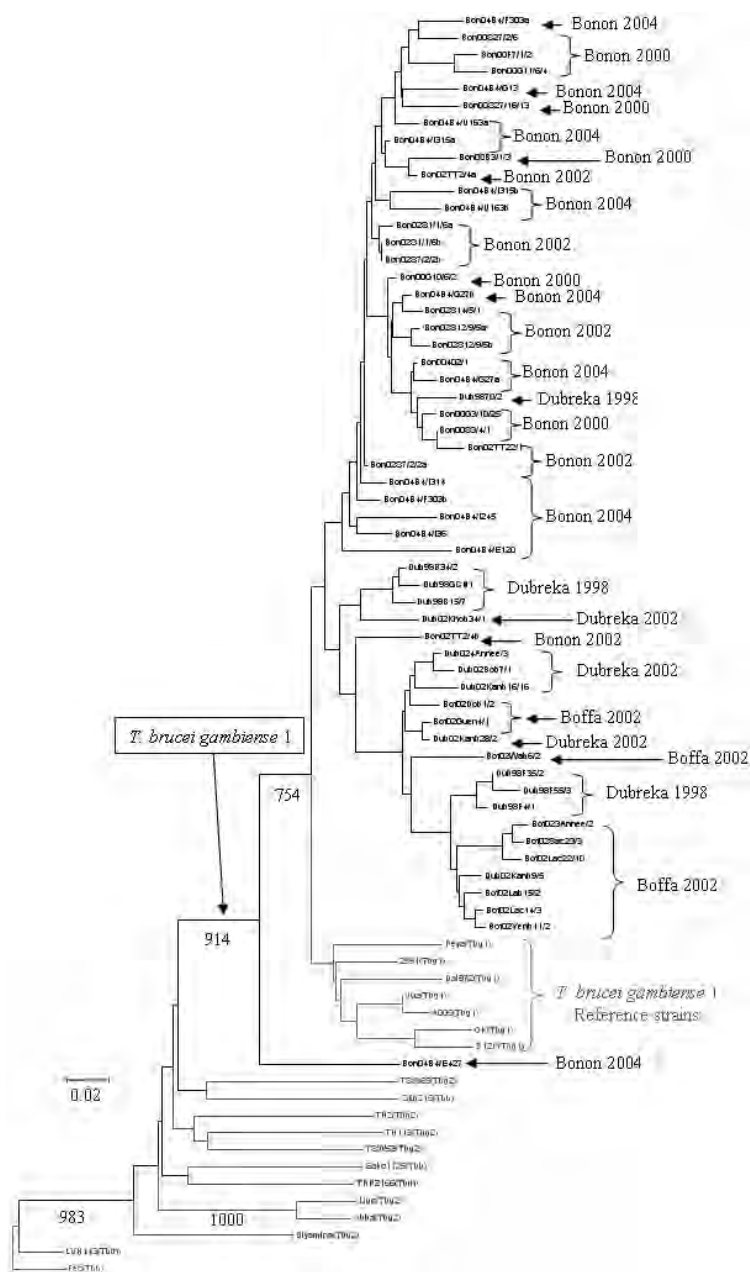
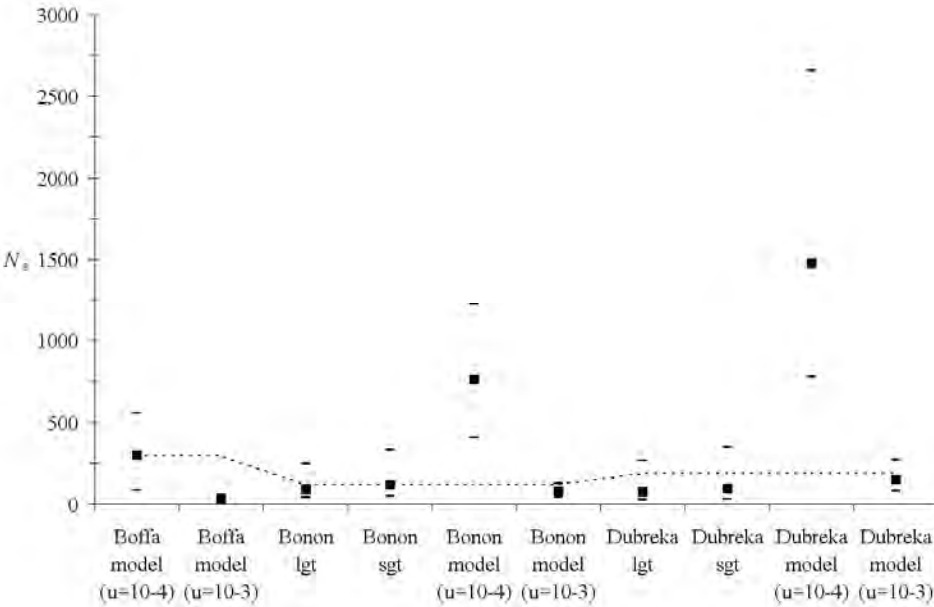


Figure 7:



**AFRICAN ANIMAL TRYPANOSOMIASIS/
TRYPANOSOMIASE ANIMALE AFRICAINE**

**EFFECT OF N'DAMA ORIGIN MARKER ALLELES ON
TRYPANOTOLERANCE IN A BC1 CATTLE
POPULATION UNDER NATURAL TSETSE AND
TRYPANOSOMOSIS CHALLENGE/**

**EFFET DES ALLELES DE MARQUEUR D'ORIGINE
N'DAMA SUR LA TRYPANOTOLERANCE AU SEIN
D'UNE POPULATION DE BETAIL ISSUE D'UNE
PREMIERE GENERATION RECROISEE (BC1) SOUS UNE
PRESSION NATURELLE DE LA MOUCHE TSE-TSE ET
DE LA TRYPANOSOMOSE**

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Résumé

Une démarche intéressante et prometteuse dans la lutte contre la trypanosomose est celle qui consiste à comprendre et exploiter la trypanotolérance développée naturellement. Les animaux trypanotolérants ont une grande capacité de maîtriser le développement de la maladie et de limiter ses effets néfastes. Pour établir des mesures de lutte contre la maladie sur la base de la trypanotolérance, une étude de l'effet des allèles de marqueur d'origine N'Dama sur la trypanotolérance a été effectuée chez des bovins recroisés (BC1), afin de déterminer dans quelle mesure la trypanotolérance a été influencée par le génome N'Dama. Cent quatre vingt douze (192) BC1 ont été exposés à la

mouche tsé-tsé et à la trypanosomiase pendant une période d'une année et ont été phénotypés pour 33 caractères trypanotolérants définis. Pour produire les génotypes BC1 et suivre le développement des allèles d'origine N'Dama à partir de leurs grands parents, l'AND issu des 192 BC1, quatre grands parents N'Dama, 4 grands-mères borana Kenyans et 13 grands parents F1 hétérozygotes (N'Dama x Borana) ont été testés pour 35 marqueurs microsatellites couvrant 5 chromosomes ciblés. Les sommes d'allèles d'origine N'Dama à travers les cinq chromosomes ont été calculées pour chaque animal en tant que score global d'allèles N'Dama et utilisés pour les classer par catégorie en groupes d'allèles N'Dama supérieur et inférieur à 30%. Pour chaque phénotype défini, les deux catégories étaient classées en termes de trypanotolérance 1 (trypanotolérant) ou 2 (susceptible) et la différence significative déterminée par un test t à deux échantillons non appariés (Genstat^R). Dans l'ensemble, le groupe supérieur d'allèles de N'Dama était beaucoup plus important ($p = 0.008$) plus trypanotolérant (classé premier dans 82% des traits) que le groupe inférieur d'allèles de N'Dama. En conclusion, on peut dire qu'il existe une corrélation positive entre le nombre d'allèles de marqueur de N'Dama hérités individuellement par les animaux BC1 et les traits individuels comme trypanotolérants et à tous les niveaux de trypanotolérance.

Summary

A promising approach to trypanosomosis control is to understand and exploit naturally evolved trypanotolerance. Trypanotolerant animals have a substantial capacity to control the development of the disease and limit its harmful effects. To infer control options for the disease based on trypanotolerance, a study of the effect of N'Dama origin marker alleles on trypanotolerance was implemented in backcross (BC1) cattle to determine the extent to which its trypanotolerance was influenced by N'Dama genome. 192 BC1 were exposed to natural tsetse and trypanosomosis challenge for a period of one year and phenotyped for 33 defined trypanotolerant traits. To generate the BC1 genotypes and trace the N'Dama origin alleles from their grand sires, DNA from the 192 BC1, four N'Dama grandsires, 4 Kenyan boran grand dams and 13 F1 heterozygous sires (N'Dama x Boran) were screened for 35 microsatellite markers spanning 5 targeted chromosomes. The sums of N'Dama origin alleles across the five chromosomes were computed for each animal as overall N'Dama allele score and used to categorise them into upper and lower 30% N'Dama allele groups. For each defined

phenotype, the two categories were ranked in terms of trypanotolerance as 1 (trypanotolerant) or 2 (susceptible) and significance determined in a two unpaired sample t-test (Genstat^R). Overall, the upper N'Dama allele group was significantly ($p = 0.008$) more trypanotolerant (ranked first in 82% of the traits) than the lower N'Dama allele group. It is therefore concluded that there is a positive correlation between number of N'Dama marker alleles inherited by individual BC1 animals and both individual trypanotolerant traits and at overall trypanotolerance levels.

Introduction

Rearing trypanotolerant animals is one way of circumventing trypanosomosis disease as such animals have a substantial capacity to control and mitigate its harmful effects. Therefore a promising approach to control this disease is to understand and exploit naturally evolved trypanotolerance. Of the trypanotolerant breeds, the shorthorn N'Dama of West Africa is the most studied and characterised. To infer better control options for the disease, we studied the effect of N'Dama origin alleles on trypanotolerance in a large BC1 population [F_1 (N'Dama x Kenya Boran) x Kenya Boran] under conditions of natural tsetse and trypanosomosis challenge. To achieve this objective, 192 BC1 progenies were introduced into a natural tsetse and trypanosomosis challenge area in six (6) batches of almost 40 animals (mixed males and females) per batch. During exposure to tsetse challenge, all BC1 were phenotyped and recorded at regular intervals for the classical trypanosomosis disease traits: parasitemia, packed cell volume; (PCV), body weight and treatments. From these basic traits, a set of 41 trypanotolerant traits were defined to assess the susceptibility of the progenies to trypanosomosis. Batch and gender effects were estimated by two-way ANOVA with batch and gender as main effects, and all data were corrected for the estimated effects. The batch and gender- corrected trait scores were "standardized" and traits for which "low" values indicated high trypanotolerance were converted into traits for which "high" value indicated high trypanotolerance by multiplication by (-1). This was done so that for all traits, positive trait scores were associated with positive effects on trypanotolerance. A "global" analysis approach was used in which these standardized scores for the 41 trypanotolerance traits were combined to give an overall "*Trypanotolerance trait Score, OTTS*"; and a combination of scores of related traits to give 4 groups of "*component trypanotolerance score*". The body weight traits; WTI, MWT, MXWT, MNWT, WTF1, WTT1 and WTC were not considered as related to

trypanotolerance and therefore were excluded from the calculation of global or component OTTS scores.

Similarly, for genotyping, DNA from 4 N'Dama grandsires, 4 Kenyan Boran grand dams, 13 F₁ sires and the 192 BC₁ progenies were screened using 35 highly polymorphic microsatellite markers spanning 5 chromosomes previously found to harbour trypanotolerant QTLs. Analysis to identify N'Dama marker alleles in the BC₁ was based on the 13 F₁ sire haplotypes which were inferred from the grandparent genotypes. In the analysis of each BC₁ progeny, the transmitted sire haplotypes were inferred and from this and the progeny genotype, the dam haplotype that was transmitted to the progeny were also inferred. From the sire haplotype transmitted, and knowing the origin of the sire haplotype, the progeny genotypes were scored as homozygous Boran (BB) coded "2", or heterozygous Boran/N'Dama (NB) coded "1". Therefore to get the "*Overall N'Dama Allele Score*", (ONAS), for each progeny, all "1s" were summed across all the 35 markers while for each of the five chromosomes, the corresponding "1s" were summed up to give the 5 "*component N'Dama allele scores*".

Based on mean number of N'Dama alleles inherited (ONAS), the BC₁ progenies were sorted into "High" and "Low" groups. The high group consisted of 64 BC₁ progenies that ranked in the highest 30% of N'dama allele score while the low group consisted of a similar number of 64 BC₁ progenies ranking in the lowest 30% of N'Dama allele score. Individual traits for the two groups were compared in terms of trypanotolerance. Similarly, standardized totals for OTTS and its component group of traits were compared to give an idea of which trait groups contributed most to OTTS. To assess the relationship of N'Dama allele score and body weight traits on overall trypanotolerance, regression/correlation of ONAS was done on OTTS while body weight traits (non trypanotolerant traits) was done separately on the two measures; ONAS and OTTS.

Results indicated that there was a positive correlation between number of N'Dama alleles inherited by an individual BC₁ progeny and trypanotolerance, at individual trait and overall trypanotolerance levels. There was also low correlation/regression of body weight traits with OTTS or ONAS implying that the N'Dama resistance alleles may not be tightly linked to the small body size, and also that the body size difference of the N'Dama and Kenya Boran is not due to a large number of genes of small effects.

It is concluded that simple monitoring of the number of N'Dama alleles at the target chromosomes in a synthetic N'Dama × Boran BC population could provide an efficient cost effective means of increasing

trypanotolerance in the synthetic, while retaining the desirable productive qualities of the Boran.

Materials and Methods

Breeding, phenotyping and phenotype data standardization

Breeding of BC1 (N'Dama × Kenyan Boran) × Kenyan Boran resource population and their management; correcting for batch and gender effects; standardization of trait values were done according to the detailed explanations in Orenge, (2010).

Backcross, BC1 sampling and DNA processing

BC1 progenies were sampled for blood while in Kapiti for DNA processing. At least 20 ml of blood was collected from the jugular vein into at least two 10 ml heparinised vacutainer tubes. During sampling, animals were restrained in a crush pen. After swabbing the area with a piece of cotton wool soaked in methylated spirit, a 19 inch gauge needle fitted with a needle holder was inserted into the jugular vein. Once the vein is properly located and blood spurts out, a vacutainer tube is pressed against the needle inside the needle holder tube and two tubes of blood were collected. The tubes were labeled with a marker pen indicating the ear tag number code of the animal, gender, date of birth, and ID number of the parents (dam and sire). The tubes with blood were slowly swirled and placed in a cool box until transported to the laboratory.

In the laboratory blood from the two tubes was pooled together into 25ml falconer tubes which were labeled exactly as the vacutainer tubes. The blood was stored at -20° C until DNA extraction. DNA was extracted using the salting out procedure, an ILRI protocol based on Sambrook *et al.*, 1998. In this procedure, 40 ml of phosphate buffer saline (PBS) without calcium or magnesium is added to a pellet of frozen blood in a 50 ml falcon tube which is then vortexed to break up the pellet. This was centrifuged at 800g x 10 min at 4 °C and the supernatant discarded. The white pellet was suspended in 9 ml of solution B. The second and third steps above were repeated before 0.5ml of proteinase K/10% Sodium dodecyl sulphate (SDS) solution was added. The mixture was incubated at 58 °C overnight (12-18 hours); 2.7 ml of saturated NaCl was added and shaken vigorously for 15 seconds before another centrifugation at 800g x 10 minutes at 4 °C. The supernatant was now transferred into a new tube and its volume measured. 2 X volume of cold absolute ethanol

was added to the supernatant and mixed gently by inversion. The precipitated DNA was removed into a new 50 ml tube containing 10 ml of 70% ethanol for washing. The clean DNA was then transferred into labeled 5 ml universal tubes and excess 70% ethanol was removed by air drying.

Individual cattle DNA concentrations were determined using a spectrophotometer (genequant®) and standardized to a uniform concentration of 200ng/microlitre by appropriate dilution with Tris EDTA (TE), and placed into 5 milliliter universal tubes properly labeled and kept at -4°C for amplification.

Selection of primers, multiplexing and amplification

Initially, a total of 100 primers covering bovine chromosomes 2, 4, 7, 16 and 17 were selected from www.marc.USDAGov/genome/genome.html and www.cgd.CsIRO.au for optimization. After optimizing, a final selection of 35 primers were made for QTL analysis (Table 1) after dropping the ones that failed to amplify consistently across the samples or lacked polymorphism. The selection of chromosomes (BTA 2, 4, 7, 16 & 17) were based on the fact that they were previously shown to contribute to bovine trypanotolerance with an additive mode of gene action (which could be revealed easily in backcross or half-sib QTL experimental design) albeit in an F₂ population experimental design (Hanotte *et al.*, 2003).

For ease of genotyping, primers were provisionally divided into 11 multiplex groups of 3-6 primers each, based on the published allele sizes and dye color (FAM for blue, PET for red, VIC for green and NED for yellow) such that primers of the same dye color could be in the same group so long as they did not overlap in allele size (Table 1). This enabled all primers in the same group to be co-loaded together providing savings in terms of time and materials.

Table 1. Summary of 35 primers optimized, dye colours, provisional group sizes, allele sizes and PCR program used

Primer	BTA	Position	Dye	Provisional group	Allele1	Allele2	PCR Program used
BM2113	2	92.1	6-Fam	1	123	143	59-54
TGLA159	4	97.3	6-Fam	1	208	236	59-54
RM006	7	22.1	Vic	1	112	126	59-54
ETH11	16		Vic	1	205	211	59-54
BM4117	2	100.9	6-Fam	2	105	117	59-54
ILSTS006	7	116	Ned	2	281	299	59-54
BM8225	16	59.6	6-Fam	2	133	145	59-54
INRA193	17	27.1	Ned	2	142	154	59-54
BMS2782	2	41.7	Ned	3	181	191	59-54
INRA048	16	73	Vic	3	255	303	59-54
BM4440	2	55	Ned	4	121	147	59-54
BM9065	7	102.4	Ned	4	162	184	59-54
INRA053	7	123.5	Vic	4	97	113	59-54
BM1862	17	80.3	6-Fam	4	191	224	59-54
BMS1116	7	30.5	Ned	5	143	151	59-54
BMS2258	7	75	Vic	5	130	162	59-54

(Table 1 continue)

Primer	BTA	Position	Dye	Provisional group	Allele1	Allele2	PCR Program used
BM719	16	78	6-Fam	5	139	161	59-54
ILSTS026	2	7.5	Pet	6	140	162	59-54
ILSTS098	2	55	Ned	6	97	123	59-54
BM7160	7	0	Pet	6	169	189	59-54
BMS2220	17	16.2	Ned	6	90	118	59-54
BMS1907	16	48.2	6-Fam	7	107	119	59-54
BMS1101	17	31.4	Pet	7	139	183	59-50
CSSM042	2	34.4	6-Fam	8	175	219	59-54
HUJ223	17	74.8	Pet	8	150	180	59-54
BM6121	16	12.2	6-Fam	9	138	164	59-54
HUJ625	16	89.5	6-Fam	9	196	224	59-54
BM305	17	51.9	6-Fam	9	101	141	59-54
BL1043	7	134.1	Ned	10	89	125	59-54
BMS1825	17	3.8	Ned	10	148	226	59-54
DIK063	4	7.1	6-Fam	11	144	166	59-54
BMS1172	4	30.8	6-Fam	11	81	103	59-54
CA067	4	51.9	Ned	11	90	102	59-54
BMS2571	4	69.7	Ned	11	158	174	59-54
BMS648	4	87.4	6-Fam	11	148	162	59-54

For each primer pair, DNA Polymerase chain reaction (PCR) optimization was done using a Gene Amp® PCR system 9700 (Applied Biosystems) thermocycler. The amplification reagents were as follows: For one reaction, 5 µl of the Reddy mix {1.25 units of Thermoprime plus DNA polymerase, 1.5m M MgCl₂, 0.2 □M dNTPs (dATP, dCTP, dGTP, dTTP) and a nucleic acid gel stain (GelRed™-10,000X in DMF) was mixed with 0.1 µl each (20 pmol/µl) of forward and reverse primers, 3.8 µl of double distilled water and 1µl of genomic DNA (20ng/µl) making a total reaction volume of 10µl (see Table 2 below).

Table 2. PCR reaction components

PCR Components	One Reaction mix (µl)
ABgene® Reddy mix	5
Forward primer (20 pmol/µl)	0.1
Reverse primer (20 pmol/µl)	0.1
Double distilled water	3.8
Total mix volume	9
DNA (20 ng/ul)	1
Total reaction volume	10

A step down or touch down program of 94 °C initial denaturation for 4 min, annealing at 59 °C-54 °C for 1 minute (at each temperature) for 30 cycles and elongation at 70 °C for 30 seconds was used for amplification as summarised in the steps below:

Step 1.

Initial Denaturation

94 °C for 3 min

Step 2

5 cycles Step down annealing temperature 1 degree/cycle (59-55)

a. 94 °C for 15 s

b. 59 °C for 30 s

c. 70 °C for 30 s

Step 3

30 Cycles constant annealing temperature

a. 94 °C for 15 s

b. 54 °C for 30 s

c. 70 °C for 30 s

Step 4

Final extension

72 °C for 10 min

To confirm PCR product sizes and that amplification of the primers had taken place, the PCR products were electrophoresed by loading 1 µl each of PCR product and a Lambda (L) standard size marker of 1000 base pairs (bp) onto a 1-2 % agarose gel embedded with 2 µl ethidium bromide (density of 1.01g/cm³ at room temperature). The gel was run at 80 volts for 1 hr (5 cm/hr).

Size confirmation was done by checking the position of the PCR product against the size product of the Lambda`standard size marker. Where there was no product recorded, amplification was taken to have failed

and amplification process was repeated at different PCR conditions until a product was obtained or failed repeatedly, in which case the primer pair was dropped.

PCR fragment analysis

PCR fragment analysis was done on the ABI PRISM® 3730 Genetic Analyzer. This is an automated capillary electrophoresis system that detects and analyzes up to 96 capillaries of fluorescently labeled DNA fragments per run. Other components in this system include a computer workstation with Microsoft® Windows® NT operating system, GeneScan® analysis software, capillary array and reagent consumables.

Sample preparation and dilution

The primers are chemically labeled with fluorescent dyes to facilitate the detection and identification of PCR fragments. Each primer is labeled with one dye but up to four dyes can be used for labeling, as noted above: FAM (Blue), VIC (yellow), NED (green) and PET (red). Samples were prepared in 96-well plates.

Each of the PCR (1 µl) products that amplified was diluted using LIZ® 500 and Formamide internal standard which was reconstituted according to the following procedure:

1. 12 µl of LIZ® 500 internal standards was added to 1000 µl of HiDi®-formamide and mixed thoroughly.
2. 9 µl of the above mixture was pipetted into a single well in a 96 well plate.
3. 1 µl of a PCR product was added to the LIZ®-formamide mixture and pipetted into the 96 well plates prepared in step 2 above.
4. The preparation was denatured at 94 °C for 3 min then place on ice, ready for running on the ABI 3730 genetic analyzer.

Sample running

Material from the samples was electrophoretically injected into thin, fused-silica capillaries filled with polymer. Electrophoresis of all samples begun simultaneously when voltage was applied across all capillaries. As the PCR product fragments migrate along the capillaries, shorter fragments move faster than longer fragments. As the fragments enter a detection cell, they move through the path of a laser beam. The

laser light causes the dye on the fragments to fluoresce and the fluorescence is captured by a charge-coupled device (CCD) camera. This converts the fluorescence information into electronic information, which is then transferred to the computer workstation for processing by the 3730 data collection software. After processing of data, it is stored in the instrument database and displayed as an electropherogram plotting relative dye concentration (y-axis) against time (x-axis) for each of the dyes used to label the fragment. Each peak in the electropherogram represents a single fragment. The positions and shapes of the electropherogram peaks were used to determine the fragment profile. The analyzed data was stored as sample files on the hard drive of the computer and analyzed data was viewed with genescan analysis software.

PCR fragment analysis on Genemapper® software version 3.7 Using GeneMapper® software version 3.7, the electropherograms were interpreted with peak detection and quality quantification used in allele calling and collation procedure. Individual genotypes were further counterchecked for consistency against known parental genotypes. The software features include the ability to generate panel data from sample files that have been added to a project and a custom plot functionality that facilitates the discrimination of DNA fragments with the same dye using custom plot colors. The analysis mainly entailed Peak detection, allele calling, peak quality quantification and result reports which were exported in a tab-delimited text file for storage and further analysis. The steps involved include creation of a new project which contains a new kit. Inside the kit is a panel consisting of markers, their dye colors and size ranges. It covers all the markers used in a group of samples and therefore several panels can be made depending on the groups of samples one has for analysis. Also in the new kit are created bins and bin sets.

Tracing N'Dama (and Boran) alleles in the BC1 and computing overall and component ND'ama allele scores

Before tracing N'Dama (and Boran) marker alleles in the BC1, the data were assessed for polymorphic information content, allele frequency, observed heterozygosity, null alleles and Hardy Weinberg Equilibrium and confirmation of integrity of animal identification was confirmed according to Marshall *et al.* 1998; Slate *et al.*, 2000 and Kalinowski *et al.*, 2007.

Tracing N'Dama (and Boran) marker alleles in the BC1 was based on the 13 F1 sire haplotypes which were inferred from the grandparent

genotypes. In the analysis of each BC1 progeny, the transmitted sire haplotypes were inferred and from this and the progeny genotype (d), the dam haplotypes (b) that were transmitted to the progeny were also inferred. From the sire haplotype (c) transmitted, and knowing the origin of the sire haplotype {(a) and (b)}, the progeny genotypes (d) were coded heterozygous Boran/N'Dama (NB) as “1” or homozygous Boran (BB) as “2” (Figures 6 & 7).

Therefore to get the overall “*N'Dama allele score*”, ONAS for each progeny, all “1s” were summed across all the 35 markers in the target chromosomes, while for each of the chromosomes, the corresponding “1s” were summed up to give the five “*component N'Dama allele scores*”.

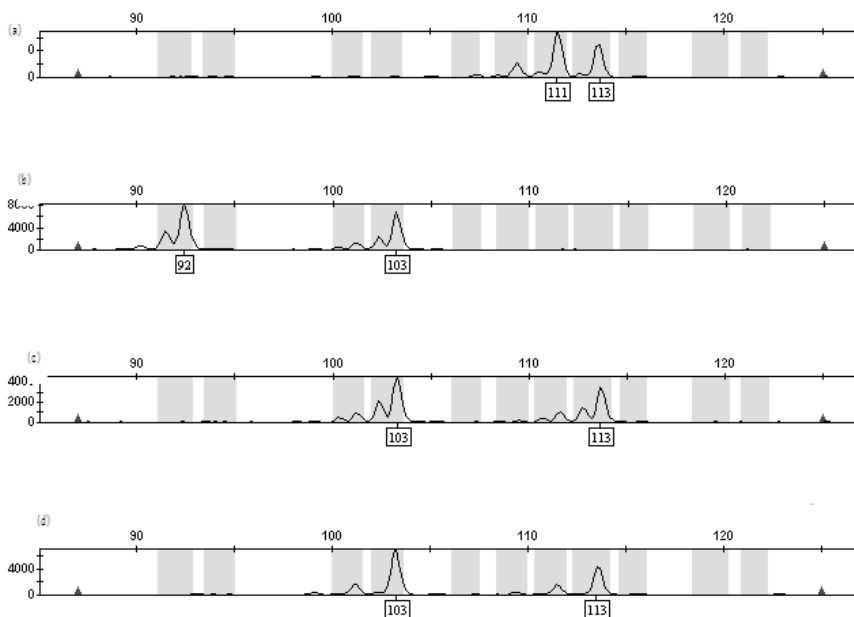


Figure 1. Tracing N'Dama allele 113 at marker BL1043 in the BC1 (d) from the N'dama grandsire (a) and Boran grand dam (b) through the heterozygous F1 sire (c).

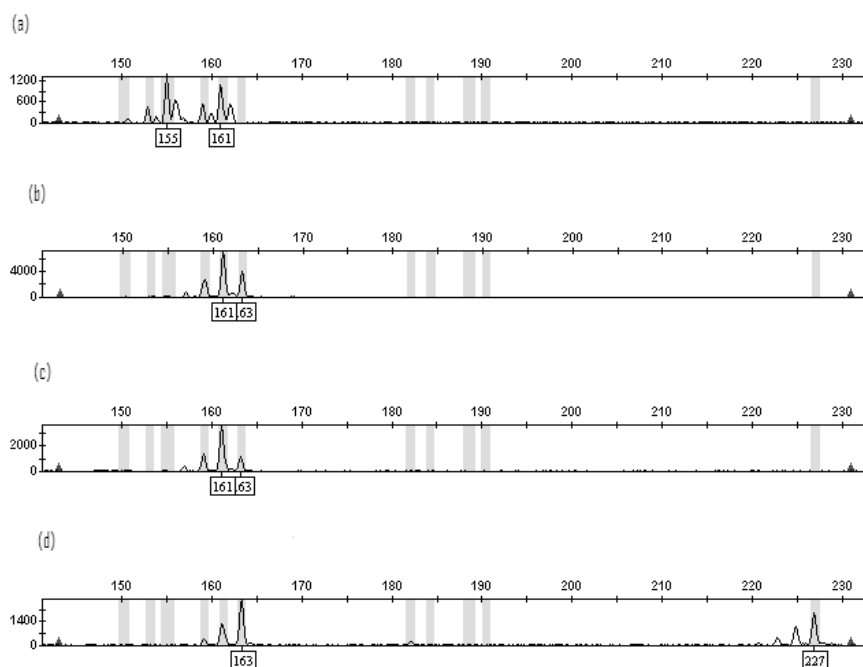


Figure 2: Tracing Boran allele 163 at marker BMS1825 in the BC1 (d) from N'Dama grandsire (a) and Boran grand-dam (b) through the heterozygous F1 sire (c).

Conversion of Low-value traits to High-value trait basis

Trypanotolerant traits (apart from basic body weight traits) in Orege, (2010) were designated either as “High-value” or “Low-value” traits on the basis of trait definition. The “High-value” trait category included traits for which a high numerical value indicated high trypanotolerance (e.g. DF1, days to first infection after exposure to tsetse challenge) while “Low-value” trait category included traits for which a low numerical value indicated high trypanotolerance (e.g. MPAR, mean parasitemia score). This categorization was for the purpose of getting an overall or component trypanotolerance score measure in an individual animal, by simply summing the standardized trait scores for that animal. Therefore, in order to calculate and have this uniform overall trypanotolerance score for each individual (without consideration of individual Low-value traits), the standardized values for the 18 Low-value traits (including traits 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 33, 34, 35, 36 and 49) were

multiplied by (-1) to convert them to the same basis as the High-value traits. In this way, positive values for all traits were associated with positive effects on trypanotolerance. The summed value across all traits was now obtained for each individual, and this represented the overall Trypanotolerance Trait Score (OTTS) of the individual.

In addition, component trypanotolerance scores were calculated according to the four major components of trypanotolerance and one of non-trypanotolerance based on the individual overall trypanotolerance score above: Parasitemia (PAR), including Traits 2, 3, 4, 5, 6, 7 and 8); Treatment (T), including traits 9, 10, 11, 12 and 13); Treatment infection cycle (IC), including traits 14, 15, 16, 17, 18, 19, 20 and 21); PCV (including traits 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 and 34); Body weight change (BWC), including traits 42, 43, 44, 45, 46 and 47; and straight body weight traits (SBWT), including traits 35, 36, 37, 38, 39, 40 and 41) respectively (see Orenge, 2010)

Classification of BC1 on the basis of N'Dama allele/trypanotolerant trait scores and correlations

On the basis of number of overall N'Dama alleles (ONAS) inherited from the F₁ heterozygous sire, BC1 progenies were classified into upper and lower 30% trypanotolerant groups. The high group consisted of 64 BC1 progenies that ranked in the highest 30% of N'dama allele score while the low group consisted of 64 BC1 progenies ranking in the lowest 30% of N'Dama allele score. Corrected (for fixed effects of gender and batch) trait values before scaling were used to generate means, standard deviation within the groups, difference between means of the two groups and standard error of the difference. Using each trait mean and from the definition of traits, each trait was ranked as 1 (more trypanotolerant) or 2 (more susceptible) for each group (high and low). Mean rank of the two groups in terms of trypanotolerance was also calculated and the corresponding test of significance between the two groups was done using a two-sample (unpaired) one sided t-test where the high N'Dama allele group was also the high trypanotolerant group.

Association of overall and component N'Dama allele score with trypanotolerant trait scores were documented using regression/correlation analysis methods in GLM SAS statistical package. Significance association tests ($p < 0.05$, $p < 0.001$) were interpreted as supporting the proposition that trypanotolerance in the BC1 population was determined

to some degree by the proportion of N'Dama genome transmitted from the F1 sire. Likewise, each Component Trypanotolerance Score was analyzed in this manner in relation to Overall N'Dama Allele Score, and Overall Trypanotolerance Score was analyzed in relation to the Component N'Dama allele scores to assess the effect of specific N'Dama chromosomal regions on trypanotolerance. Also computed were trait-trait correlation matrixes of the traits used in the study.

Results and discussion

Classification and selection of BC1 individuals on "ONAS", "OTTS" basis and comparison of trypanotolerance

Table 3 shows the effect of N'Dama origin allele score (ONAS) on trypanotolerance in BC1 to determine its relation to N'Dama genome. The BC1 individuals were classified and selected to High groups (upper 30%) and Low groups (low 30%) on the basis of overall N'Dama alleles scores (ONAS). The batch and gender corrected mean standardized values of the various traits in the two groups were compared. Based on ONAS criteria and considering 41 trypanotolerant traits, mean ranks of the high and low groups were 1.21 and 1.79 respectively, thus the high group was significantly more trypanotolerant ($p < 0.001$ by two unpaired sample t-test) than the low group. On an individual trait basis, the upper group ranked first in terms of trypanotolerance in 34 out of the 41 trypanotolerant traits considered. A very high statistical significance ($p < 0.001$) of the difference between high and low group was found for only four traits i.e. NINF, NT, DC2A and DC12. A high statistical significance ($p < 0.05$) was found in seven traits i.e. NIT, MB, DT1, DC1A, DCIB, DT2 and MPC between the two groups. An almost statistical significant ($p < 0.1$) was found in three traits i.e. DF2, PCI and WTIT1. Therefore out of the 34 traits in which the high group ranked first, only 15 were statistically significant (with at least $p < 0.1$). These are the traits one may be interested in selecting to get a highly trypanotolerant herd based on the overall N'Dama allele score for the five target chromosomes in the BC1 cattle population. The low group ranked first in 6 out of 41 trypanotolerant traits with only one trait (PCIF1) almost statistically significantly ($p < 0.1$). Thus the overall pattern of results from this table showed that N'Dama markers origin alleles were positively associated with an overall and individual trait trypanotolerance levels in the BC1 population under natural tsetse and trypanosomosis challenge.

Table 3: Comparison of groups having high and low overall number of N'Dama alleles (ONAS) according to trait. Mean, mean actual corrected trait value in the High or Low group, respectively. D and SE(D) difference between group means (High – Low) and SE of the difference. D', difference between groups standardized against overall trait mean and standard deviation. R/S: R, High ONAS group is more resistant than the Low ONAS group; S, High group is more susceptible than the Low group.

No.	Trait	Mean		D	SE(D)	p-value	D'	R/S
		High	Low					
1	ONAS	19.45	6.64	12.81	0.55	<0.001		
2	STR	18	20.7	-2.69	1.19	0.026	0.37	1**
3	STRTV	14.57	16.24	-1.66	0.92	0.078	0.3	1*
4	STRTC	3.43	4.46	-1.03	0.62	0.099	0.27	1*
5	TPS	44.33	51.06	-6.73	2.81	0.018	0.38	1**
6	MPAR	2.6	2.5	0.1	0.09	0.257	-0.2	2
7	%PARD	36.17	37.91	-1.75	2.16	0.752	0.05	1
8	NINF	4.48	5.35	-0.88	0.29	0.003	0.48	1**
9	NT	4.74	6.02	-1.28	0.33	<0.001	0.67	1***
10	NT1	2.07	2.24	-0.17	0.23	0.448	0.14	1
11	NIT	0.75	0.73	0.02	0.03	0.604	0.1	1
12	MNT	0.1	0.1	0	0.02	0.875	0.03	2
13	MNTI	0.04	0.04	0	0.02	0.998	0	"="
14	DF1	13.38	11.06	2.32	1.74	0.184	0.2	1
15	DT1	30.95	13.06	17.88	6.99	0.012	0.41	1**
16	DC1A	44.58	24.14	20.44	14.25	0.004	0.46	1**
17	DF2	34.56	27.39	7.17	4.02	0.078	0.3	1*
18	DCIB	63.73	37.95	25.78	9.01	0.005	0.48	1**
19	DT2	33.99	19.72	14.28	10.5	0.177	0.23	1
20	DC2A	66.77	46.13	20.64	11.8	0.084	0.31	1*

Table 3 (Continue)

No.	Trait	Mean		D	SE(D)	p-value	D'	R/S
		High	Low					
21	DC12	110.31	67.16	43.15	14.25	0.003	0.52	1**
22	PCI	32.42	31.49	0.93	0.81	0.254	0.2	1
23	MPC	24.39	23.66	0.74	0.33	0.026	0.36	1
24	MXPC	33.56	31.86	1.7	0.61	0.006	0.48	1**
25	MNPC	15.85	16.18	-0.34	0.48	0.482	-0.02	2
26	PCSR	31.28	30.26	1.02	0.73	0.165	0.26	1
27	PCF1	26.72	26.1	0.62	0.86	0.472	0.13	1
28	PCT1	17.42	17.24	0.18	0.41	0.665	0.07	1
29	PCF2	25.33	23.54	1.79	0.82	0.03	0.42	1**
30	PCT2	17.68	17.12	0.56	0.45	0.22	0.25	1
31	PCIF1	-6.62	-6.07	-0.55	1.01	0.591	0.09	2
32	PCF1T1	-8.58	-8.06	-0.52	0.82	0.527	0.12	2
33	PCIT1	-15.13	-14.02	-1.12	0.91	0.221	0.21	2
34	PCT1F2	8.44	6.84	1.6	0.8	0.048	0.36	1**
42	WTIF1	-4.9	-11.91	7.01	4.2	0.099	0.33	1*
43	WTIT1	-18.56	-32.4	13.83	5.7	0.019	0.63	1**
44	WTC	-5.5	-6.33	0.83	3	0.783	0.05	2
45	WTC-W	-0.4	-0.2	-0.2	0.17	0.237	-0.19	2
46	WTF1T1	-10.29	-12.26	1.98	2.95	0.506	-0.26	1
47	WTT1F2	0.12	-4.5	4.62	5.74	0.432	0.38	1

Table 4: Comparison of total standardized score of groups having High and Low overall number of N'Dama alleles (ONAS) according to Overall standardized Trypanotolerance Trait Score (OTTS) and its components. N, number of traits in the trait group. D' and SE(D') difference between High and Low ONAS groups in standardized trait score and its standard error. No., number of traits in the trait group. Mean D', average D' /trait for the trait group. R/S: R, High ONAS group is more resistant than the Low ONAS group; S, High group is more susceptible than the Low group.

Trait group	N	High	Low	D'	SE(D')	p-value	R/S	Mean D'
ONAS		19.45	6.64	12.81	0.55	<0.001	1***	
OTTS	38	3.90	- 3.97	7.87	1.90	<0.001	1***	0.207
PAR	7	0.85	- 0.81	1.66	0.72	0.022	1**	0.237
PCV	13	1.74	- 0.89	2.62	1.06	0.015	1**	0.202
SBWT	7	0.05	0.56	-0.51	0.84	0.542		
TIC	8	0.70	- 1.80	2.50	0.72	<0.001	1***	0.313
TREAT	5	0.27	- 0.51	0.78	0.58	0.181	1	0.156
BWC	6	0.34	0.03	0.31	0.43	0.475	1	0.052

*** $p < 0.001$

** $p < 0.05$

* $p < 0.1$

P-values were obtained after performing a two-sample (unpaired) one sided t-test where the high N'Dama allele group and high OTTS value is also the high trypanotolerant group.

Table 4 gives the standardized totals for OTTS and its component group traits according to ONAS. The standardized values were used because of combining different traits for comparison purposes. The mean standardized D values gave an idea of which trait groups contributed most to OTTS. Based on this comparison, the high group was very highly statistically more trypanotolerant ($p < 0.001$) than the lower group in terms of ONAS, OTTS and TIC while it was highly significant ($p < 0.05$) in parasitemia and PCV groups of traits. There was however no statistical significance between the groups in TREAT and BWC

component traits. The picture presented here shows that PAR, PCV and TIC component traits significantly contributed to the Overall Trypanotolerance Trait Score of the animal (OTTS) and these are the traits to be considered in selecting for a trypanotolerant herd based on ONAS.

Overall trypanotolerance on overall N'Dama allele score over the 5 target chromosomes

To further document the association between the two categories of classifications, OTTS data points were regressed onto those of ONAS over the 5 target chromosomes. Results revealed a very high significant relationship ($p<0.001$) between the two categories (Figure 3), although regression/ correlation coefficients were low (0.489 and 0.241 respectively). This results attests to the strong association of N'Dama origin alleles to the overall BC1 cattle trypanotolerance as indicated in the scattergram below.

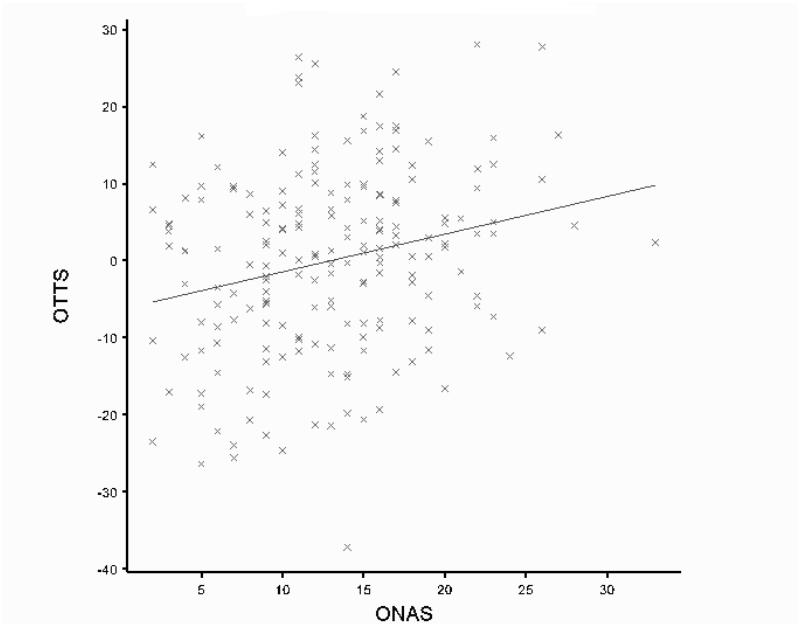


Figure 3: Scattergram of distribution of individual points for Overall trypanotolerant trait scores (OTTS) onto N'Dama allele score (ONAS); correlation coefficient = 0.241 and slope (regression coefficient), $b = 0.489$

Body weight component (mean weight, minimum weight and maximum weight) and maximum body weight of the BC1 on overall N'Dama allele score (ONAS) and Overall trypanotolerant trait scores (OTTS)

To have a better idea of the overall relationship of body weight and trypanotolerance, body weight components (mean weight, minimum weight and maximum weight) of the BC1 were regressed onto ONAS and OTTS. In the first case, regression and correlation coefficients were very low and not statistically significant ($p=0.153$).

Regression of actual values (not standardized values) of maximum body weight on overall N'Dama allele score had no correlation (0.004) and regression coefficient of 0.25, with no statistical significance at all

All these analyses are consistent in showing a strange aspect of the absence of effect of N'Dama alleles on body weight as revealed by very low correlation and regression values. The very low correlation and regression of body weight group and maximum body weight on "ONAS" is potentially important. It implies that N'dama resistance alleles are not tightly linked to the small body size, and also that the body size difference of the N'Dama is not due to a large number of genes of small effect. Also it seems to imply that the weight difference between N'Dama and Boran is not due to the chromosomes that carry trypanotolerance alleles. Equally important is also that weight difference between N'Dama and Boran may be due to only a small number of QTLs.

Overall trypanotolerance trait scores (OTTS) on N'Dama allele score components

Regression analysis to evaluate the effect of specific chromosomal N'Dama alleles components on overall trypanotolerance of the BC1 as given by overall trypanotolerance score (OTTS), revealed a significant association with BTA 4 N'Dama allele components ($p = 0.043$, regression coefficient of 0.962, correlation of 0.15) and highly significance association ($p=0.004$, regression coefficient of 0.962, correlation of 0.21) with BTA17. Correlation coefficients between OTTS and the two N'Dama allele components (BTA 4 and BTA 17) that showed significance association were statistically similar (0.15 and 0.21

respectively). This would mean that BTA 4 and BTA 17 exerted some substantial control on the overall trypanotolerance of the BC1 population. It is interesting to note that one of these chromosomes, BTA 17 revealed tremendous contribution to overall cattle trypanotolerance in the F2 population in the artificial challenge (Hanotte et al., 2003).

Regression of component trypanotolerance scores on Overall N'Dama allele score

Regression of various trypanotolerance score components on ONAS to show effect of the overall N'Dama genome on various trypanotolerant group traits revealed a significance association affecting treatment infection cycles ($p=0.045$) and an almost significance association with PCV($p = 0.094$) with overall N'Dama allele scores. Regression coefficients were 0.197 and 0.131 respectively for the two groups of traits, with overall N'Dama allele score. Correlation between treatment-infection cycles and PCV with overall N'Dama allele score were low and statistically similar (0.16 and 0.14 respectively while correlation between these trait groups was also moderately low, 0.34.

This is plausible since treatment-infection cycle group of traits were measured mainly based on PCV group of traits and hence the two were moderately related.

Table 5: Regression/correlation results and *p*-values of component trypanotolerance scores on BTA2, 4, 7 16 & 17 component N'Dama allele scores respectively

Chromosome N'Dama allele component	Component trypanotolerant trait score	Correlation coefficient	Regression coefficient	<i>p</i>-value
BTA2	PAR	-0.119	-0.222	0.101
	PCV	0.156	0.404	0.031
	TIC	0.051	0.105	0.485
	TREAT	0.105	0.148	0.147
	BWC	0.001	0.001	0.990
	BWT	-0.175	-0.365	0.015
BTA4	PAR	0.085	0.196	0.239
	PCV	0.147	0.468	0.043
	TIC	0.086	0.220	0.234
	TREAT	0.036	0.063	0.617
	BWC	0.037	0.052	0.615
	BWT	0.051	0.130	0.486
BTA7	PAR	0.044	0.075	0.548
	PCV	0.16	0.382	0.026
	TIC	0.091	0.173	0.210
	TREAT	0.760	0.098	0.296
	BWC	0.006	0.006	0.935
	BWT	0.109	0.208	0.132
BTA16	PAR	-0.004	-0.007	0.954
	PCV	-0.056	-0.131	0.438
	TIC	0.084	0.157	0.245
	TREAT	0.003	0.004	0.967
	BWC	0.018	0.019	0.799
	BWT	-0.006	-0.012	0.930
BTA17	PAR	0.193	0.314	0.007

	PCV	0.111	0.250	0.126
	TIC	0.081	0.146	0.263
	TREAT	0.004	0.005	0.959
	BWC	0.069	0.069	0.345
	BWT	-0.011	-0.019	0.883

Regression of component trypanotolerance trait scores on component N'Dama allele scores and correlations

Table 5 shows regression analysis results of different trypanotolerance trait score components onto various chromosomal N'Dama allele score components.

N'Dama allele score components of BTA 2 had an almost significant ($p = 0.089$) control on parasitemic group of trypanotolerant traits and a highly statistical significant ($p = 0.011$) control of body weight group of traits. BTA 4 N'Dama allele component did not exert any significant control on any of the trypanotolerant trait components while BTA 7 N'Dama allele components exerted an almost significant influence on body weight group of traits. N'Dama allele components of BTA 16 exerted an almost significant ($p = 0.092$) control of change in body weight group of traits during infection. N'Dama allele components of BTA 17 had a very high significant ($p = 0.007$) control of parasitemic group of trypanotolerant traits and an almost significant control ($p = 0.087$) on treatment-infection cycle group of trypanotolerant traits. The above regression analysis findings

agrees in principle with previous study of F2 population (Hanotte *et al.*, 2003) where it was shown that body weight group of traits were controlled by alleles in BTA 2, 7 and 16 while BTA 17 mainly controlled parasitemic traits.

Table 6 shows a Pearson correlation matrix of overall and component trypanotolerant trait scores (OTTS, PAR, PCV, TIC, TREAT, BWC) and non trypanotolerant component of straight body weight traits (BWT) and overall N'Dama allele score (ONAS) in the five target chromosomes of BC1 cattle population. There were high correlations of OTTS with treatment-infection cycle ($r = 0.66$), PCV ($r = 0.72$) and TREAT ($r = 0.63$), moderate low correlations ($r = 0.37$) with parasitemic (PAR) groups of trypanotolerant traits. This finding implied that overall trypanotolerant trait scores were influenced proportionately by these traits. OTTS also had moderately low but positive correlation of 0.21 with BWC component traits meaning that the latter had low moderate influence on the former. PCV component trait score had a moderate positive correlation of 0.37 and 0.28 with treatment-infection cycle, TIC and TREAT components trait scores, respectively. This is plausible as most of the treatments were based on PCV thresholds. At the same time PCV and TIC had no or negative correlation with BWC as

expected. There was generally lack or low correlation between ONAS and trypanotolerant trait components.

Table 6: Correlation coefficients of 5 trypanotolerance and one non-trypanotolerance component scores with one another and with overall trypanotolerance score and overall N'Dama allele score.

	ONAS	OTTS	PAR	PCV	BWT	TIC	TREAT	BWC
ONAS	1							
OTTS	0.241**	1						
PAR	0.086	0.373***	1					
PCV	0.206**	0.72***	-0.031	1				
BWT	-0.014	-0.037	-0.235	0.19*	1			
TIC	0.163	0.661***	0.032	0.287***	-0.059	1		
TREAT	0.09	0.633***	-0.028	0.378***	0.006	0.416***	1	
BWC	0.055	0.211**	0.066	-0.01	-0.113	-0.094	0.01	1

* P<0.1 *** p<0.05 ***p<0.001

In conclusion, results clearly show a strong association of Overall trypanotolerance with number of N'Dama alleles inherited. The results also show that control of Parasitemia and PCV group of traits contributes greatly to the overall trypanotolerance of the animal. These traits have been associated with trypanotolerance in the past (Murray *et al.*, 1981). Monitoring of number of N'Dama alleles inherited in the target chromosomes may be an efficient and cost effective means of improving trypanotolerance in a Boran based backcross in the humid and sub-humid tsetse infested areas of East Africa.

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**ENQUETES PRELIMINAIRES SUR LA
TRYPANOSOMOSE BOVINE ET LA
CHIMIORESISTANCE DANS LE CERCLE DE SIKASSO
AU MALIEN PRELUDE A UNE ACTION DE LUTTE
ANTIVECTORIELLE/**

**BASELINE SURVEY ON BOVINE TRYPANOSOMOSIS
AND CHEMORESISTANCE IN THE SIKASSO CERCLE
OF MALI AS A PREAMBLE TO A VECTOR CONTROL
OPERATION**

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Summary

The Sikasso circle lies in the cotton belt of Mali, in which an ILRI/BMZ project and its partners have been conducting trypanocide resistance characterisation and control studies since 2002. Several resistance and high prevalence foci have been detected within Sikasso. The project is supporting community-led vector control efforts to reduce trypanosome transmission rates and use of trypanocides to contain and eventually reverse trypanocide resistance. A baseline epidemiological evaluation was conducted before commencement of vector control to generate data to form the basis of assessing the outcome of the vector control initiative.

The baseline data collected included trypanosome prevalence, tsetse densities and trypanocide resistance levels. Surveys were conducted in 8 villages, 4 from the area selected for vector control and 4 others from the planned control area. A sample of 100 cattle from each village was bled and blood examined using the buffy coat technique (BCT) for trypanosome detection. Tsetse densities were established by deploying traps along rivers in sites most epidemiologically at risk for 24 hours before the tsetse catches were separated into their respective species and sexes and counted. Resistance levels were assessed by treating infected cattle with diminazene (3.5 mg/kg) or with isometamidium (0.5mg/kg) and monitoring for trypanosome parasite relapses through parasitological checks conducted at day 14 and day 28 post-treatment. Trypanosome prevalence ranged between 11 and 16% in the site intended for vector control and 14 and 19% in the planned control area with *Trypanosoma congolense*, causing more than 70% of infections in both areas. Tsetse apparent densities ranged between 4 and 20 flies/trap/day in the vector control zone and 4 - 15 flies/trap/day in the non-control zone. Two tsetse species, *Glossina palpalis gambiensis* and *G. tachinoides* were found in the catches, and the first was the dominant species. Diminazene treatment failure rate was 33% and 26% for the vector control and non control zones respectively, during day 14 check. Isometamidium registered 61% and 41% failure rate at day 28 post treatment in the control and non-control zones, respectively. The situation as hereby established largely justifies the current vector control operation in which the collected data will be useful for the assessment of the end result of this operation.

Key words: Bovine trypanosomosis, chemoresistance, vector control, Sikasso, Mali.

Résumé

Le cercle de Sikasso se situe au cœur de la zone cotonnière du Mali, où, depuis 2002, s'effectuent des recherches sur la caractérisation et la gestion de la résistance aux trypanocides vétérinaires. Ces recherches, réalisées par le projet ILRI/BMZ et ses partenaires, ont permis de détecter, dans le cercle de Sikasso, plusieurs foyers de chimiorésistance ainsi que des prévalences élevées de la trypanosomose chez les bovins. Une lutte antivectorielle s'appuyant sur la participation communautaire y a été recommandée pour contenir la résistance et éventuellement obtenir

sa réversion à travers une réduction du niveau de transmission des infections et de celui des traitements trypanocides. Avant le démarrage de cette action, une évaluation épidémiologique a été réalisée en vue de l'établissement de données de base devant servir à vérifier les résultats de la lutte. Ces données portent essentiellement sur la prévalence des infections, l'abondance des tsé-tsé et les niveaux de résistance des trypanosomes aux trypanocides. Les enquêtes ont été réalisées dans 8 villages du cercle de Sikasso dont 4 choisis en zone de lutte et 4 en zone témoin. La prévalence de l'infection a été mesurée sur un échantillon de 100 bovins par village, utilisant la technique du « buffy coat » pour la recherche de trypanosomes. L'abondance des tsé-tsé a été évaluée par piégeage durant 24 heures le long des rivières à des points considérés comme épidémiologiquement dangereux. Les insectes capturés ont ensuite été triés selon l'espèce et le sexe, puis dénombrés. Quant aux niveaux de résistance, ils ont été évalués par la recherche de rechutes parasitologiques chez des sujets infectés et traités, soit au Diminazène (3.5 mg/kg), soit à l'Isometamidium (0.5mg/kg) et examinés à Jour 14 et Jour 28 après traitement.

Les prévalences observées ont varié entre 11 et 16 p.100 dans la zone prévue pour la lutte et entre 14 et 19 p.100 dans la zone prévue pour servir de témoin avec une prédominance de *Trypanosoma congolense*, cause de plus 70p100 des infections dans les deux zones. Les densités de tsé-tsé (DAP) y ont varié respectivement entre 4 et 20 gl/p/j et entre 4 et 15 gl/p/j. Les captures contenaient deux espèces, *Glossina palpalis gambiensis* et *Glossina tachinoides*, avec une prédominance de la première. Les taux d'échecs de traitement dans la zone prévue pour la lutte et dans celle prévue pour servir de témoin ont été respectivement de 33p.100 et 26 p100 pour le diminazène à J14, et de 61p.100 et 41p.100 pour l'isometamidium à J28.

La situation observée justifie largement une action de lutte antivectorielle dans le cercle de Sikasso et les données recueillies serviront de référence pour juger des résultats de cette action.

Mots-clés, Trypanosomose bovine, chimiorésistance, lutte antivectorielle, Sikasso, Mali.

Introduction

Le projet ILRI/BMZ a réalisé, entre 2002 et 2006, des études sur la résistance aux trypanocides dans certaines zones cotonnières de l'Afrique

de l'Ouest. Ces études ont permis de mettre en évidence plusieurs foyers de chimiorésistance dans le cercle de Sikasso, au Mali (1,2). En conséquence, le même projet renouvelé pour une deuxième phase de trois ans (2006-2009) a décidé d'entreprendre une lutte contre les vecteurs dans un secteur d'environ 500 km² dans le cercle de Sikasso à la frontière entre le Mali et le Burkina Faso. Cette lutte antivectorielle se réalise contre *Glossina palpalis gambiensis* et *G.tachinoides*, et avec la participation des communautés. *G. morsitans submorsitans*, jadis présente dans la zone, a disparu (3), suite à la dégradation de son habitat et à la raréfaction de la grande faune sauvage. La lutte contre le vecteur a été conçue pour pouvoir évaluer son aptitude à limiter la propagation de la résistance, et éventuellement, à induire sa réversion, à travers la réduction de la transmission et la fréquence des traitements trypanocides.

L'étude, objet du présent rapport, a pour but de dresser la situation de départ en ce qui concerne l'abondance des tsé-tsé, la prévalence de l'infection et les niveaux de résistance aux trypanocides. Les données générées seront utilisées pour évaluer l'efficacité de la lutte au plan entomologique, parasitologique et de la résistance en les comparant à celles qui seront générées au cours et à la fin des opérations de lutte.

Matériel et Méthodes,

L'étude comprend une enquête transversale destinée à mesurer les densités des tsé-tsé et les prévalences des infections dues aux trypanosomes chez les bovins, et une enquête longitudinale destinée à déterminer le taux des infections résistantes aux traitements trypanocides.

Enquête transversale

Cette enquête a été réalisée, entre Novembre et Décembre 2007, dans 4 villages sentinelles choisis dans la zone de lutte (secteur Est) et 4 autres villages témoins choisis dans une zone distante de quelques dizaines de kilomètres et où aucune lutte antivectorielle n'est opérée (secteur Ouest). L'enquête comprend un volet entomologique et un volet parasitologique.

Entomologie

Pour mesurer les densités de tsé-tsé, 8 pièges ont été placés, pendant 24 heures, le long des galeries traversant les terroirs des 8 villages étudiés à

des points épidémiologiquement dangereux, identifiés avec l'appui des populations. Les captures ont permis de calculer les densités moyennes (nombre de mouches/piège/j) par village et par secteur. Elles ont aussi permis de déterminer la fréquence relative des différentes espèces de tsé-tsé présentes dans la zone.

Parasitologie et Hématologie

Dans chacun des 8 villages retenus, 100 bovins représentant entre 33-100% du troupeau villageois ont été examinés pour la recherche de trypanosomes et pour la détermination des valeurs de l'hématocrite (PCV). La méthode utilisée est celle du « Buffy coat » (4) appliquée à des échantillons sanguins prélevés à la veine jugulaire dans des tubes contenant un anticoagulant (EDTA). La lecture des PCV et l'examen au microscope des échantillons sanguins ont été réalisés sur place, en utilisant un groupe électrogène pour alimenter les microscopes et la centrifugeuse à hématocrite. Au total 798 bovins ont été ainsi examinés. Pour chaque animal trouvé infecté de trypanosomes, des gouttes de sang ont été déposées sur papier filtre pour des tests ADN à réaliser ultérieurement en vue de détecter des marqueurs de résistance.

Enquête longitudinale:

Cette enquête, réalisée en Décembre 2007, a pour but de détecter des échecs de traitement chez les animaux trouvés infectés au cours de l'enquête transversale et traités soit au Diminazène (Veriben, Sanofi), soit à l'Isoméamidium (Trypamidium, Merial). En effet, dans chaque village concerné par l'enquête transversale les positifs parasitologiques ont été partagés au hasard entre deux groupes dont l'un a été traité avec du Diminazène (3.5 mg/kg, sol. de 7%, en i.m) et l'autre avec de l'Isoméamidium (0.5mg/kg, sol. 1%, i.m). Pour estimer le poids des animaux, nous avons procédé à la mesure du périmètre thoracique. Les mesures obtenues ont été converties en poids, grâce à un abaque conçu pour le zébu local, génotype largement dominant dans les troupeaux villageois.

Des contrôles parasitologiques ont été réalisés aux 14^{ème} et 28^{ème} jours (J14 et J28), suivant les traitements, et les infections détectées ont été enregistrées.

Pendant ces deux contrôles, du sang de chaque animal, positif ou non, a été déposé sur papier filtre et conservé pour des analyses ultérieures d'ADN.

Tous les animaux positifs au cours de ces contrôles ont été traités au Diminazène à la double dose (7mg/kg)

Pour le Diminazène dont la durée de protection est d'environ 3 semaines, le taux d'échec a été calculé au contrôle de J14

Pour l'isoméтамidium dont la durée de protection pour une dose de 0,5 mg/kg est estimée à 2 mois, le calcul du taux d'échec été effectué au contrôle de J28.

Pour le calcul des taux d'échec (Nb positifs/Nb traités), les positifs sont comptés une seule fois au cours du suivi. Les bovins disparus avant d'être positifs sont exclus de l'effectif de même que ceux absents au premier contrôle et négatifs au deuxième contrôle. Par contre les bovins positifs à l'un des contrôles et absents à l'autre sont maintenus dans l'effectif. Un taux d'échec de 25 p.100 ou plus est considéré comme un indicateur de résistance.

Résultats

Enquête transversale,

Entomologie

Deux espèces de glossines ont été capturées le long des galeries ; il s'agit de *Glossina palpalis gambiensis* et de *G.tachinoides* avec une prédominance de la première qui constitue environ les deux tiers des captures. Les densités moyennes, toutes espèces confondues ont été de 10,87 gl/piège/j dans le secteur Est et 7,25 gl/piège/j dans le secteur Ouest (voir Tableau I).

Tableau I: Enquête transversale entomologique, novembre-décembre 2007, Sikasso

Secteur/sites	<i>G.tachnoides</i>	<i>G.palpalis</i>	Total capturé	DAP (8 pièges)
Secteur Est				
Kafela	26	27	53	6.625
Finibougou	64	94	158	19.75
Farako	37	63	100	12.5
Ziebougou	2	35	37	4.625
Total	129 (37%)	219 (63%)	348 (100/%)	10,87
Secteur Ouest				
Diassadiè	22	43	65	8.125
Waibera	47	72	119	14.875
Kapala	1	15	16	2
Tiogola	22	10	32	4
Total	92 (40%)	140 (60%)	232 (100%)	7,25

Parasitologie et Hématologie,

Prévalence et espèces de trypanosomes:

Dans tous villages concernés par l'étude, la prévalence peut être considérée comme élevée, car supérieure à 10%. Comme indiqué dans le tableau II, les prévalences ont varié entre 11 et 16%, avec une moyenne de $13,9\% \pm 3,5$ dans le secteur Est et entre 14-19%, avec une moyenne de $17,5\% \pm 3,8$ dans le secteur Ouest. La comparaison de ces prévalences ne montre pas de différence significative entre les deux zones au risque de 5%.

T.congolense a été l'espèce la plus fréquemment rencontrée représentant 74,5% des infections dans le secteur Est et 71,4% dans le secteur Ouest. Le reste des infections est dû à *T.vivax*. *T. brucei* n'a pas été détectée au cours des enquêtes, et toutes les infections observées étaient monospécifiques.

Tableau II: Enquête transversale parasitologique, novembre-décembre 2007, Sikasso

Secteur /sites	Effectif bovins examinés	Positifs	% prevalence
Secteur EST			
Kafela	100	14	14
Finibougou	100	16	16
Feraku	96	14	14.6
Ziebougou	100	11	11
Total	396	55	13.9
Secteur Ouest			
Dissadie	100	19	19
Ouahibera	100	18	18
Kapala	100	14	14
Tiogola	100	19	19
Total	400	70	17.5

Hématocrite et anémie:

Si l'on considère comme anémiés tous les bovins présentant un PCV < 25%, on peut dire que dans le secteur Est 30 % des bovins examinés sont anémiés contre 36 % dans le secteur Ouest, la différence entre les deux zones n'est pas significative au risque de 5%. Le pourcentage global de bovins anémiés est d'environ 33%.

La comparaison entre le pourcentage de bovins anémiés et infectés d'une part, et d'autre part le pourcentage de bovins anémiés et indemnes, a donné les résultats suivants,

- 72 anémiés parmi les 125 bovins infectés (57,6 %)
- 190 anémiés parmi les 671 bovins indemnes (28,3%)
- $\varepsilon = 6,4$, différence fortement significative ($P < 0,001$)

Si l'on considère que 28,3% des bovins infectés présentent une anémie d'origine non trypanosomienne, on peut estimer que près de 30% des bovins infectés ont une anémie d'origine trypanosomienne.

Enquête longitudinale:

Tableau III: Enquête longitudinale sur la résistance aux trypanocides, 2007, Sikasso

Secteur /sites	Pos. J0	Pos. J14	Pos. J28	Infections
DIMINAZENE				
Secteur Est				
Kafela	7	5	1	6
Finibougou	7	1	1	2
Farako	7	2	0	2
Ziebougou	5	1	0	1
Total pos.	26	9	2	11
% Echecs		34,6	7,7	42,3
Secteur Ouest				
Dissadie	9	2	1	3
Waibera	9	2	5	7
Kapala	7	3	1	4
Tiogola	9	2	1	3
Total pos.	34	9	8	17
% Echecs		26,5	23,5	50
ISOMETAMIDIUM				
Secteur Est				
Kafela	7	1	4	5
Finibougou	8	4	1	5
Farako	7	2	1	3
Ziebougou	6	3	0	3
Total pos.	28	10	6	16
% Echecs		35,7	21,4	57
Secteur Ouest				
Diassadie	10	3	2	5
Waibera	9	0	1	1
Kapala	7	3	0	3
Tiogola	9	2	2	4
Total pos.	35	8	5	13
% Echecs		23	14	37

Pour le diminazène administré à la dose de 3,5 mg/Kg en solution de 7% et en injection intramusculaire, les taux d'échec calculés au 14^{ème} jour après le traitement ont été de 34,6% dans la zone prévue pour la lutte et de 26,5% dans la zone prévue pour servir de témoin.

En ce qui concerne l'Isométnidum utilisé à la dose de 0.5 mg/Kg en solution de 1% et en injection intramusculaire, les taux d'échec sont de 57% dans la zone prévue pour la lutte et 37% dans la zone devant servir de témoin.

Il n'y a pas de différence significative, au risque de 5%, entre les deux secteurs du point de vue de la résistance au Diminazène, comme à l'Isométnidum.

Discussion et Conclusion

Les différences apparentes observées entre les chiffres, dans les deux zones, en ce qui concerne les prévalences des infections, les pourcentages de sujets anémiés et les niveaux de résistance aux trypanocides, ne sont pas statistiquement significatives. On peut donc dire que les groupes de villages choisis dans les deux secteurs sont comparables au départ.

Les résultats parasitologiques confirment la forte transmission de la trypanosomose bovine dans la zone avec des prévalences situées entre 10 et 20%. Ces prévalences élevées, observées avant l'intervention rendent plus aisée la mesure de la réduction de la transmission après intervention ; il est donc important que de telles enquêtes soient réalisées à la période de l'année où le risque est à son maximum, saison post-pluvieuse dans le cas de la zone de Sikasso.

Trypanosoma congolense constitue l'espèce dominante, représentant au moins 70% des infections, le reste étant dû à *T. vivax*. L'absence virtuelle de *T. brucei* pourrait être due à la fois à sa rareté et à la faible sensibilité de la technique utilisée particulièrement pour cette espèce de trypanosome généralement responsable de faibles parasitémiés chez les bovins.

La fréquence relative des différentes espèces pourrait se trouver modifiée à la fin de la lutte, notamment dans le secteur Est où l'abaissement de la transmission cyclique pourrait favoriser la transmission mécanique de *T. vivax*.

Les infections trypanosomiennes sont cause d'environ 30% des anémies constatées chez les bovins infectés et contribuent au taux relativement élevé de bovins anémiés qui est d'environ 33%. D'autres facteurs, notamment alimentaires ou les maladies dues aux helminthes et aux tiques, pourraient aussi y avoir contribué. La comparaison des pourcentages d'animaux anémiés avant et après la lutte et entre les deux

secteurs permettraient de juger de l'impact de la lutte sur cet indicateur très important de l'état de santé des troupeaux.

En ce qui concerne les tsé-tsé, tous les sites en sont infestés avec des densités moyennes variant entre 7 et 11 mouches par piège et par jour dans les deux secteurs. Les captures contiennent seulement deux espèces de glossines, *Glossina palpalis gambiensis* et *G.tachinoides* avec une nette prédominance de la première. Ces densités nous paraissent suffisantes pour faciliter la mesure du degré de suppression des populations de tsé-tsé à la fin de la lutte. Le piégeage de 24 heures, insuffisant pour les prospections entomologiques, peut être intéressant pour des évaluations rapides ciblées sur les points de contacts fréquents entre le bétail et la tsé-tsé.

La méthode utilisée pour évaluer les taux d'échec des traitements trypanocides a révélé des niveaux de résistance importants dans les deux secteurs de la zone d'étude pour les deux trypanocides qui connaissent un usage courant au Mali (Diminazène et Isométydium).

La lutte antivectorielle à entreprendre est supposée aboutir à une réduction de la transmission et du nombre de traitements trypanocides. Il est espéré que la situation de faible risque trypanosomien qui en résulterait puisse, si elle était maintenue, aboutir aussi à une « réversion » de la résistance aux trypanocides, comme cela a été le cas de la résistance à la chloroquine après l'arrêt des traitements au Malawi (5).

Mais la réduction du nombre de bovins infectés, du fait de la lutte, pourrait affecter la pertinence de cette méthode; et, dans ce cas, il serait absolument nécessaire de recourir à des tests moléculaires pour détecter les souches résistantes.

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**OCCURRENCE OF DIMINAZINE, HOMIDIUM AND
ISOMETAMIDIUM RESISTANT TRYPANOSOMA
CONGOLENSE STRAINS ISOLATED FROM CATTLE OF
THE Ghibe Valley of South-Western Ethiopia/**

**PRESENCE DE SOUCHES DE TRYPANOSOMA
CONGOLENSE RESISTANT A LA DIMINAZINE, A
L'HOMIDIUM ET L'ISOMETAMIDIUM ISOLEES DU
BETAIL DE LA VALLEE DE Ghibe ET DES LOCALITES
DU LAC ABAYA, AU SUD-OUEST DE L'ETHIOPIE.**

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Résumé

Une enquête sur le développement de la résistance aux médicaments que sont l'acéturate de diminazène, l'homidium bromide et le chlorure d'isométymidium, par les isolats du *Trypanosoma congolense* de la vallée de Ghibe et de la région du Lac Abaya, au sud-ouest de l'Ethiopie, a été menée dans une structure exempte de mouches, en six groupes de cinq génisses de 12 à 20 mois pour chaque localité, d'avril à juillet 2006. Les isolats des deux zones étaient obtenus à partir d'un pool de cinq bovins parasitémiqes pour le *T.congolense* pour chaque isolat.

Deux régimes de dosage ont été utilisés pour chacun des trois médicaments trypanocides, de 3,5mg/kg poids corporel de et 7mg/kg, 1mg/kg et 2mg/kg, 0,5mg/kg et 1mg/kg pour l'acéturate de diminazène, l'homidium bromide et le chlorure d'isométymidium, respectivement. Des régimes de dosage élevé de chaque médicament ont été utilisés pour identifier les souches résistantes.

Deux isolats ont développé une résistance multiple aux trois trypanocides examinés dans ce test. Il a également été clarifié que l'acéturate de diminazène ne peut pas être utilisé pour éliminer les infections dues au *T.congolense* dans les deux localités pour la

détermination de la fréquence de l'infection pour évaluer la réponse de l'espèce au défi de la trypanosomose dans des conditions naturelles. L'homidium bromide n'a littéralement aucun effet sur les deux isolats, alors que l'acéturate de diminazène et le chlorure d'isoméтамidium avaient des effets partiels et temporaires, en ce sens qu'ils soulagent les animaux affectés, avec une légère supériorité de la diminazène (49% d'efficacité sur les isolats du Lac Abaya et 40% sur les isolats de Ghibe) sur l'isoméтамidium (33% d'efficacité sur les isolats de Ghibe et 28% sur le Lac Abaya).

Les conclusions ci-après ont été tirées de cet exercice,

1. La trypanosomiase dans les deux localités ne peut pas être combattue de manière efficace avec l'utilisation de médicaments uniquement. Aussi, il a été recommandé de tester l'approche de la lutte intégrée, notamment la lutte contre le vecteur et l'utilisation d'espèces trypanotolérants.
2. Aucun des trois médicaments testés ne pouvait être utilisé dans les deux localités comme des pairs sanatives

Il est proposé qu'une enquête plus poussée soit effectuée pour évaluer les régimes de dose double et triple des différents trypanocides à intervalles variables, avec un seul médicament ou des combinaisons de médicaments potentiels. D'après ce test, il a également été jugé essentiel d'utiliser le protocole d'infection -- traitement simultané pour élucider si l'échec médicamenteux est dû à l'inaccessibilité des parasites sequestrés ou bien au développement d'une vraie résistance du parasite aux médicaments.

Mots clés, *Vallée de Ghibe ; Lac Abaya; résistance au médicament ; Trypanosoma congolense ; isolat ; dose discriminatoire ; pair sanative.*

Abstract

This study reports on an investigation on drug resistance development to Diminazine Aceturate, Homidium Bromide and Isometamidium Chloride by *Trypanosoma congolense* isolates from the Ghibe valley of South-Western Ethiopia. The trial was conducted in a fly-free facility in six

groups of five heifers (each 12-20 months of age) from April to July 2006. The isolates were obtained from a pool of five *T. congolense* parasitaemic individual cattle from each site. Two dosage regimes were used from each of the three trypanocidal drugs, 3.5mg/kg and 7mg/kg, 1mg/kg and 2mg/kg and 0.5mg/kg and 1mg/kg body weight for diminazine aceturate, homidium bromide and isometamidium chloride, respectively, with the higher dosage regimes in each drug used for discrimination of drug-resistant strains. The results showed that the isolates have developed multiple drug resistance to all the three trypanocides tried. It also became clear that diminazine aceturate cannot be conclusively used to clear *T. congolense* infections for the determination of infection frequency. Homidium bromide literally did not have any effect on the isolates whereas diminazine aceturate and isometamidium chloride had partial and temporary effect of providing relief to the affected animals with diminazine being slightly superior (49% effective) over isometamidium (33% effective). It was concluded that Trypanosomosis in the area cannot be effectively controlled using these trypanocidal drugs alone. Hence, integrated control approaches including vector control and use of trypanotolerant breeds are recommended. Furthermore, none of the three drugs tested could be used as sanative pairs. It is therefore suggested that further investigations are necessary to evaluate double and triple dose regimes of different trypanocides at varying intervals with single or combinations of potential drugs. Concurrent or simultaneous infection and treatment protocol is recommended to elucidate the unclear situation of whether the treatment failure was due to inaccessibility of sequestered parasites to drugs or due to actual development of resistance by the parasite against the drugs.

Keywords; Drug resistance; T. congolense; discriminatory dose; sanative drug pairs; Ethiopia

Introduction

One of the main problems in the effective control of African Trypanosomosis is the development of resistance by the different species and strains of trypanosomes against any regularly or continuously used drug to control them in affected hosts (animals and humans). Reports are available on the development of trypanocidal resistance by many species of trypanosomes including *T. congolense* in which the phenomenon is commonly encountered (Pinder and Authie, 1984; Sones, 1988;

Sutherland *et al.*, 1991; Ainanshe *et al.*, 1992; Codjia *et al.*, 1993, Mulugeta *et al.*; 1997; Eisler *et al.*, 2001).

Authenticated reports on drug resistance development in Ethiopia date back to the 1970s (Scott and Pegram, 1974; Langridge, 1976) with detection of resistant strains of *T. congolense* against homidium bromide or ETHIDIUM®. This was followed by widespread reports from different parts of the country on the development of resistance to almost all trypanocidal drugs in use at the time such as SURAMIN® or NAGANOL®, TRYPAMIDIUM® or SAMORIN®, BERENIL® and quinapyramine sulphate (ANTRYCIDE®) or quinapyramine sulphate+chloride (ANTRYCIDE PROSALT) (Scott, 1974; Lemecha, 1981 unpublished report; NTTICC, 1986; ILRAD, 1991; Codjia *et al.*, 1993; Rowlands *et al.*, 1995; MoARD, 2004). Multiple drug resistance development by *T. congolense* strain isolates from cattle of the Ghibe valley was confirmed by ILRI scientists since 1989 (Codjia *et al.*, 1993; Mulugeta *et al.*, 1997) and the phenotype was found to be maintained over a long period, as similar situations were expressed by the different molecular karyotypes of *T. congolense* isolates of the same locality between July 1989 and February 1993 (Mulugeta *et al.*, 1997).

A more recent observation on response of *T. congolense* strains to treatment with diminazine aceturate indicated that the parasites are not sensitive to normal curative dose of 3.5mg/kg body weight in cattle, as substantiated by frequent relapses in experimental animals of a joint breed comparison trial for trypanotolerance being conducted by the Ethiopian Institute of Agricultural Research (EIAR) and the International Livestock Research Institute (ILRI) under natural tsetse-trypanosomosis challenges in the Ghibe valley of southwestern Ethiopia. In this research project, which monitors, *inter alia*, the packed cell volume (PCV) values, the number of infected animals (prevalence rates) and the number of treatment assistance each animal and each breed requires at specific intervals, it became increasingly difficult under field (natural) conditions to determine whether detection of trypanosomes in the blood of infected animals after trypanocidal treatment was a new infection or actually a relapse of the previous infection due to failure of the applied drug to clear the parasite. This study was, therefore, conducted to assess the efficacy of diminazine aceturate, homidium bromide and isometamidium chloride commonly used to control cattle trypanosomosis in the country and to evaluate the appropriateness of the use of diminazine aceturate in eliminating trypanosome parasites for experimental animals of the breed

comparison study in order to make accurate estimates of the number of infections and treatment requirements per animal and per breed.

Materials and Methods

*Location and collection of *T. congolense* isolates*

Five animals (A231, G187, S60, S160 and Y23) showing repeated relapses (recurring infections) from the breed comparison trial for trypanotolerance in the Ghibe valley, 230 km south-west of Addis Ababa, were selected after prior checking for *T. congolense* infection using dark ground, phase contrast (DG/PC) buffy coat technique (BCT) according to Murray *et al.*, (1977). Five to ten ml of blood was collected from the jugular vein of each of the five animals aseptically into anticoagulant (Ethyl-diamine tetra-acetic-acid-EDTA) treated vacutainers, which were immediately put in an ice box. The blood on ice in vacutainers was taken within less than 12 hours to the National Animal Health Research Centre at Sebeta, near Addis Ababa. The blood in the five vacutainers was put in screw-cupped 200 ml bottles under aseptic condition of laminar flow cabinet and immediately warmed up (incubated) in a water-bath at 37°C. From the pooled blood of five donors, wet blood film was prepared and examined under the low power objectives (X25 and X40) of the microscope to check for viability (movement) of the isolates, in a manner similar to earlier work by Sinyangwe *et al.* (2004). On the same day 5ml of this pooled blood was intravenously inoculated with disposable syringes and needles into two donor heifers that were previously unexposed to tsetse-borne trypanosomes for which they were checked at least twice before inoculation and kept in fly-proof facility. These two animals were used as donors for the trial on the Ghibe isolate. This isolate was named NAIL-TcG (denoting National Animal Health Research Centre-NAHRC, and International Livestock Research Institute-ILRI *Trypanosoma congolense*, Ghibe).

Experimental animals and treatments

Seventy yearling (12-20 months old) heifers belonging to the local Abyssinian Shorthorn Zebu were physically examined and rectally checked for temperature values in the tsetse-free highland of Sululta locality, some 25 km north of Addis Ababa, before they were purchased for this trial. The animals were ear-tagged, weighed, deticked with

Tactic[®] and dewormed by broad spectrum anthelmintics (Albendazole[®] and Fasinex[®]) after they were brought to the NAHRC premises at Sebeta. They were also vaccinated against pasteurellosis, anthrax and blackleg and treated against tick-borne haemoparasites with long-acting oxytetracycline. The animals were allowed to be acclimatized to the new environment for about a month before being subjected to the trial. After matching for weight (and physical conditions where possible), the experimental heifers were divided into six treatment sub-groups of five animals each, and three heifers as controls.

The treatment structure was as follows:

BERENIL[®] 3.5mg/kg body weight dose, with 5 animals;
BERENIL[®] 7mg/kg body weight dose, with 5 animals;
ETHIDIUM[®] 1mg/kg body weight dose, with 5 animals;
ETHIDIUM[®] 2mg/kg body weight dose, with 5 animals;
SAMORIN[®] 0.5mg/kg body weight dose, with 5 animals
SAMORIN[®] 1mg/kg body weight dose, with 5 animals, and
Control sub-group, no trypanocidal treatment, with 3 animals.

As the sub-grouping based on matched weight and physical conditions were completed, the subgroups were assigned to the 6 treatment regimes at random as stipulated above. Mean initial weight was 73 ± 13 kg. Weight was taken to estimate drug dosage for each animal in each of the groups.

All animals were kept indoors in four fly-proof rooms (barns), with animals inoculated with the Isolates being kept in separate rooms after the final fitness check-up and allocated into the different experimental regimes. All the animals were similarly provided with water and hay *ad libitum*. Any mortality in each of the sub-groups was necropsied, if cadaver was not severely decomposed due to long time lapse after death, to clarify its cause and to keep record of any lesions

Isolate inoculation into experimental animals

When the parasitaemia of the donor animals rose to 10^6 /ml of blood as estimated by the wet film (BCT), they were aseptically bled from jugular vein into vacutainers, which were emptied into sterile 200ml bottles. The blood was then diluted with a mixture of phosphate buffered saline (PBS) and distilled water at a ratio of 2:3 according to Murray *et al.*

(1983). The inoculums contained 5×10^5 to 10^6 /ml of *T. congolense* organisms, as observed in the buffy coat film under the microscope, from which 5ml was inoculated intravenously via the jugular vein into each animal of all the groups including controls.

The animals were monitored every other day for detection of the parasite, rectal temperature, PCV values and any other conditions manifested by the animals. The animals were daily observed for any clinical symptoms and, if necessary, appropriate measures were taken accordingly.

Selected drugs and treatment regimes

The three commonly used drugs for tsetse-borne trypanosomosis control in the country comprising diminazine aceturate (BERENIL[®], Intervet, Germany; VERIBEN[®], CEVA Sante Animale), homidium bromide (ETHIDIUM[®], Laprovvet, France) and isometamidium chloride (SAMORIN[®], M&B; VERIDIUM[®], CEVA Sante Animale) were used at recommended normal and high or discriminatory dose rates in a similar procedure to the work of Mbwapbo *et al.* (1999) in mice, in Tanzania, and of Geerts *et al.* (1999), again in mice on isolates of *T. congolense* from Kenya, Tanzania and Zambia. Thus, diminazine aceturate (BERENIL[®] and VERIBEN[®]) was used at 3.5mg/kg and at 7mg/kg body weight of a 7% concentration as normal curative and as discriminatory doses, respectively. Homidium bromide (ETHIDIUM[®]) was used at 1mg/kg body weight and at 2mg/kg body weight of a 2.5% concentration as normal curative and discriminatory (prophylactic) doses, respectively, and isometamidium chloride (SAMORIN[®]) at 0.5mg and 1mg/kg body weight of a 1% solution as a normal curative/prophylactic and discriminatory (prophylactic) doses, respectively. All drugs were intramuscularly administered deep in the neck and/or rump (hip). As the main objective was to detect whether or not the *T. congolense* strains in the area retained or developed resistance to the trypanocidal drugs currently in use in the country, no attempt was made to reveal the level of resistance at variable dose ranges outside of recommended dosages.

The first treatment regimes started in all experimental groups (except the controls) with the first appearance of trypanosome isolate in the blood of any of the syringe-inoculated animals without waiting for appearance of the parasites in the rest of the groups inoculated with the same isolate on the same day.

Following the administration of respective drugs and drug regimes, each animal was monitored every other day (except week-ends and holidays) for parasitaemia and PCV values using BCT and for temperature variation and any other condition that could be attributable to trypanosome infection and treatment.

Re-treatment to relapses of first treatments was carried out when all animals in the respective treatment sub-group showed recurring parasitaemia. Relapses from lower dose level of each of the drugs were retreated with high doses of same drugs. When relapses occurred to high dose levels of a given drug, high dose levels of alternative drugs amongst the three drugs was used for re-treatment. Normal dose, i.e. 3.5mg/kg body weight of BERENIL[®], for instance, was retreated with discriminatory dose of 7mg/kg body weight BERENIL[®]. Relapses from high (discriminatory) BERENIL[®]-dose group were retreated with high (discriminatory) dose of SAMORIN[®] or VERIDIUM[®]. Relapses to normal dose regime of 0.5mg/kg body weight of SAMORIN[®] were retreated with high dose regime of 1mg/kg body weight of the same drug. Relapses to high SAMORIN[®] dose regime were retreated with high dose of BERENIL[®]. Although treatment regime for ETHIDIUM[®] was similarly planned, relapse treatments did not utilize this drug as it was found to have little effect, if any, on the isolates. The alternate use of drugs against relapses at high dose level was to reveal if the *T.congolense* contained mixtures of susceptible and resistant strains to BERENIL[®] and SAMORIN[®] as there appeared a sharp drop in parasitaemia up to 5-7 days post-treatment. Moreover, alternate application of BERENIL[®] and SAMORIN[®] at high dose levels was employed to assess if these two drugs could still be used as sanative pairs in the area from which the parasites were isolated and possibly elsewhere.

Data analysis

Data was transcribed into computer using the Microsoft Office Excel[®] 2003 software and chi-square (χ^2) test was used to check the statistical significance of differences between the treatment groups in the proportion of post-treatment clearance and relapses to the different drugs and variable dosage regimes at different stages (here chi square was used to qualitative analysis of infected and uninfected groups proportions).

Results

The *T. congolense* strain isolates from the locality has shown marked resistance to the three drugs, *i.e.* diminazine aceturate, homidium bromide and isometamidium chloride, tested in this trial at the standard recommended dosage levels in cattle. This result reconfirmed previous findings (ILRAD, 1991; Codjia *et al.*, 1993) on isolates in the Ghibe valley. In particular, Homidium bromide (ETHIDIUM®) appeared to have very little effect, if any, on the development of *T. congolense* in the infected host (Table I). The 3.5mg/kg body weight dose regime of diminazine aceturate (BERENIL®) showed significantly weaker ($p < 0.001$) initial clearance capacity of parasitaemia and faster post-treatment relapses of *T. congolense* than its higher (discriminatory) dose regime of 7mg/kg body weight. The 3.5mg/kg body weight diminazine aceturate showed its highest trypanocidal effect on the fifth day post-treatment with 80% of treated animals testing negative to BCT. However, all animals in this treatment regime turned *T. congolense* positive by the eighth day post-treatment. BERENIL® discriminatory dose appeared to have cleared *T. congolense* up to the eighth day post-treatment, but by the 12th day post-treatment all animals were found positive to the parasitological (BCT) test (Table I). Last relapse to discriminatory isometamidium chloride dose was on the 17th day post-treatment (dpt).

Considering results from the application of sanative pair procedure, the following observations were made:

- i. Diminazine aceturate (BERENIL® or VERIBEN®) sanative (7mg/kg) dose to normal (3.5mg/kg) BERENIL® or VERIBEN® dose relapsed on the 11th dpt similar to that observed with BERENIL® on SAMORIN® or TRYPAMIDIUM® normal dose on the same *T. congolense* isolate;
- ii. BERENIL® sanative to SAMORIN® discriminatory dose took 15 dpt for total relapse of all treated animals in the sub-group;
- iii. Relapse to BERENIL® sanative on SAMORIN® normal dose was complete by the 10th dpt, and
- iv. Relapse to BERENIL® sanative to SAMORIN® discriminatory was complete by the 12th dpt.

Table I. Treatment response of *Trypanosoma congolense* isolate from the Ghibe valley (NAIL-TcG)

Group	N o	Tc inocul. date	1 st treatment date	% relapse 7dpt	% relapse 14dpt	% relapse 21dpt	2 nd treatm. date	Drug Used	% relapse 7dpt	% relapse 14dpt	% relapse 21dpt
Berenil N*	5	5/25/2006	5/29/2006	100	100	100	6/10/2006	B.S.D	0	100	100
Berenil D**	5	5/25/2006	5/29/2006	0	100	100	6/15/2006	S.S.D .	Not cleared	60	100 on 17 st dpt
Ethidium N	5	5/25/2006	5/29/2006	100	100	100	6/10/2006	B.S.D	20	100	100
Ethidium D	5	5/25/2006	5/29/2006	100	100	100	6/15/2006	B.S.D .	0	100	100
Samorin N	5	5/25/2006	5/29/2006	60	100	100	6/15/2006	B.S.D	20	100 on 11 th dpt	100
Samorin D	5	5/25/2006	5/29/2006	80	80	100 on 17 th dpt	6/15/2006	B.S.D .	20	80	100 on 15 th dpt

Key. N*=normal dose, D**=Discriminatory dose, dpt=days post-treatment, B.S.D. =Berenil sanative dose, S.S.D. =Samorin sanative dose

Excluding the nearly ineffective influence of homidium bromide, the overall efficacy of diminazine aceturate and isometamidium chloride on *T.congolense* isolates of the area from the day of treatment (day one) up to complete relapse of all individuals in each group could be summarized as follows:

- i. Diminazine aceturate 40% (or 30 results out of 75 examinations) turned negative
- ii. Isometamidium chloride 33% (or 27 results from 82 examinations) turned negative

Obviously, all the three drugs were found to be ineffective to control *T. congolense* infected cattle in the area. However, diminazine aceturate and isometamidium chloride appeared to provide temporary relief to the animals from the disease at variable degrees with diminazine aceturate having slightly better remedial effect than isometamidium chloride. There was no significant ($P > 0.05$) difference in effect between diminazine aceturate and isometamidium chloride on Ghibe isolate. There was also no significant ($P > 0.05$) difference of diminazine aceturate on *T. congolense* isolates from the area.

One out of 30 experimental animals died during the course of the trial, and it belonged to homidium bromide normal. The dead animal had high parasitaemia, very low PCV values (anemia), poor body condition and pallor of mucous membranes. Post-mortem lesions were dominated by edema of dependent parts of the body, joints, liver and lungs. Straw

colored pericardial fluid was also encountered together with adhesion with epicardium and adjacent organs. The death appeared to be due to the effects of *T. congolense* infection. It is important to mention that the animals were provided with only good quality dry hay without any supplementation of green fodder or concentrate.

Discussion

This trial reaffirmed and consolidated the widely held opinion on the seriousness of drug resistance development by the pathogenic species of tsetse-borne trypanosomes in general and of *T. congolense* infections in particular affecting several species of livestock in Ethiopia, in line with previous reports by Pinder and Authie (1984), Rowlands *et al.* (1990), ILRA (1991), Ainanshe *et al.* (1992), Codjia *et al.* (1993), Mulugeta *et al.* (1997), Diall *et al.* (2005) and Jamal *et al.* (2005).

In this trial, it was unequivocally made clear that the three most commonly employed trypanocidal drugs for controlling tsetse-borne cattle trypanosomosis, i.e. diminazine aceturate, isometamidium chloride and homidium bromide cannot completely cure animals from *T. congolense* infections in the Ghibe valley at both lower and upper dosages recommended by the manufacturers of the tested drugs. It was also not possible to establish effective and curative doses on both *T. congolense* isolates within dose ranges recommended by the manufacturers of the three drugs. Furthermore, these drugs cannot be used as sanative pairs to clear the resistant *T. congolense* strains in the area as relapses were encountered on all the experimental cases within one month post treatment.

Earlier studies (ILRAD, 1991; Mulugeta *et al.*, 1997) also reported that *T. congolense* isolates from the Ghibe valley had developed multiple resistances to the three drugs, i.e. homidium, isometamidium and diminazine. The report by ILRAD (1991) indicated that 12 of the 13 isolated *T. congolense* parasites from two sites (Ghibe and Tolley) in the Ghibe valley had developed resistance to the three drugs. Mulugeta *et al.* (1997) reported 10 randomly collected *T. congolense* isolates from cattle at Ghibe in February 1993 were all savanna-type strains of *T. congolense* and all were found to be resistant to BERENIL[®] (diminazine aceturate), Samorin[®] (isometamidium chloride) and Novidium[®] (homidium chloride) at doses of 7mg/kg, 0.5mg/kg and 1mg/kg body weight, respectively, for over a long period.

Some recent investigations (Fyumagwa and Silayo, 2003; Munstermann *et al.*, 2003; Silayo *et al.*, 2005) support that repeated applications of trypanocides on resistant strains of trypanosomes at specific intervals could improve their efficacy. Kyumagwa and Silayo (2003) reported that even putative drug-sensitive stock of *T. congolense* IL 3575 relapsed after treatment with diminazine aceturate at 3.5mg/kg body weight, but cured by the same drug at 7mg/kg dose while drug-resistant *T. congolense* ADRI was not cured either at doses of 3.5mg/kg or 7mg/kg body weight in swine. This drug-resistant strain of *T. congolense* (ADRI) is reported to have been effectively treated by triple application of diminazine aceturate at 7mg/kg body weight at 48-hour intervals. Silayo *et al.* (2005) also reported that they were able to effectively cure mice from drug-resistant *T. congolense* infection with diminazine aceturate at 21mg/kg two-dose application in 48 hour-intervals. This same parasite was said to have resisted single treatment by diminazine aceturate at 7mg/kg body weight dose showing the superiority of two-dose application over that of single application.

Combination of drugs and concurrent application is also suggested by some investigators to treat drug-resistant trypanosome strains (Joshua and Babalola, 1983). High level of parasitaemia with resistant *T. congolense* strains was assumed to affect drug efficacy implying that drug efficacy trials should be made on animals with moderate parasitaemia or moderate trypanosome challenge.

We believe that the experimental animals were subjected to a considerably high trypanosome challenge, as the inoculums of each animal received [5ml (5×10^5 - 10^6 /ml)] is believed to be much higher than what they would have acquired and then developed from natural inoculation of infective tsetse fly. Although there is no doubt about the existence of drug-resistant *T. congolense* strains in the isolates from the area, it appears necessary to do more research on the issue to come up with some practical proposition on trypanosomosis containment including the sound utilization of trypanocidal drugs, which are more popular and more practical to clients and to veterinary personnel than other available control measures.

The application of available drugs at specific intervals and the combination of drugs to be alternated or to be concurrently applied under defined conditions warrant further investigation. It is also very useful to

clarify whether the perceived drug resistance is real or due to limited contact of drugs with the parasite that might have lodged in sites inaccessible to the drugs in some body tissues and organs. Some investigators (Whitelaw *et al.*, 1985; Silayo *et al.*, 1992) reported that they came across instances in which relapses to trypanocidal treatments were not due to development of drug resistance by the trypanosomes *per se*, but instead, due to inaccessibility of trypanocidal drugs to some body parts such as the brain where the parasite is sequestered. Simultaneous infection and treatment with the drugs under scrutiny may elucidate the situation of drug inaccessibility and parasite actual response to the full strength of the trypanocide in the body.

Conclusion

Results of this trial confirmed that drug resistance prevails in the study area at presumably very high frequency, seriously impeding trypanosomosis containment endeavors with trypanocidal drugs alone, which make imperative the application of some measures of vector control in the area to reduce losses in production and animals. It also emerged that diminazine aceturate cannot be conclusively used to estimate frequency of *Trypanosoma congolense* infection per specified period for evaluating breed differences in trypanotolerance traits as re-infection cannot be distinguished from relapses to treated infections under natural challenge especially if the detected parasite is of the same species with the pretreatment parasite. Therefore selection and utilization of livestock breeds less affected by the pathogenic effects of trypanosomes needs to be intensified wherever possible in tsetse-trypanosomosis endemic areas.

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Appendix

Anim. Code	Group	Wt.(Kg)	Innocul. Date	1 st Treat. Date	Drug	Days post 1 st treatment											2 nd treat. date	Wt. at 2 nd treatment	Drug Type	Drug Dose	Days post 2 nd treatment													
						1	2	3	4	5	6	7	8	9	10	11																		
																					1	2	3	4	5	6	7	8	9	10	11	12	13	14
59	B.N	50	5/25/2006	5/29/2006	2		+		-			+		+		+	6/10/2006	50 B.S. D	4		+		-		-			+		+				
67	B.N.	69	5/25/2006	5/29/2006	2.		+		-			+		+		+	6/10/2006	69 B.S. D	5.84		+		-		-			-		+				
60	B.N.	69	5/25/2006	5/29/2006	2.		+		+			+		+		+	6/10/2006	69 B.S. D	5.52		+		-		-			+		+				
63	B.N.	81	5/25/2006	5/29/2006	3.		+		-			+		+		+	6/10/2006	81 B.S. D	6.52		+		-		-			+		+				
25	B.N.	97	5/25/2006	5/29/2006	3.		+		-			+		+		+	6/10/2006	97 B.S. D	7.76		+		-		-			+		+				
13	B.D.	65	5/25/2006	5/29/2006	5.		-		-			-		+		+	6/15/2006	65 T.S. D.	6.5		+		+		+			+		+		-		+
6	B.D.	73	5/25/2006	5/29/2006	5.		-		-			-		+		+	6/15/2006	73 T.S. D.	7.3		+		+		+			+		-				+

[illegible]

Key: B.N.=Berenil (Veriben) normal dose; T.N.=Trypanidium (Samorin or Veridium) normal dose; E.N.=Ethidium normal dose; B.D.=Berenil (Veriben) discriminatory dose; T.D.=Trypanidium (Samorin) discriminatory dose; E.D.=Ethidium discriminatory dose; B.S.D.=Berenil (Veriben) sanative dose; T.S.D.=Trypanidium (Samorin) sanative dose

FIELD DETECTION AND EVALUATION OF TRYPANOCIDAL DRUG RESISTANCE IN THE SISSALA EAST DISTRICT OF NORTHERN GHANA/

DETECTION SUR LE TERRAIN ET EVALUATION DE LA RESISTANCE AU MEDICAMENT TRYPANOCIDE DANS LE DISTRICT EST DE SISSALA, AU NORD DU GHANA

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Résumé

Des études sur le terrain ont été menées pour détecter et évaluer la résistance aux médicaments trypanocides par les trypanosomes infectant le bétail dans le District Est de Sissala, au Nord du Ghana. Une étude transversale initiale sur la prévalence et les densités de mouches tsé-tsé dans 14 villages de la zone couverte a été menée pour identifier les sites de prévalence élevée et les foyers suspectés. La prévalence du trypanosome varie considérablement entre les villages, avec deux sites, Kunchogu (22%) et Yugantu (8%) qui enregistrent une prévalence relativement élevée, sur la base de la technique sur couche leuco-plaquettaire de contraste de phase (BCT). Les densités de mouches tsé-tsé variant également de zéro à 6,8 mouches par piège et par jour. Dans le site à risque plus élevé, une étude longitudinale a été menée de Mai à Juillet 2008. 100 bovins ont été choisis au hasard dans le troupeau du village, et ces bêtes ont été marquées à l'oreille et réparties en deux groupes, 50 ont été traitées avec du chlorure d'isométylthiazolium (ISMM) à 1 mg/Kg de poids corporel et 50 ont été gardées pour le groupe témoin. Les deux groupes ont été examinés à des intervalles de 14 à 56 jours,

pour les parasites trypanosomes, à l'aide de la technique BCT. Au cours de la période de suivi, 8 animaux (16%) ont été diagnostiqués positifs dans le groupe témoin, alors que 6 animaux (12%) ont été diagnostiqués positifs dans le groupe traité. Une analyse statistique utilisant le test de Risque Relatif (RR) a montré un taux minimal d'échec de protection de 28% avec les traitements à l'ISMM. Cette étude, ainsi que des données des pays voisins, montre que la résistance aux trypanocides est une menace à prendre au sérieux au Ghana. Les éleveurs de bétail du District Est de Sissala sont particulièrement vulnérables, car la plupart des bovins sont des zébus sensibles à la trypanosomose et les trypanocides demeurent la principale stratégie de lutte contre la trypanosomose du bétail.

Summary

Field studies were conducted to detect and assess trypanocidal drug resistance of trypanosomes infecting cattle in the Sissala East District of northern Ghana. An initial cross-sectional survey of trypanosomosis prevalence and tsetse densities in 14 villages in the study area were carried out to identify high prevalence sites (suspected 'hot spots'). The trypanosome prevalence varied considerably between villages, with two sites, Kunchogu (22%) and Yugantu (8%) recording a relatively high prevalence based on the phase-contrast buffy coat technique (BCT). Tsetse densities also varied from zero to 6.8 flies per trap per day. In the highest risk site, a longitudinal study was conducted from May to July 2008. A total of 100 cattle were randomly selected from the village herd, these cattle were ear-tagged and assigned into two groups, 50 were treated with isometamidium chloride (ISMM) at 1 mg/Kg body weight and 50 were left to serve as control. Both groups of cattle were screened at intervals of 14 days until 56 days for trypanosome parasites using BCT. During this follow up period 8 animals (16%) became positive in the control while 6 animals (12%) were found positive in the treated group. A statistical analysis using the Relative Risk (RR) test showed a minimal protection failure rate of 28% with ISMM treatments. This study shows together with evidence from neighbouring countries that trypanocide resistance is a threat which must be taken seriously in Ghana. Livestock farmers in the Sissala East District are particularly vulnerable because most of the cattle are trypano-sensitive Zebus and trypanocidal drugs remain the main strategy for the control of cattle trypanosomosis.

Introduction

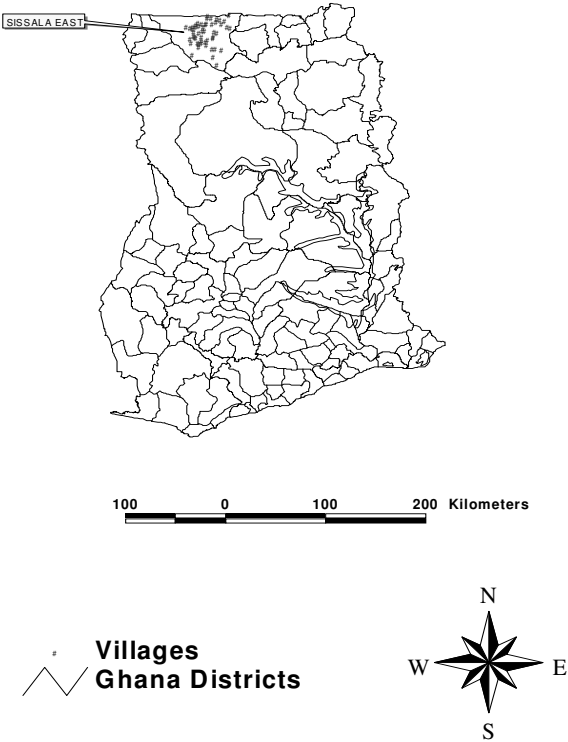
Trypanosomosis is a major constraint to animal health in Sub-Saharan Africa. The control of African bovine trypanosomosis continues to depend in most endemic areas on the use of mainly isometamidium and diminazene. All of these trypanocidal drugs have been in widespread use for over four decades, and the development of drug resistance in the cotton zone of West Africa was first confirmed in Burkina Faso by Authié (1984), Clausen *et al.* (1992) and McDermott *et al.* (2003) and recently in Mali by Diall *et al.* (2003). Anecdotal evidence suggests that the problem of trypanocide resistance may be present in other areas of the cotton zone of West Africa not yet investigated. “Drug resistance was first reported in the 60’s and recent studies provide evidence of the extent and impact of the problem (Diall *et al.*, 2003) as well as trends in key socio-economic drivers (excessive and improper use of drugs, agricultural intensification and shifts to trypanosusceptible cattle) that will continue to increase the risk and spread of resistance. The dependence of smallholder farming on trypanocides, the serious negative effects of drug resistance and the fact that resistance will worsen and spread if nothing is done, warrant action to prevent and contain the problem of drug resistance” (Randolph, 2006). Trypanosome chemoresistance in the cotton zone of West Africa has been for many years a subject of research for German funded research project. There is a need to evaluate the situation in other cotton production areas in the neighbouring countries in order to come out with a more complete picture of trypanosomosis and trypanocidal drug resistance distribution across the cotton belt of West Africa.

This West African trypanocide resistance project is a collaborative research project between the International Livestock Research Institute (ILRI) and research partners in Germany and West Africa, funded by the German Federal Ministry of Economic Cooperation and Development (BMZ). After having evidenced the presence of resistance in Burkina Faso, Mali and Guinea, the project is engaged in resistance evaluation activities in northern Ghana. The main objectives of this research work are: to study the risk factors of trypanosomosis and chemoresistance in northern Ghana; to detect suspected trypanosomosis high risk sites and evaluate trypanocide resistance and to initiate trypanocidal drug resistance risk communication process in Ghana.

Materials and Methods

Description of study area

The study area is located in the Sissala East District in the north-western part of Ghana. The area under study falls within the cotton belt of West Africa. The research area is endowed with Guinea Savannah agro-ecological systems, more draught animals and wooded savannah vegetation with many wetlands patches and small strips of gallery forest along river banks. The study area has a sub-humid climate with two main seasons: a dry season from October to May and a wet season from June to September. Zebu cattle predominate in the Sissala East District of northern Ghana.

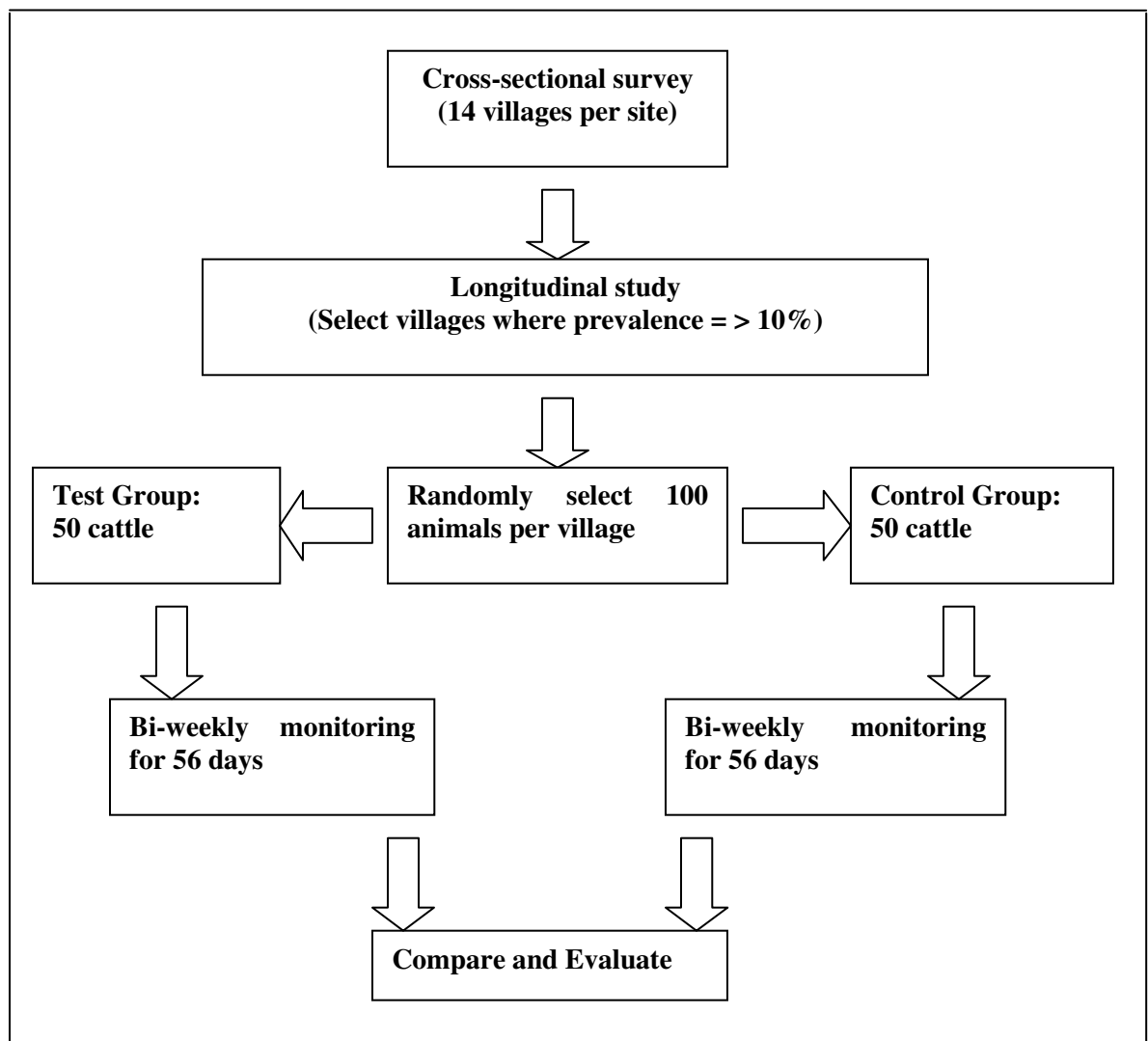


Livestock population in the study area

Livestock census conducted in 2007 by the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC-Ghana) showed that the Sissala East District has 22,658 cattle, 14759 goats, 8,634 sheep, 559 donkeys, 497 pigs and 7 horses (PATTEC - Ghana (2007) Livestock Census: unpublished data).

Study Design

The field surveys were conducted in fourteen (14) villages within the Sissala East District of northern Ghana during the period April – July 2008. They followed the recommended research protocol for field evaluation of trypanocidal drug resistance (Diall, 2005) which involves a cross-sectional survey of disease challenge (Entomology), disease prevalence (Parasitology) and drug use followed by a longitudinal study in cattle to assess treatment failures:



Cross-sectional surveys (CSS)

Parasitology (Trypanosome species and prevalence of infections)

Prior to the cross-sectional surveys, 14 villages were selected. The Sissala East District of northern Ghana has 56 villages; the selection was based on administrative criteria and livestock numbers. Within each selected village, we marked the maximum number of cattle aged one year or above with a crayon and individual numbers were assigned to each animal. Later, the individual cattle numbers were written on pieces of paper and folded up to conceal the numbers written on them. All the pieces of paper were then put in a hat and 50 of the pieces of paper were selected blindly and the animals with corresponding number were included in the sample. While bleeding in the first village, some members of the parasitological team proceeded to mark animals of the second village and submitted corresponding numbers for selection and bleeding the next day and so on. Cattle were bled from the jugular vein using EDTA-Vacutainer tubes (Becton Dickinson & Co.) plus precision glide needles and examined for trypanosomes using the buffy coat technique (BCT) (Murray *et al.*, 1977). The microscopy was done on site shortly after bleeding the animals using a portable Yamaha generator to provide electricity. For each animal examined the age, sex, breed and information about general health status were recorded.

Trypanocidal drug use in the Sissala East District

Information concerning the use of trypanocides was obtained from livestock farmers during the cross-sectional surveys.

Entomology (Tsetse species and densities)

Staff of the Veterinary Services conducted entomological surveys in the dry season (May 2008) along the Sissili River and its tributaries in the Sissala East District of northern Ghana. A total of 30 biconical traps (Challier and Laveissière, 1973) were deployed at an interval of about 100 metres along 3 trapping sites in the study area. The coordinates of each trap position were recorded with a global positioning system (GPS). Tsetse flies were collected from each trap after 24 hours and sorted according to species and sex.

Longitudinal study (LS):

After the cross-sectional surveys, Kunchogu and Yugantu villages emerged as the hot spots for evaluation of trypanocidal drug resistance in the Sissala East District of northern Ghana. These villages were subjected to block treatment study from May to July 2008. A total of 100 cattle were randomly selected from the village herd, the cattle were ear-tagged and assigned into two groups, 50 were treated with isometamidium chloride (Trypamidium-Samorin®) at 1 mg/ kg body weight and 50 were left to serve as control. Both groups of cattle were screened at intervals of 14 days until 56 days for trypanosome parasites using BCT. Animals of the control group found positive at any of those checks were treated with diminazene aceturate (Berenil®) at 3.5 mg/ kg body weight. Animals from the isometamidium treated group found positive from Day 14 to 56 were curatively treated the same way as the control group.

Additionally, 2.5 ml of blood from all trypanosome positive cattle was inoculated intravenously into young goats. This same blood was also spotted and dried on Whatman 903® Protein Saver Cards for detection of genetic markers of resistance in trypanosome isolates.

Data analysis

The evaluation of Isometamidium Chloride (ISMM) resistance is based on the comparison of cumulative incidence of infections (risk) between the test group and the control group. The difference observed is then analysed using a statistical test (the test of Relative Risk (RR)). Data required for this test are the number of positive and negative animals recorded during the longitudinal monitoring in the test and control group (Diall, 2005).

Initiation of trypanocide resistance risk communication process in Ghana

To initiate the trypanocide risk communication process, a national workshop was organized by the Tsetse and Trypanosomosis Control Unit (TTCU) in collaboration with the International Livestock Research Institute (ILRI). The workshop was held in Accra, Ghana, at the Veterinary Services Directorate during the period 5 – 6 November 2008.

In attendance were twenty-five (25) participants representing the Animal Production Directorate (APD), Animal Research Institute (ARI), Central Veterinary Laboratory (CVL), the Directorate of Agricultural Extension Services (DAES), Environmental Protection Agency (EPA), Food and Drugs Board (FDB), Ghana Atomic Energy Commission (GAEC), Ghana Broadcasting Corporation (GBC), Ghana Cotton Company (GCC), Ghana Standards Board (GSB), Ghana Veterinary Medical Association (GVMA), Information and Audio Visual Unit of the Ministry of Food and Agriculture (IAVU-MOFA), Project Planning Monitoring and Evaluation (PPM & E), the University of Cape Coast (UCC), the University of Ghana (UG), the Veterinary Council of Ghana (VCG) and the Veterinary Services Directorate (VSD).

During the workshop all participants agreed that trypanocidal drug resistance is an important issue and they also gratefully acknowledged the implementation of the workshop.

Results

Cross-sectional surveys:

Cross-sectional parasitological surveys in the study area

The trypanosome species and prevalence for each of the 14 villages in the study area are presented in the Table 1 below: 83.3% (5/6) of all trypanosome infections were due to *T. vivax* and 16.7% (1/6) due to *T. congolense*. The trypanosome prevalence varied considerably between villages, with two sites, Kunchogu and Yugantu emerging as suspected 'hot spots' (prevalence of 22% and 8% respectively).

Table 1: Trypanosome species and prevalence of infections in the Sissala East District

Village	№ of cattle bled	Trypanosomes detected		
		Species	Positive(s)	Prevalence (%)
Kunchogu	50	<i>T. vivax</i>	11	22.0
Yugantu	50	<i>T. vivax</i>	4	8.0
Banu	42	<i>T. congo</i>	1	2.4
Kroboi	50	<i>T. vivax</i>	1	2.0
Tumu	50	<i>T. vivax</i>	1	2.0
Pina	50	-	1	2.0
Fachuboi	50	-	0	0.0
Guosi	50	-	0	0.0
Du-East	50	-	0	0.0
Dolibizon	50	-	0	0.0
Bujan	50	-	0	0.0
Chinchan	50	-	0	0.0
Wuru	50	-	0	0.0
Basissan	50	-	0	0.0

Drug use levels in selected villages

Answers provided by livestock farmers concerning the use of trypanocides during the cross-sectional surveys indicated that farmers do treat their cattle with trypanocides. However, studies conducted in Mali and Guinea have shown that farmer's responses to question on the level of trypanocide treatments can be subjective. Therefore, only the trypanosomosis prevalence was used to identify suspected 'hot spots' of drug resistance (Diall, 2005).

Cross-sectional entomological surveys in the study area

The tsetse species and densities for the study sites are presented in the Table 2 below: Almost all the tsetse flies caught along the Sissili River and its tributaries in northern Ghana were *Glossina tachinoides*, except 6 flies caught at a tributary of the Sissili River were unidentified tsetse flies. Tsetse densities also varied from zero to 6.8 flies per trap per day.

Table 2: Tsetse species and densities in the Sissala East District, Ghana

Traps deployment sites		Tsetse capture				AD	
		<i>G. morsitans</i> <i>G. palpalis</i> <i>G. tachinoides</i>					
M	F	M	F	M	F		
Kunchogu river Poruku		0	0	0	0	22	40 6.8
Gbele forest reserve		0	0	0	0	1	0 0.1
Basissan Sissili river		0	0	0	0	0	0 0.0

AD: apparent density (mean number of tsetse per trap per day)

M: Male

F: Female

Longitudinal studies:

Bi-weekly monitoring of the effect of Isometamidium

At the highest risk sites (Kunchogu and Yugantu), a longitudinal study was conducted from May to July 2008 to evaluate trypanocide treatment failures. At Kunchogu, 6 animals became positive in the isometamidium treated group while 8 animals were found positive in the control group during the follow up period.

In contrast, Yugantu village never recorded any trypanosome positive case during the fortnight parasitological checks which unexpectedly ended at Day 28 for lack of cooperation from livestock farmers.

Diminazene aceturate treatment failures

At Kunchogu village we observed some treatment failures to diminazene when we treated fourteen (14) cattle that became infected during the longitudinal study, the animals were followed up for 6 weeks and two (2) infections relapsed.

Assessment of ISMM resistance in the study area using the test of Relative Risk (RR)

A statistical analysis using the Relative Risk test which is based on confidence intervals showed a minimal protection failure of 28% with

isometamidium treatments. A study site is considered a suspected hot spot of isometamidium resistance when the minimum protection failure rate is above 25% (Table 3).

Table 3: Assessment of ISMM resistance using the test of Relative Risk (RR)

Parameter	Test Group	Control Group
The Risk (R)	0.12	0.16
The relative Risk (RR)	0.75	
The Relative Risk Reduction (RRR)	25%	
The lower limit of the 95 % confidence interval of RR	0.28	
The maximum protection rate provided by ISMM	72%	
Minimum protection failure rate	28%	

Discussions

Comparison of results with previous work

From this study, we found the following cross sectional and longitudinal study results:

North-West Ghana, Sissala East District: 83.3% (5/6) of all trypanosome infections were due to *T. vivax* and 16.7% (1/6) due to *T. congolense*. Trypanosomosis is mostly transmitted by *G. tachinoides*. Trypanosomosis prevalence is relatively low (zero to 22%). Suspected trypanocide resistance to isometamidium and some diminazene treatment failures were observed in 1 out of 14 studied villages. Complementary laboratory tests are yet to confirm the resistance. Most of the cattle kept in this area are trypano-sensitive zebus.

A number of field studies have been conducted in the cotton zone of West Africa and they have demonstrated varied levels of drug resistance between and within study sites. Trypanocidal drug resistance was first confirmed in Burkina Faso by Authié (1984), Clausen *et al.*, (1992) and McDermott *et al.*, (2003), next in Mali by Diall *et al.*, (2003) and recently in Mali, Guinea by Talaki *et al.*, (2006).

Recent studies have confirmed the existence of a marked increasing trend of trypanosomosis risk and drug resistance from eastern Guinea across southern Mali to the south-western part of Burkina Faso (Talaki *et al.*, 2006 and Randolph, 2007).

North-East Guinea, Mandiana Prefecture: Only two tsetse species prevail in the area: *G. palpalis gambiensis* (dominant) and *G. morsitans submorsitans*. Detected trypanosome infections were due to low-pathogenic *T. brucei*. Trypanosomosis risk is generally low. Trypanocide resistance was suspected in 1 village out of 30 studied in the area. Trypanotolerant Ndama cattle are the main bovine species kept (Randolph, 2007).

Southern Mali, West Sikasso: *G. palpalis gambiensis* and *G. tachinoides* predominate in the area. Trypanosome infections were due to *T. congolense* (dominant) and *T. vivax*. Trypanosomosis risk is generally low, but resistance was confirmed in 1 village out of 34 studied (Randolph, 2007).

Southern Mali, East Sikasso: Tsetse distributions and trypanosome populations are similar to west Sikasso. Trypano-sensitive zebu cattle are very common in the area. High levels of resistance have been confirmed in 7 villages out of 34 studied in this zone (Randolph, 2007).

South-West Burkina Faso, Kenedougou Province: Earlier studies had confirmed high trypanosomosis risk and high levels of trypanocide resistance in the Kenedougou Province (McDermott *et al.*, 2003). Results of additional surveys carried out by the West African trypanocide resistance project in 8 villages still confirmed the presence of high levels of resistance and disease (Randolph, 2007).

A longitudinal or block treatment study was conducted at the highest risk sites (Kunchogu and Yugantu) from May to July 2008 to evaluate trypanocide treatment failures. At Kunchogu village, six (6) animals became positive in the isometamidium treated group while eight (8) animals were found positive in the control group during the follow up period. In contrast, Yugantu village never recorded any trypanosome positive case during the bi-weekly parasitological checks which was discontinued at Day 28 for lack of cooperation from cattle owners. Once again, at Kunchogu village we observed some treatment failures to

diminazene when we treated fourteen (14) cattle that became infected during the longitudinal study, the animals were followed up for 6 weeks and two (2) infections relapsed.

Recommendations

This field test (block treatment with isometamidium) has shown a suspected drug resistance to isometamidium and some treatment failures to diminazene in the Sissala East District of northern Ghana. It is useful to confirm the results of field studies of resistance to trypanocidal drugs using laboratory studies of trypanosome isolates. This could be done using drug sensitivity studies with small ruminants (goats) in a fly-proof stable at an appropriate facility such as the International Centre for Livestock Research Development in the Sub humid zone (CIRDES), Bobo-Dioulasso, Burkina Faso. Furthermore, the use of the Polymerase Chain Reaction – restriction fragment length polymorphism (PCR-RFLP) to detect genetic markers of resistance in trypanosome strains is a faster molecular tool for drug resistance (Delespaux et al., 2008).

Conclusion

In conclusion, the block treatment test and the analysis of the longitudinal field data using the Relative Risk (RR) test showed a suspected drug resistance to isometamidium chloride in 1 out of 14 villages studied in the Sissala East District of the Upper West Region of Ghana.

The entomological survey and the use of trypanocidal drugs in the study area have supported the parasitological findings at Kunchogu village, the highest trypanosomosis prevalence site.

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DETERMINATION OF DIMINAZENE ACETURATE IN ANIMAL TISSUES BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)/

DETERMINATION DE L'ACETURATE DE DIMINAZENE DANS LES TISSUS DES ANIMAUX PAR LE DOSAGE D'IMMUNOABSORBANT LIE AUX ENZYMES (ELISA)

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Résumé

L'acéturate de diminazène fait partie des rares médicaments pour le traitement de la trypanosomose animale. L'utilisation généralisée de ce médicament en Afrique peut porter à la présence de résidus de diminazène indésirables dans la viande destinée à la consommation humaine, au-delà des limites maximums permises. Il est par conséquent nécessaire de mettre au point des méthodes efficaces pour la détection des résidus du médicament dans les tissus comestibles des animaux. Cette étude a évalué une technique immuno-enzymatique (ELISA) pour la détection de diminazène dans les différents tissus des chèvres. Le diminazène a été administré par voie intramusculaire à cinq chèvres saines avec une dose de 3.5mg/kg de poids corporel, alors que deux chèvres non traitées ont été utilisés comme des témoins. Toutes les chèvres étaient sacrifiées sept jours après l'administration de diminazène. Le foie, le muscle et le rein prélevés en tant qu'échantillon ont été collectés chez toutes les sept chèvres et analysés pour trouver le niveau de diminazène. Les principaux niveaux de diminazène dans le muscle squelettique, le foie et le rein des cinq chèvres étaient les suivants, 0,75µg/g ± 0,14µg/g, 32,05µg/g ± 5,7µg/g et 4,29µg/g ± 0,66µg/g respectivement. Il y a eu des différences significatives ($p < 0,05$) dans les niveaux de diminazène dans le foie, le rein et le muscle squelettique des animaux individuels. Les niveaux de diminazène dans le foie et le rein étaient similaires à ceux précédemment signalés par les autres chercheurs utilisant des méthodes chromatographiques. Toutefois, comparée à ces

méthodes, la technique ELISA est plus rapide, a une grande capacité et est d'usage plus facile. La méthode ELISA de diminazène est par conséquent une méthode plus efficace pour s'assurer la sécurité sanitaire des aliments par rapports aux résidus de ce médicament.

Summary

Diminazene aceturate is one of the few drugs for treatment of animal trypanosomosis. The extensive use of the drug in Africa may lead to the presence of undesirable diminazene residues in meat for human consumption, above the allowed maximum limits. There is therefore need to develop cost effective methods for detecting residues of the drug in edible animal tissues. This study evaluated an enzyme-linked immunoassay (ELISA) for detecting diminazene in different goats tissues. Diminazene was administered intramuscularly to five healthy goats at a dose of 3.5mg/kg body weight, while two untreated goats were used as controls. All the goats were sacrificed at seven days after administration of diminazene. Liver, muscle and kidney sampled were collected from all the seven goats and analysed for diminazene. The mean diminazene levels of the five goats in skeletal muscle, liver and kidney were: $0.75\mu\text{g/g} \pm 0.14\mu\text{g/g}$, $32.05\mu\text{g/g} \pm 5.7\mu\text{g/g}$ and $4.29\mu\text{g/g} \pm 0.66\mu\text{g/g}$ respectively. There were significant differences ($p < 0.05$) in diminazene levels in liver kidney and skeletal muscle of the individual animals. Diminazene levels in liver and kidney were similar to those previously reported by other researchers using chromatography methods. However, compared to these methods, ELISA is faster, has a high throughput, and easier to run. Diminazene ELISA is thus a cost effective method for ensuring the safety of food from residues of the drug.

Introduction

Diminazene aceturate is a widely used drug for the treatment of animal trypanosomosis. This drug belongs to the diamidine group of chemotherapeutics that have been shown to have antiprotozoal, antifungal and antibacterial effects (Kettner 2007, Anne *et al.*, 1980).

Although the effect of diminazene on humans is not well documented, diminazene has been shown to cause mutation to yeast cells of *Saccharomyces cerevisiae* (Mahler and Perlman, 1973). Studies have also shown that diminazene binds to both DNA and RNA (Pilch, 1995). Othman and Sahar (2004) observed cytogenetic effects on cultured river-

buffalo lymphocytes treated with diminazene. They were able to show that the number of cells with different types of structural chromosomal aberrations, including breaks, gaps, deletions, fragmentations and centric fusions were increased significantly compared to the control. Frequency of sister chromatid exchanges and the formation of micronuclei in all lymphocyte cultures treated with different doses of diminazene were increased significantly and depended on dose. When an animal is treated with an antimicrobial drug, a selective pressure is applied to all bacteria exposed to the drug. Bacteria that are susceptible to the antimicrobial are killed or put at a competitive disadvantage, while bacteria that have the ability to resist the antimicrobial have an advantage and are able to grow more rapidly than more susceptible bacteria. In addition, bacteria can become resistant when resistance genes are passed from a resistant bacterium to a sensitive one. Thus, antimicrobial agents may increase the prevalence of resistant bacteria among both target pathogens and normal bacterial flora (Tollefson and Flynn, 2002). Similar effects cannot be ruled out for diminazene considering that it may possess antibacterial properties. Residues of the drug can have a significant adverse impact, on access to markets for food products of animal origin due to their perceived hazardous effects on health of consumers. To mitigate the risk to public health, the FAO/WHO joint expert committee has come up with maximum residue levels (MRLs) for diminazene in skeletal muscle, liver and kidney of 500 µg/kg, 12,000 µg/kg and 6,000 µg/kg respectively (Codex Alimentarius, 1995, WHO, 1994). The MRL or drug tolerance is the maximal level of concentration of residues permitted in animal tissue at the time of slaughter and is intended to ensure that the residue has no harmful effect. Diminazene residues may be present in meat, when after administration to animals the withdrawal time in relation to MRL is not taken into consideration. Cost effective and efficient methods of analysis capable of detecting diminazene in tissues below the MRLs are essential in ensuring compliance with these statutory requirements. Among the methods that have been employed in the determination of diminazene levels ELISA may become more popular because of its ease, accuracy, and the availability of inexpensive reagents. This work reports the use of an enzyme-linked immunosorbent assay (ELISA) to determine levels of diminazene in tissues of treated animals.

Materials and Methods

Experimental animals

Seven male goats of mixed Maasai breeds and aged about one year were purchased from Kiserian in Ngong division, Kajiado district, Kenya. The animals were brought to our Trypanosomiasis Research Centre (TRC) laboratories in Muguga. The goats were allowed to acclimatize in doors for three weeks. During this period they were drenched for endoparasites with Nilzan[®] plus (3.0% oxycyclozanide, 1.5% levamisole and 0.382% colbalt sulphate) from Coopers Ltd., Nairobi, Kenya and allowed free access to hay, green fodder (Napier grass) salt lick and water. The animals weighed between 19 kg and 25 kg. Diminazene was administered intramuscularly to five healthy goats at a dose of 3.5mg/kg body weight. Two Diminazene free goats were included as controls. The animals were sacrificed seven days after drug administration and liver, skeletal muscle, kidney and serum samples collected.

Sample preparation

Ten gram control samples of muscle liver and kidney were weighed in five replicate in 50ml plastic tubes and cut into small pieces using a pair of scissors. One milliliter of a 500ng/ml solution of diminazene in distilled water was added into each tube and left for ten minutes for the drug to penetrate into the tissues. Similar sets of samples were weighed from tissues of healthy goats injected with diminazene at a dose rate of 3.5mg/kg body weight. To all the samples was added 18 ml and 19ml of borax buffer 0.1M pH 9.7 (for spiked and incurred tissues respectively) and mixture macerated using an Ultra-turax tissue homogenizer. The macerator rod was rinsed with 1ml buffer which was added to the extraction tube making a total volume of 20 ml. The extract was centrifuged at 1,800 x g for 20 minutes using a Megafuge 1.0 (Hereus sepattech, Germany) and supernatant decanted into clean 50 ml tubes for storage. The supernatant was frozen overnight to precipitate some of the proteins, thawed and centrifuged again before use. The extraction process for diminazene free blank tissue samples was carried out in a similar way. The serum was prepared by leaving the blood at 37°C for two hours and overnight at 4°C. This was followed by centrifugation as for tissue samples.

Assay for diminazene levels

The levels of diminazene in different tissues were determined by enzyme-linked immunosorbent assay according to the method of Karanja et al. (in press) as briefly explained below. A 100µl of hyper-immune serum diluted with coating buffer was pipetted into wells of microtitre plate (96well Immulon 4® Dynatech labs, Chantilly, USA) and incubated at 4°C overnight followed by freezing at -20°C. Plates were then thawed and washed five times using 0.3ml/well of phosphate buffered saline containing Tween 20 (PBST). 100µl of tissue extract and the diminazene-enzyme conjugate were added to each well. The plates were incubated overnight at 4°C. The washing step was repeated to remove the unbound compounds. A 100µl of substrate solution comprised of hydrogen peroxide and a chromophore, 3,3',5,5'-tetramethylbenzidine (TMB) was then added to each well. The enzymatic reaction was stopped after 10 minutes by addition of 100µl of 1M orthophosphoric acid. Absorbance values were measured at 450 nm using a 96-well microtitre plate reader (Immunoskan PLUS Type 314 of Labsystems, Finland). The concentrations of analyte in samples were read directly from the calibration curve generated using diminazene standards in buffer. The interpolated concentrations were corrected for drug recovery by a factor calculated for each tissue based on diminazene recoveries from the spiked tissues. Diminazene levels were also determined in serum collected from all the goats at the time of slaughter.

Results

The results of diminazene levels in goat tissues obtained using an ELISA method are presented. A standard curve used in the extrapolation of diminazene concentrations in skeletal muscle tissues is shown in figure 1. Similar curves were obtained for liver and kidney (not shown).

The diminazene recoveries from the spiked liver, muscle and kidney were 84% ± 9%, 77% ± 6.6% and 76% ± 6.7%. The diminazene levels corrected for recovery in goat skeletal muscle, liver and kidney seven days after injection were as shown in Figure 2. The mean (±SD) diminazene residue levels were, skeletal muscle 0.75µg/g ± 0.14µg/g, liver 32.05µg/g ± 5.7µg/g, kidney 8.1µg/g ± 4.5µg/g. The mean diminazene serum level was 0.27µg/ml ± 0.05µg/ml.

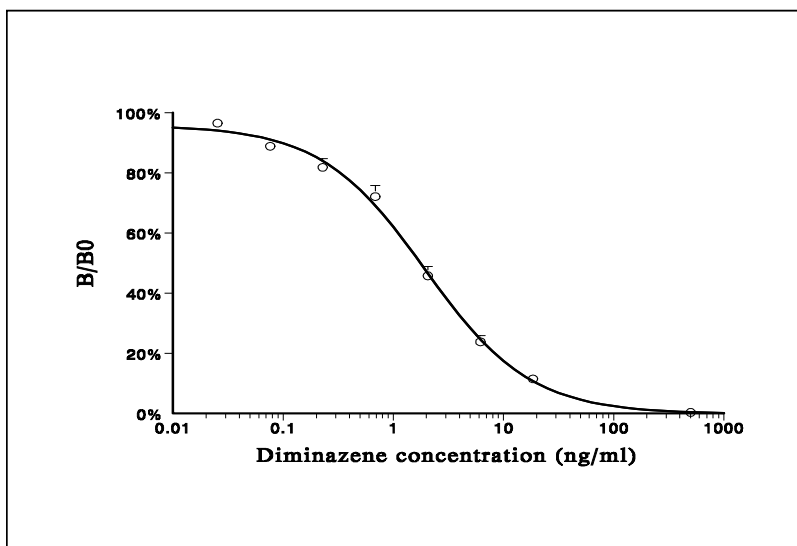


Figure 1: Diminazene calibration curve for skeletal muscle tissue extracts

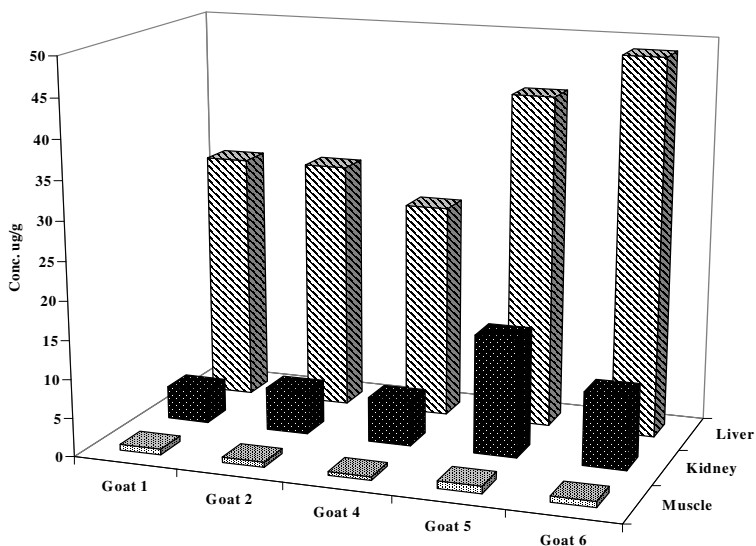


Figure 2: Diminazene levels ($\mu\text{g/g}$) in goat muscle, liver and kidney one week after intramuscular drug administration at a dose rate of 3.5 mg/kg bwt

Discussion

There were significant differences ($p < 0.05$) in diminazene tissue levels of the individual animals. Similar individual differences were observed by (Miller et al., (2005) after diminazene was administered to dogs. These differences can be explained by the intraindividual variation in genetic factors that influence drug metabolism (Anthony, 1998, Alvares, 1979). Variation in diminazene levels in water deprived and non-water deprived goats were observed by Onyeyili *et al.* (2002). Similar variations may arise if the animals in a study take varying amounts of water for whatever reasons. The diminazene levels in serum, muscle liver and kidney were all significantly different ($p < 0.05$) at the time of slaughter. Diminazene levels in liver and kidney were similar to 40.53 $\mu\text{g/g}$ and 3.27 $\mu\text{g/g}$ respectively determined in rabbits by Gilbert (1983), for a similar time period. However, the levels in liver were lower than the 75 $\mu\text{g/g}$ found in calves by Kellner *et al.* (1985). The diminazene concentration levels in all tissues were still higher than the 12 $\mu\text{g/g}$, 0.5 and 6 $\mu\text{g/g}$ maximum residue limit (MRL) of liver, muscle and kidney respectively one week after administration. No diminazene was detected in the control tissue samples. It can be stated unequivocally that ELISA method determines diminazene in tissues with the same degree of accuracy as the liquid chromatography methods employed by Aliu and Odegaard, (1985), Murilla and Kratzer (1989) and Kaur *et al.* (2000). ELISA however, is not as resource demanding and is faster and well suited for monitoring and screening edible animal products for diminazene residues. This is in sharp contrast to the methods currently in use that are lengthy, require costly instrumentation and highly trained personnel. In conclusion it can be stated that this study has indicated that ELISA method can be employed for determining concentration levels of diminazene in meat. However, to fully evaluate the usefulness of the method for monitoring and screening meat for diminazene residues, more tissues should be collected at different intervals post drug administration and from different animal species.

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**POOR QUALITY AND FAKE TRYPANOCIDAL DRUGS, A
REAL THREAT FOR A SUSTAINABLE AND
PROFITABLE LIVESTOCK PRODUCTION IN SUB-
SAHARA AFRICA/**

**MAUVAIS QUALITE ET TRYPANOCIDE CONTREFAITS,
UNE REELLE MENACE POUR LA DURABILITE ET LA
PROFITABILITE DE LA PRODUCTION ANIMALE EN
AFRIQUE AU SUD DU SAHARA**

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Résumé

Les trypanosomoses animales constituent probablement la plus importante contrainte pour une agriculture et un développement rural durable en Afrique Sub-saharienne. L'impact général des trypanosomes sur les productions agricole et animale est estimé à 4,75 milliards USD par an. Chaque année, près de 35 millions de dose de trypanocides, l'équivalent de 35 millions USD, sont administrés aux ruminants domestiques. Toutefois, cette estimation ne tient pas compte du marché illégal des trypanocides qui peut représenter 60 à 90% dans beaucoup de pays africains.

Plusieurs études et enquêtes ont été conduites pour analyser la qualité chimique des différents trypanocides vendus en Afrique. Ces produits ont été analysés conformément aux protocoles internationaux convenus.

Un grand nombre de trypanocides qu'on trouve sur les marchés africains sont de mauvaise qualité ou simplement contrefaits. Moins de la moitié des échantillons de diminazène (n=100) sont conformes aux spécifications courantes de la pharmacopée, c'est-à-dire 95-105% du libellé d'étiquette.

De même, la non-équivalence et la présence de faux produits ont été trouvées dans un grand nombre de marques et lots différents d'isométymidium et de chlorure d'homidium.

L'utilisation de trypanocides de mauvaise qualité ou contrefaits a des conséquences graves à la fois en santé animale et pour la sécurité alimentaire (produits chimiques inconnus, indésirables et leurs résidus dans la chaîne alimentaire). L'utilisation de trypanocides de mauvaise qualité entraîne la résistance des trypanosomes. Un nombre restreint de composés efficaces sont toujours disponibles et les perspectives de développement de nouvelles molécules sont très très limitées.

Le besoin de contrôler la qualité des trypanocides vendus en Afrique est très important.

Un partenariat entre la FAO et l'IFAH (*International Federation of Animal Health Companies*) a été mis en place pour permettre le contrôle des trypanocides dans deux laboratoires d'analyse chimique indépendants en Afrique Sub-Saharienne.

Summary

Trypanosomiasis is arguably the most important animal health constraint to sustainable and profitable agriculture and rural development in sub-Saharan Africa. The overall impact of trypanosomiasis on crop-livestock production has been estimated at US\$ 4.75 billion/year. Each year, approximately 35 million doses of trypanocides are administered to domestic ruminants, corresponding to \$US35 million. However, this figure underestimates the unofficial trypanocide market of unregistered products which in many African countries are estimated at 60 to 90 per cent. Different studies and market surveys were carried out to analyse the chemical-analytical quality of all the different trypanocidal drugs found in different markets in Africa. The drugs were analysed following agreed protocols in the International arena. A great number of trypanocidal drugs found in African markets are of poor quality or even fake. Less than half of diminazene samples (n = 100) were found to comply with the usual pharmacopoeial specification of 95-105% of label claim. Also the non-equivalence and the presence of fake products were demonstrated in an important number of different brands and batches of isometamidium and homidium chloride samples. The use of poor quality and fake trypanocides has severe implications for both animal health and food safety (unspecified, unwanted

chemicals and their residues in the food chain). The use of poor quality trypanocides induces trypanosome resistance. A restricted number of effective compounds are still available and prospects for the development of novel molecules are meagre. There is a tremendous need to control the quality of trypanocidal drugs in Africa. A partnership between FAO/IFAH (International Federation of Animal Health companies) is created to enable the control of trypanocidal drugs in 2 independent chemical-analytical laboratories in Sub-Sahara Africa.

Introduction

Animal production is currently evolving at a fast pace in Africa, from extensive, pastoralist systems to semi-intensive and intensive farming units and from backyard poultry rearing to complete integrated (compartmentalized) avian industry.

Also the mentality and the education level of farmers are changing rapidly, which creates the awareness to adapt and modify their production from “quantity farming” (headcounts, status symbol) to “sustainable quality farming”. This should lead to produce high quality animal products and to create export opportunities which, in turn, results in a high added value for the livestock sector outputs.

Due to the relatively overall high prices for meat and milk products, the animal production industry is in a tremendous transformation/transition phase, from small farms to medium-large scale and intensive production units.

To be able to produce high quality livestock products, farmers increasingly need high quality and effective veterinary drugs for the prevention and treatment of their high value animals. This is very stimulating livestock production environment for the industry which allows to an expansion of the commercialization of quality veterinary products in Africa. In fact, the changing of the African market from a very strong price orientated to a quality based market demands also a quality chain, including the use of certified good quality veterinary drugs.

Quality Control of trypanocidal drugs

Diminazene Aceturate preparation: Material and methods

- Tetley J. et al. (2002): **Non-conformance of Diminazene preparations to manufacturer's label claims: an extra factor in the development of parasite resistance.** In: *Integrated Control of Pathogenic trypanosomes and their Vectors*, 5:24-25.

104 samples of diminazene preparations, representing 19 different brands were obtained by FAO from 11 participating countries in Africa.

The origin of supply sources such as private pharmacies, veterinary clinics, unregulated open markets and government supply systems was investigated.

Tolerance chemical analysis limit of active principle ± 10 percent.

Content of diminazene samples were determined by a validated HPLC method.

Results

68 percent of samples were within the ± 10 percent tolerance limit.

24 percent of the samples were falling below 90 percent of the label claim.

8 percent of the samples were above the upper limit.

Comparison of all the products obtained from governmental sources and those obtained from private circuit, such as private pharmacies/chemical shops, veterinary clinics and the unregulated open markets showed no significant differences in the content of diminazene aceturate.

Isometamidium preparation: Material and methods

- Schad G.J. et al. (2007): Development and validation of an improved HPLC method for the control of potentially counterfeit isometamidium products. In *Journal of Pharmaceutical and Biomedical Analysis*, 46: 45-51.

Development and validation of a simple HPLC method for the quantification of the major constituent (M&B4180A) and the major related substances (M&B4250, M&B38897 and M&B4596) of Isometamidium.

The validated method has been applied to a number of commercial available samples of isometamidium based products which were obtained from the open market in West Africa.

Results

Table 1: Results of analysis of various commercial isometamidium products

	Manufacturer	Wt. of contents of sachet	M&B4596	M&B38897	M&B4250	M&B4180A
Innovator Specifications ^(a) (mg) for 125 mg sachet			6.25–12.5	12.5–25.0	12.5–25.0	68.75–81.3
Trypamidium TM	Merial, Lyon, France	125 mg 3000 mg	11.3 mg 5.6 mg	16.4 mg 9.1 mg	17.6 mg 15.8 mg	74.0 mg 64.4 mg
Kelamidium (batch n°8898)	Kela, Hoogstraten, Belgium					
Innovator Specifications ^(a) (mg) for 1g sachet			50–100	100–200	100–200	550–650
Inomidium	Inouko	1.04 g	60.3	65.5	99.0	653.5
Lobidium	LOBS,	1.04 g	55.7	79.3	125.4	602.6
Isometamidium	PKM	1.30 g	92.4	145.6	143.6	773.8
Veridium TM	Ceva,	1.08 g	50.8	99.5	103.1	640.2
Veridium TM	Ceva,	1.08 g	62.6	86.4	82.1	679.3
Kelamidium	Kela,	2.15 g	79.2	37.4	15.3	419.0
Kelamidium	Kela,	1.86 g	72.8	114.3	127.3	608.7

^(a) Based on the innovator specification of M&B4180A (55–65%), M&B4596 (5–10%), M&B38897 (10–20%) and M&B4250 (10–20%).

Veterinary Theses on Quality of Veterinary Drugs from the Inter-State Veterinary School, Dakar, Senegal

- Ndjana F.M. (2006): Study of the Distribution and Quality of Veterinary Drugs in Cameroon.
- Walbadet, L. (2007): Study of the Distribution and Quality of Veterinary Drugs in Senegal.

Material and methods

Market survey of different therapeutical groups of veterinary drugs collected in different regions in Cameroon and Senegal were analyzed by LACOMEV, Senegal (Control Laboratory for Veterinary Drugs).

Drugs were obtained as well as from the official markets (legal) and from the unregulated open markets (parallel markets).

Tolerance limit of active principle ± 10 percent.

Results

Table 2: Rate of NON-CONFORMANCE by Therapeutic Group in Cameroon

Therapeutic group	Number of samples analyzed	Number of NON-CONFORMANCE samples	Percentage of NON-CONFORMANCE
Trypanocidal drugs	11	11	100
Anthelminthics and endectocides	23	12	52
Antibiotics	14	10	71
Total	48	33	69

Table 3: Rate of NON-CONFORMANCE by sampling sector in Cameroon

Sampling sector	Number of Samples analyzed	Number of NON-CONFORMANCE samples	Percentage of NON-CONFORMANCE
Official market (legal)	28	18	64
Parallel market	20	15	75
Total	48	33	69

Table 4: Rate of NON-CONFORMANCE by type of veterinary drugs in Senegal

Molecule	Number of samples analyzed	Number of NON-CONFORMANCE samples	Percentage of NON-CONFORMANCE
Albendazole	15	2	13
Diminazene	10	7	70
Oxytetracyclins	15	14	93,
Ivermectine	15	14	93
Total	55	37	67

Table 5: Rate of NON-CONFORMANCE by sampling sector in Senegal

Sampling sector	Number of Samples analyzed	Number of NON-CONFORMANCE samples	Percentage of NON-CONFORMANCE
Official market (legal)	29	20	69
Parallel market	26	17	65
Total	55	37	67

Other surveys conducted in different African countries

In Mali (Abiola 2001) showed NON-CONFORMANCE rate of 43 percent

In Benin-Togo (Akoda 2001) showed NON-CONFORMANCE rate of 48 percent

In Mauritania (Abiola 2002) showed NON-CONFORMANCE rate of 59 percent

In Chad (Abiola 2005) showed NON-CONFORMANCE rate of 61 percent

Situation in East Africa

Even if there are no officially published surveys available concerning the quality of veterinary drugs in East Africa, anecdotal evidence suggests the existence of a similar situation as in West and Central Africa.

Consequences of the use of poor quality trypanocidal drugs are:

- NO efficacy (=> [RESISTANCE])
- UNDER DOSING (=> RESISTANCE)
- Products contain high amount of chemical “by-products”:
 - => TOXICITY for the animals
 - => RESIDUES in MILK and MEAT
 - => toxic for the consumers
 - => very dangerous for FOOD QUALITY and FOOD SECURITY(a serious public health concern)

Several of the above mentioned drugs are “officially” registered in some African countries, but it has to be mentioned that still in most African countries REGISTRATION AUTHORITIES are weak or efficient at a sub-standard level. For instance, quite often, personnel are limited in number (only one person in charge to control the appropriateness of documentation provided and/or available. In addition, in many cases, the personnel is not able (or does not have the means) to control the quality of the documentation and that of the samples accompanying the file for Quality Control.

Trypanocidal drugs from ethical companies have real, scientifically and technically proven registration files.

Analytical dossier of the active ingredient and excipients are supplied.

Clinical dossier to prove the EFFICACY and SAFETY (shelf life, withdrawal period for milk, meat; safety for animals, for the environment and for the consumers).

Quality Control dossier:

- of active ingredients
- of excipients
- quality control during production chain
- quality Control of finished and final product.
 - quality packaging for safe conservation (guarantee for indicated shelf life).

Trypanocidal drugs from ethical companies are registered in all countries where trypanosomiasis is a problem, also in those where they have High Qualified Registration Authorities

If you are using trypanocidal drugs from ethical pharmaceutical companies, follow the recommendations of the manufacturer about dosage, administration, treatment regimes, etc ...and do not try to invent “new” dosages, etc....

Very recently it was orally reported that dromedaries infected with *T. evansi* in France were recommended by an “EXPERT Committee” to be treated with Melarsomine (Cymelarsan) at 10 times the recommended dose, other “experts” recommended 3 times the dose and some others recommended to slaughter ALL the dromedaries. While other “experts” claimed to recommend the use of three different molecules to cure the animals (knowing that there are still only two different molecules available, i.e. melarsomine and quinapyramine).

GOOD VETERINARY PRACTICE OF TRYPANOCIDAL DRUGS

There is a very strong tendency to use more and more DIMINAZENE based products as the preferred chemical drug to treat sick animals at the detriment of ISOMETAMIDIUM to prevent and to treat trypanosomiasis in endemic areas.

Prophylactic and curative treatments (isometamidium) PREVENT the onset of the disease in livestock, with consequent reduced or no production losses (healthy animals for healthy, good quality production).

Treat only with DIMINAZENE (curative treatment) the diseased animals (poor general condition => loss of productivity).

Many clinical studies carried out with curative products (diminazene) or with curative+prophylactic products (isometamidium) show the economic benefits of treatment with trypanocidal drugs. But ALL studies show much better productivity data (growth rate, milk and meat production, fertility and reproduction) when ISOMETAMIDIUM based products were used.

FAO-IFAH Project

To assist the African governments, the veterinary services, private veterinarians, farmers and livestock community as a whole to have on the market good quality standard trypanocide products replacing poor quality, counterfeit/fake trypanocides a partnership has been signed between FAO and IFAH. This partnership aims, *inter alia*, at:

- Developing reliable methods to control the quality of trypanocidal drugs
- Creating two chemical-analytical laboratories in Africa (one in West Africa and one in East Africa) to control the quality of drugs which are circulating and/or commercialized in the different countries.

Once these two laboratories will become operating, we encourage everyone involved in the use of trypanocidal drugs to send samples to these independent laboratories for quality control testing.

The results of these analyses will be published as large as possible with the name of the products and the name of the manufacturer, so that everyone will be, or can be, aware of the quality of the drugs he is using.

Conclusions and Recommendations

- Be critical with the results of a large number of studies published when they observed “resistance “against trypanocidal drugs, because in several studies poor quality or fake drugs were used. This practice may have induced the resistance.
- Be aware that in Africa, large fraction of veterinary drugs, including trypanocides, sold on the local markets could be of poor quality, fake/counterfeit.
- Use always high quality drugs for the treatment of trypanosomiasis and always respect the recommendations of the manufacturer to avoid resistance. You have to know that there are no real new trypanocidal drugs under development; hence, we have to use the existing ones in the best possible way.
- In trypanosomiasis endemic areas prefer the use curative+prophylactic treatments (isometamidium).
- If you are not sure about the quality of the trypanocidal drugs you are using, please send them to the FAO-IFAH project for quality control.

Only the use of **HIGH QUALITY DRUGS** and **GOOD VETERINARY PRACTICE** will create sustainable, profitable and safe animal

production (healthier livestock, increase of productivity, increase of fertility, increase of food safety) and this will enable a very significant increase in the LIFE STANDARD of African farmers.

CYMELARSAN EFFECTIVELY CURES TRYPANOSOMES IN DOURINE INFECTION AND WITH NO RELAPSE/

LE CYMELARSAN GUERIT EFFECTIVEMENT LES TRYPANOSOMES DANS L'INFECTION DE DOURINE SANS RECHUTE

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Résumé

Des études sur la sensibilité au Trypanocide ont été menées pour évaluer l'efficacité du Diminasan® et du Cymelarsan® contre les souches Dodola 713/943 et 834/940 du *Trypanosoma* chez des souris et des chevaux infectés pour les besoins de l'expérimentation. Le Diminasan® à des doses allant de 3,5 mg/kg à 28 mg/kg et le Cymelarsan® à des doses de 0,25 mg/kg et 0,5 mg/kg de poids corporel n'ont guéri aucune des souris. Il y avait une relation claire entre la dose et le temps moyen de rechute observé chez les souris. Les souris traitées avec de faibles doses ont rechuté après un temps plus bref que chez les souris traitées avec des doses plus fortes. Les souris traitées avec des doses plus fortes de Cymelarsan® (1,0 mg/kg et 2,0 mg/kg de corps corporel) ont été guéries et la parasitémie n'a pas été détectée pendant 60 jours. Deux groupes de chevaux composés de deux animaux, étaient chacun infecté par la souche

Dodola 834/940 du *Trypanosoma* et traités une seule fois avec du Cymelarsan® à une dose de 0,25 mg/kg en solution de 0,5 % et une dose de 0,5 mg/kg en solution de 1 %, respectivement. Le Cymelarsan® à 0,25 mg/kg et 0,5 mg/kg de poids corporel élimine la parasitémie dans les 24 heures qui suivent le traitement et aucun des animaux n'a connu une rechute pendant les 60 jours d'observation. La sensibilité de la souche d'un trypanosome particulier au Cymelarsan® a également été confirmée par l'amélioration relative des moyennes de PCV des chevaux, suite au traitement. Une différence significative au plan statistique ($p < 0.01$) dans les niveaux moyens de PCV des chevaux traités avec du Cymelarsan® a été observée entre le 20^{ème} jour au pic de la parasitémie et le 40^{ème} jour et le 60^{ème} jour d'observation. Les niveaux moyens de PCV des chevaux dans le groupe témoin ont baissé progressivement dans les 60 jours d'observation. Les résultats de l'étude actuelle seront importants pour la conception de mesures de contrôle de la dourine.

Summary

Trypanocidal sensitivity studies were conducted to assess the efficacy of Diminasan® and Cymelarsan® against *Trypanosoma* species 713/943 and 834/940 Dodola strains in experimentally infected mice and horses. Diminasan® at doses from 3.5 mg/kg to 28 mg/kg and Cymelarsan® at doses of 0.25 mg/kg and 0.5 mg/kg body weight failed to cure any of the mice. There was a clear dose dependent relationship in the mean time of relapse observed in mice. Mice treated with lower doses relapsed after a shorter time than mice treated with higher doses. Mice treated with higher doses of Cymelarsan® (1.0 mg/kg and 2.0 mg/kg body weight) got cured and parasitemia was not detected for 60 days. Two groups of horses containing two animals each were infected with *Trypanosoma* 834/940 Dodola strain and treated only once with Cymelarsan® at a dose rate of 0.25 mg/kg as a 0.5 % solution and 0.5 mg/kg as a 1 % solution, respectively. Cymelarsan® at 0.25 mg/kg and 0.5 mg/kg body weight cleared parasitemia within 24 hours post treatment and none of the animals showed any relapse throughout the 60 days of observation. The sensitivity of the particular trypanosome strain to Cymelarsan® was also supported by the relative improvement in the mean PCV levels of horses following treatment. A statistically significant difference ($p < 0.01$) in the mean PCV levels of horses treated with Cymelarsan® was observed between day 20 at peak parasitemia and days 40 as well as 60 of observation. The mean PCV levels of horses in the control group progressively decreased within the 60 days of observation. The results

obtained from the present study will be important for designing effective control measures of dourine.

Introduction

The causative agent of dourine *Trypanosoma equiperdum* differs from other mammalian trypanosomes due to the fact that is primarily a tissue parasite and transmitted directly from one animal to another of the same species during coitus (Stephen, 1986). In practice, diagnosis is based on clinical evidence supported by serology (Alemu et al., 1997; Hagos, 2005). Since 1982 *T. equiperdum* has not been isolated in the world. Moreover, the available *T. equiperdum* strains in different veterinary diagnostic laboratories were found to be rather related to *T. evansi* than to *T. equiperdum* (Claes et al., 2003). Consequently, it appears difficult to identify what is causing dourine. In deed, we have also observed in Ethiopia that dourine can be caused by *Trypanosoma evansi* (data not shown). Diagnosis of the disease becomes more complicated in an area where other types of trypanosomosis like surra or nagana occur. Moreover, isolation of *T. equiperdum*, the causative agent of dourine in horses, by standard parasitological techniques is usually difficult, due to low numbers of parasites in the blood or tissues fluids (Mulligan, 1970).

Nowadays, dourine is dealt with international legislative measures imposed by the World Organization for Animal Health (OIE) aimed at isolation, castration or slaughtering of complement fixation test (CFT) positive horses (Zablotskij *et al.*, 2003). There are no officially approved drugs to treat horses suffering from dourine although some older publications mentioned experimental treatment of horses with suramin and neoarsphenamine (Novarserobezol; Ciuca, 1933) or quinapyramine sulphate (Vaysse and Zottner, 1950). Evidence from *in vitro* drug sensitivity tests on *T. equiperdum* (Zhang et al., 1992; Brun and Lun, 1994) indicates that suramin, diminazene, quinapyramine and cymelarsan[®] are effective, although no reports on clinical efficacy have been published. Hence, further *in vivo* studies should be conducted using experimental infections with parasites isolated from cases of equine trypanosomosis. In Ethiopia, horses are treated against equine trypanosomosis on irregular basis when trypanocidal drugs are available and even such treated animals show frequent relapses. A survey conducted in the Arsi-Bale highlands of Ethiopia revealed that 53/60 (88.33%) of the interviewed animal owners and professionals claimed that horses treated against equine trypanosomosis show frequent relapses

and generally treatment is not effective enough to cure clinical cases (Hagos, 2005).

Thus, in order to prevent the phenomenon of frequent relapses in dourine cases and maintain the efficacy of the available trypanocidal drugs, it is important that chemotherapeutic regimens are rationalized on the basis of the drug sensitivity of trypanosome strains in a given locality.

It was also found difficult to adopt *T. equiperdum* into laboratory animals (Brun et al., 1998). Since *T. equiperdum* is a tissue parasite naturally found in equines, its establishment in the blood of laboratory animals is extremely difficult. Hence, animal inoculation is of little use as a routine method of diagnosis because it is very difficult and often impossible to obtain a first passage. Mice, rats, rabbits and dogs are susceptible to infection with *T. equiperdum*, once it has been adapted in laboratory animals. Different routes of infection such as subcutaneous, intra peritoneal, intravenous, intraurethral and intravaginal transmission, were tested and all gave rise to clinical signs of dourine (Stephen, 1986; Claes et al., 2003). However, in a recent report, blood and genital washes from antigenaemic horses did not lead to infections when inoculated into mice and puppies (Alemu *et al.*, 1997; Hagos, 2005). Therefore, the present study was aimed at adapting *Trypanosoma spp* causing equine trypanosomosis (Dodola strains) in mice and assessing the therapeutic efficacy of trypanocidal drugs in experimentally infected mice and horses with Dodola strains of the Arsi-Bale highlands of the Oromyia Region in Ethiopia.

Materials and methods

Parasite strains

The *Trypanosoma* parasites were isolated by the Woo technique (Woo, 1970) from two naturally infected mares 713 and 834 with chronic clinical signs of equine trypanosomosis in Dodola district of the Bale highlands Oromyia Regional State, Ethiopia in August 2008. Different characteristic signs of equine trypanosomosis were observed in the two mares including vaginal oedema and discharge, presence of depigmented scars over the external genitalia, partial dragging and stiffness of the hind legs, incoordination and loss of body condition. Subsequently mares 713 and 834 were transported to Debre Zeit and housed in a fly proof stable. In order to maintain these trypanosome strains, two stallions 940 and

943, healthy and parasitologically (Woo test) and serologically (CATT/*T. evansi*) negative were purchased from the central highlands of Ethiopia (Ginchi district: 75 kms west of Addis Ababa and Cheffe Donsa district: 32 kms north of Debre Zeit) and infected by intravenous route with 100 ml fresh whole blood obtained from mares 834 and 713, respectively. The above strains were conventionally named as 834/940 and 713/943 Dodola strains.

Drug sensitivity studies in mice

Mice adaptation of strains

Swiss white mice, 8 weeks old, weighing 20-25 gram, were obtained from the breeding colony of the National Veterinary Institute (NVI) at Debre Zeit and maintained on a commercial pelleted ration and water ad libitum. They were housed in a conducive environment at the laboratory.

Ten mice were allotted to two groups and infected with blood containing 834/940 or 713/943 Dodola strains, respectively. The mice were daily injected intraperitoneal (i.p.) with 0.2 ml blood for seven consecutive days. In order to adapt the strains to mice the immune system was suppressed by daily administration of 0.33 ml of Dexamethasone Sodium Phosphate (Dexamethason[®], Tal: Sanand, Dist: Ahmadabad 382 210, INDIANA) i.p. for seven consecutive days immediately following infection of the mice.

Experimental design

Drug sensitivity studies were conducted on mice infected with 713/943 or 834/940 Dodola strains, respectively using Bis (aminoethylthio) 4-melaminophenylarsine dihydrochloride (Cymelarsan[®], Lot B 09108A, MERIAL- 17, rue Bourgelat 69002 Lyon-France) and diminazene diaceturate (Diminasan[®], Batch DG/20337 Kuipersweg 9, 3449 JA Woerden, Holland). For each drug, sensitivity studies were performed on 20 mice randomly divided into four experimental groups of five animals each. The groups (I – IV) formed the infected groups treated with different doses of trypanocidal drug. Fifth group (V) with five mice served as untreated infected control for both drugs. Cymelarsan[®] was administered i.p at doses of 0.25 mg/kg (standard dose in camels), 0.5 mg/kg, 1.0 mg/kg and 2.0 mg/kg body weight. Diminasan[®] was given i.p. at doses of 3.5 mg/kg (standard dose in cattle), 7.0 mg/kg, 14.0

mg/kg and 28.0 mg/kg body weight. Treatment was administered once parasitemia peaked 1-3 days post infection. Mice were weighed on a digital balance for calculation of drug dosages. The mice were monitored every other day for up to 60 days for the presence of trypanosomes by wet smear examination of blood obtained by tail bleeding. The control groups received the same placebo volume of sterile distilled water by i.p. route.

Drug sensitivity studies in horses

Experimental Protocol

Six adult horses parasitologically (Woo test) and serologically (CATT/*T. evansi*) negative were selected from the central highlands of Ethiopia as indicated above. The animals were transported and kept under closed confinement in a fly proof stable.

The six horses were divided into three treatment groups of two horses each designated as Group I and II and one control group III. Horses were infected with 834/940 Dodola strain cryopreserved in liquid nitrogen. About 50,000 trypanosomes per ml were inoculated directly into the jugular vein. After peak parasitemia, Cymelarsan[®] was administered by deep intramuscular route in the middle third of the neck. Horses in group I and II were treated once at a dose of 0.25 mg/kg as a 0.5 % solution, and 0.5 mg/kg as 1 % solution, respectively. Animals in the control group remained untreated.

Measured parameters

Experimental animals were monitored every other day for 60 days for parasitemia and packed cell volume (PCV). Blood samples were collected by bleeding animals from marginal ear veins into paired heparinized microhaematocrit capillary tubes sealed at one end with creastaseal (Hawaksly, England) and centrifuged at 12,000 rpm for 5 minutes (Woo, 1970). Each microhaematocrit tube was examined microscopically for trypanosomes at the buffy zone by placing the tubes in a viewing chamber (Woo, 1970). Moreover, the buffy coat was spread on clean slide and examined under a 40 x objective microscope by buffy coat method (Murray *et al.*, 1977) and intensity of infection was graded from 0 to 6 as per the standard scores described by Paris *et al.* (1982).

Data analysis

Experimental animals considered cured when no trypanosomes were observed for 60 days post treatment. The mean PCV levels of horses and relapse interval of mice were calculated over the course of the experiment. The statistical significance of any differences in the mean PCV levels of horses in treatment and control groups was determined between 20, 40 and 60 days after infection using student's t-test at a 95 % confidence limit.

Results

Mice adaptation

The mice that were challenged daily for seven consecutive days with Dexamethason[®] developed parasitemia. First parasitemia was evident within five days and peaked in 3-5 days later. It was possible to serially passage *Trypanosoma spp* every 4-5 days by i.p. transfer of 0.2 ml blood into the immunocompetent mice. In the first three passages parasitemia peaked at 5 days of post infection with + 5 score. From the fourth serial passages onwards parasitemia peaked every 3 days of post infection with +6 score. Twenty three serial passages were done and cryostabilates prepared at passages 2, 7, 15, 19 and 23 and kept in liquid nitrogen.

Drug sensitivity studies in mice

The results of the drug sensitivity studies in mice are summarized in Table 1 and 2. Diminasan[®] at doses ranging from 3.5 mg/kg to 28 mg/kg body weight failed to cure any of the mice infected with 713/943 or 834/940 Dodola strains. Mice infected with 713/943 or 834/940 Dodola strains treated with Cymelarsan[®] at doses of 0.25 mg/kg and 0.5 mg/kg also failed to cure the infection. There was a clear relationship between the time of relapse and the dose of the drug used. Mice treated with lower doses relapsed after a shorter time than mice treated with higher doses. On the contrary, cymelarsan at doses of 1 mg/kg and 2 mg/kg body weight effectively cured all mice regardless of the strain used for 60 days of observation.

Drug sensitivity studies in horses

Six horses were infected and became parasitologically positive (+ 2 score, 1-10 trypanosomes per preparation) in the Woo test 13 to 15 days post infection with 10^3 to 10^4 trypanosomes per ml. Parasitemia peaked (+ 6 score, swarming trypanosomes per field) in horses 17 to 20 days post infection (Table 3). Based on the results of drug sensitivity testing in mice Cymelarsan[®] was selected to be used in the horse experiment. Single shot treatment with Cymelarsan[®] 0.25 mg/kg and 0.5 mg/kg body weight was administered to horses of group I and II, respectively 20 days post infection. Treated horses in group I and II cleared parasitemia within 24 hours post treatment. None of the treated horses relapsed post treatment. The mean PCV levels of horses in group I and II treated with Cymelarsan[®] showed improvement starting from day 20 of observation (Figure 1). There was a statistically significant difference ($p < 0.05$) in mean PCV levels of horses in group I and II between days 20 peak parasitemia and 40 of observation. Similarly, there was also a statistically significant difference ($p < 0.05$) in mean PCV levels at days 20 peak parasitemia and 60 of observation (Table 4). Conversely, there was a progressive depression in the mean PCV levels of horses in the control group III (Figure 1).

Discussion

One of the characteristics *T. equiperdum* is that it is often impossible to obtain a first passage in mice or any other rodent when infected with blood (Stephen, 1986; Claes et al, 2003) and genital washes of antigenaemic horses (Alemu *et al.*, 1997; Hagos, 2005). In adapting trypanosomes in mice the immune system was suppressed with Dexamethason[®]. Dexamethasone is one of the frequent glucocorticoides used for experimentally induced immunosuppression. In high doses they have a profound anti-inflammatory and immunosuppressive effect, whereas low physiological concentrations rather have an immunostimulatory function (Wiegiers et al., 1993; Wiegiers and Reul, 1998).

Cymelarsan[®] was found to be very effective against *T. brucei brucei*, *T. equiperdum* and *T. evansi* in camels, buffalo, goats and pigs (Zweygarth and Kaminsky, 1990; Lun et al., 1991; Otsuly et al., 1992; Zhang et al., 1992; Zweygarth et al., 1992). The arsenical compound contains the trivalent arsenic element with a markedly reactive arsenoxide group. The

presence of arsenoxide confers the physicochemical ability of lipid solubility that allows passage across the blood brain barrier (BBB) (Peppin and Milord, 1994). The arsenical compound melarsoprol revealed the remarkable ability to cross the BBB and kill *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* parasites residing in the CSF.

In camels Cymelarsan[®] was effective against *T. evansi* infections at the standard recommended dose of 0.25 mg/kg body weight (Musa *et al.*, 1994). The results from our study showed that only at higher doses of 1.0 mg/kg and 2.0 mg/kg body weight Cymelarsan[®] was effective to clear Dodola strains in mice with no relapses for 60 days. However, Cymelarsan[®] at doses of 0.25 mg/kg and 0.5 mg/kg body weight failed to cure the infection. The inability of Cymelarsan[®] to clear parasitemia in mice as well as other domestic animals at the standard dose of 0.25 mg/kg for *T. evansi* in camels has previously been reported. For instance Cymelarsan[®] was ineffective in buffaloes treated at doses ranging from 0.25 mg/kg to 3 mg/kg (Lun *et al.*, 1991), in goats treated at a dose of 0.3 mg/kg (Zweygarth *et al.*, 1992), in mice treated at doses of 0.25 mg and 0.5 mg/kg (Syakalima *et al.*, 1995) and in cattle treated at a dose of 0.5 mg/kg (Payne *et al.*, 1994). This ineffectiveness of the drug may suggest the suggestion made by Zweygarth *et al.* (1992) that the recommended dose should be applied for the treatment of camels only and that higher doses are needed to treat *T. evansi* in smaller animals. Indeed, in our study mice treated with Cymelarsan[®] at doses of 0.25 mg/kg and 0.5 mg/kg body weight could have been underdosed as where the metabolic weight of camels versus mice should be taken into consideration. Therefore, we used higher doses in mice which appeared to be effective to clear parasitemia.

It is known that Diminasan[®] is less effective *in vivo* against trypanosomes of the subgenus Trypanozoon than *T. congolense* and *T. vivax* (Mulligan, 1970). On the contrary, a relative efficacy of Diminasan[®] on *T. equiperdum* isolates was observed following *in vitro* drug sensitivity test (Zhang *et al.*, 1992; Zhang and Baltz, 1992; Brun and Lun, 1994). From our study Diminasan[®] at doses from 3.5 mg/kg to 28.0 mg/kg body weight failed to cure 713/943 or 834/940 Dodola strains in mice. Hence, the dose necessary to cure mice might be greater than 28.0 mg/kg body weight. But higher doses of Diminasan[®] can cause severe toxicity rather than curing infection in mice (Gilbert and Newton, 1982). Drug failure could be attributed to the relatively rapid excretion of

the drugs (Mulligan, 1970). On the other hand, it could also be attributed to the pharmacokinetics of the drug where Diminasan[®] can not cross the blood brain barrier and somatic tissues due to this fact it can not be the curative drug for trypanosomes with tissue affinity. Indeed, the efficacy of Diminazene aceturate against *T. brucei* involving central nervous system infections was found to be ineffective as parasitemia returned rapidly after few days of treatment where central nervous system was demonstrated to be the source of relapsing infections (Jennings et al., 1979; Jennings et al., 1980).

The occurrence of nervous symptoms and lesions in horses suffering from dourine is associated with the presence of parasites in cerebrospinal fluid (Barrowman, 1976). Also in horses suffering from surra nervous system involvement was demonstrated. This infection was characterized by severe neurological abnormalities such as progressive ataxia, head tilt, nystagmus and cranial nerve deficits. *T. evansi* tryposmastigote were also detected in the cerebrospinal fluid using cytology (Berlin et al., 2009). In our experimental study using strains from chronic field cases of equine trypanosomosis, Cymelarsan[®] cured infections with no relapses for 60 days post infection. Relative improvement in PCV levels of animals also appeared to reflect the efficacy of the trypanocidal drug used in our experiment. Moreover, eight chronic clinical field cases of equine trypanosomosis in the highlands of Arsi-Bale of Ethiopia treated with Cymelarsan[®] 0.25 mg/kg body weight were cured. So far no relapses and parasites were found after revisiting and testing of the animals six and twelve months following initial treatment (data not shown).

Currently a stamping out strategy is imposed by the World Organization for Animal Health (OIE) with slaughtering of seropositive horses where treatment is prohibited (Zablotskij et al., 2003). However, it is not economically feasible to apply strict test and slaughter policy to control dourine in developing countries. In such countries the contribution of equines is much diversified and plays a prominent position in transport and agriculture. More than 60 % of all horses on Earth are found in developing countries (Pritchard et al., 2005). Besides, it will be worth treating clinical cases to alleviate the disease, enable animals to perform well and thereby reduce mortality. From the results of this study, it could be recommended to the OIE to replace the current strategy of eradication by an appropriate drug treatment with Cymelarsan[®].

Control of animal trypanosomosis depends mainly on chemotherapy with the available trypanocidal drugs (Holmes, 1997). In conclusion, drug sensitivity studies on Dodola strains isolated from horses with chronic equine trypanosomosis indicated that Cymelarsan[®] is quite effective in curing infections with no relapses for up to 60 days. This depicts the sensitivity of the field isolates to Cymelarsan[®] and the potential scenario with regard to the control of equine trypanosomosis by chemotherapy. Hence, the results of the present study will pave a way to envisage effective control measures against equine trypanosomosis.

Table 1: Sensitivity of 713/943 Dodola strain to Diminasan[®] and Cymelarsan[®] in mice.

Drugs	Doses (mg/kg)	No. mice treated/relapsed	Mean relapse interval in days $\pm$ SD
Diminasan [®]	3.5 mg/kg	5/5	1.25 $\pm$ 0.043
	7.0 mg/kg	5/5	1.5 $\pm$ 0.5
	14.0 mg/kg	5/5	4.5 $\pm$ 1.22
	28.0 mg/kg	5/5	6 $\pm$ 1.94
Cymelarsan [®]	0.25 mg/kg	5/5	6.5 $\pm$ 1.83
	0.50 mg/kg	5/5	7.5 $\pm$ 1.72
	1.0 mg/kg	5/0	Cured
	2.0 mg/kg	5/0	Cured
Control	Distilled water	5/5 ^a	Died 3-5 days post infection

No. Number, ^a Treated/Died

Table 2: Sensitivity of 834/940 Dodola strain to Diminasan[®] and Cymelarsan[®] in mice.

Trypanocidal drug	Doses (mg/kg)	No. mice treated/relapsed	Mean interval in days $\pm$ SD	relapse
Diminasan [®]	3.5 mg/kg	5/5	1.5 $\pm$ 0.54	
	7.0 mg/kg	5/5	2 $\pm$ 0.65	
	14.0 mg/kg	5/5	3.5 $\pm$ 1.32	
	28.0 mg/kg	5/5	4 $\pm$ 1.89	
Cymelarsan [®]	0.25 mg/kg	5/5	6 $\pm$ 1.73	
	0.50 mg/kg	5/5	6.5 $\pm$ 1.22	
	1.0 mg/kg	5/0	Cured	
	2.0 mg/kg	5/0	Cured	

Table 3: Details of the pattern of parasitemia in terms of days required for first and peak parasitemia and treatment post infection.

Horse tag no. and group	No. of days of		
	first parasitemia	peak parasitemia	treatment post infection
Group I	14	17-20	20
Group II	13	18-20	20
Group III	15	18-20	-

Table 4: Change in the mean PCV levels of horses experimentally infected with 834/940 Dodola strain and treated with 0.25 mg/kg (group I) and 0.5 mg/kg (group II) Cymelarsan[®] and untreated control (group III).

Groups	Mean PCV levels			
	time of infection	peak parasitemia (day of treatment)	time after treatment	
	Day 0	Day 20	Day 40	Day 60
I	32 ^{a*}	24 ^b	30 ^a	34 ^a
II	34 ^a	20 ^b	28 ^a	32 ^a

III	33 ^a	24 ^b	20 ^b	16 ^b

* Mean PCV values with different superscripts showed statistically significant difference ($p < 0.05$)

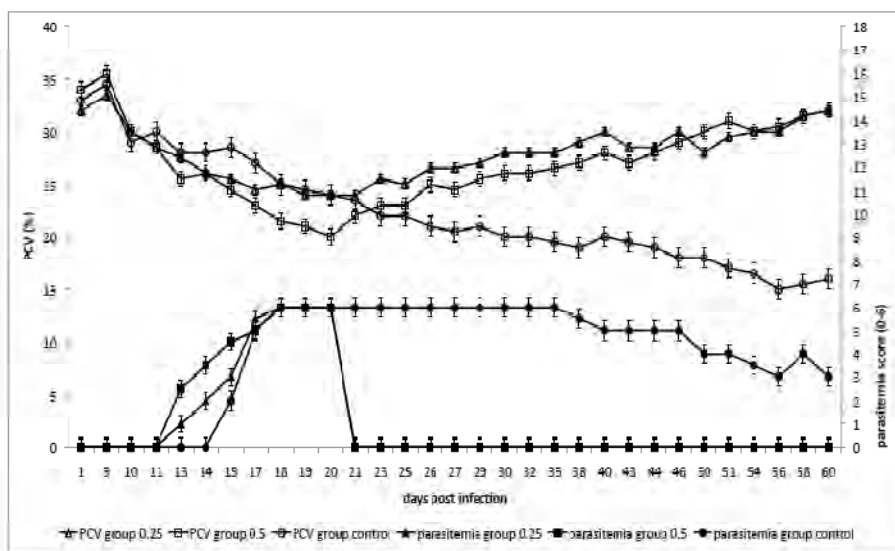


Figure 1: Profiles of mean PCV and parasitemia levels of experimentally infected horses with trypanosomes 834/940 Dodola strains and treated with cymelarsan 0.25 mg/kg (Δ) and 0.5 mg/kg ($\square$) body weight and untreated control ($\circ$).

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**EVALUATION CHEZ LES BOVINS DE LA REPONSE
ANTICORPS DIRIGES CONTRE LES ANTIGENES
SALIVAIRES DE GLOSSINES COMME MARQUEUR
D'EXPOSITION DES TROUPEAUX**

**BOVINE ANTIBODY RESPONSE DIRECTED AGAINST
GLOSSINA SALIVA: AN EPIDEMIOLOGICAL MARKER
OF CATTLE EXPOSURE TO TSETSE BITES**

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Summary

Our objective is to develop a sero-epidemiological tool to measure the exposure of cattle to tsetse bites based on the host antibody response directed against *Glossina*'s total salivary antigens. The anti-saliva (IgG) response against *Glossina palpalis gambiensis* saliva was assessed by ELISA on 102 bovine sera from Burkina Faso: 48 were sedentary bovine from a tsetse free area (North) and 54 were from a tsetse infested area (South-West). High anti-saliva responses were detected in cows from the tsetse infested area ($p=0.001$) and showed temporal variation. The response was significantly higher during the hot dry season ($p=0.0004$) when animals are most exposed to *Glossina* bites during watering at gallery forests along permanent streams. Furthermore, there was a strong positive association between the anti-saliva response and the risk of being infected by trypanosomes ($p=0.004$). These results show that the

anti-saliva response may be an interesting marker of exposure of cattle to tsetse bites. However, some animals from the tsetse free area had also elevated anti-saliva responses, suggesting the existence of immune cross-reactivity with salivary proteins from other hematophagous arthropods. We are currently carrying out experimental exposure of cows to the bite by different hematophagous vectors in order to identify *Glossina* specific antigens to develop a more specific serological tool. This tool could be used in African trypanosomosis endemic areas to (i) identify highly exposed herds towards which vector control should be directed and to (ii) assess the efficiency of entomological control measures.

Key words: Animal African Trypanosomosis (AAT), *Glossina*, saliva, marker of exposure

Résumé

L'objectif de cette étude est de développer un outil séro-épidémiologique de mesure du contact bovins/tsetse basé sur la réponse IgG dirigée contre les antigènes salivaires de glossines. 102 sérums de bovins du Burkina Faso ont été analysés par ELISA afin d'évaluer la réponse anti-salive de *Glossina palpalis gambiensis*, 48 bovins provenaient d'élevages sédentaires d'une zone sans glossine au Nord du pays et 54 d'une zone infestée au Sud-Ouest. Des réponses anti-salive plus élevées ont été détectées chez les animaux provenant des zones infestées ($p=0,001$) et ces réponses étaient plus fortes pendant la saison sèche quand les animaux fréquentent régulièrement les forêts galeries pour s'abreuver ($p=0,0004$). Par ailleurs nous avons pu montrer une association significative entre les réponses anti-salive élevées et le risque d'être infecté par les trypanosomes ($p=0,004$). Ces résultats indiquent que la réponse anti-salive chez les bovins semble être un bon marqueur d'exposition à la pique par les glossines. Cependant des réponses anti-salive élevées ont également été détectées chez certains bovins du Nord, ce qui suggère l'existence de réaction croisée avec des protéines salivaires d'autres arthropodes hématophages. Afin d'identifier les antigènes salivaires spécifiques de glossines, des expositions expérimentales de bovins à différents vecteurs hématophages sont en cours en conditions contrôlées pour le développement d'un outil sérologique plus spécifique. Cet outil pourrait être utilisé pour (i) identifier en zone d'endémie les troupeaux les plus fortement exposés et cibler les zones prioritaires pour la lutte et (ii) pour l'évaluation de l'efficacité de la lutte anti-vectorielle.

Mots clés, Trypanosomose Animale Africaine (TAA), glossines, salive, marqueur d'exposition

Introduction

Les trypanosomoses africaines, maladies parasitaires vectorielles transmises essentiellement par les glossines, affectent à la fois l'homme et les animaux et constituent une contrainte majeure pour le développement en Afrique subsaharienne. Le Centre International de Recherche-Développement sur l'Élevage en zone Subhumide (CIRDES) est depuis longtemps impliqué dans la lutte contre la trypanosomose bovine en Afrique de l'Ouest tant au niveau de l'évaluation épidémiologique des zones à risque que dans l'implémentation de programmes de lutte intégrée contre les glossines (PAAT, PATTEC,...)^{1,2}. Les techniques actuelles d'évaluation du risque entomologique (captures par piégeage) sont lourdes en moyens humains et financiers; et la couverture de vastes zones géographiques est très coûteuse.

Récemment un intérêt croissant est porté sur l'étude de la réponse de l'hôte vertébré contre les composés salivaires des insectes vecteurs hématophages³. En effet, lors du repas sanguin ces insectes injectent au site de la piqûre un mélange de composés salivaires actifs permettant la réalisation d'un repas sanguin efficace en inhibant la réponse hémostatique de l'hôte. Certaines de ces protéines salivaires sont immunogéniques et induisent une réponse immunitaire de l'hôte (réponse inflammatoire, réponse humorale, ...). Il a ainsi pu être montré que la présence d'anticorps spécifiques dirigés contre certains composés salivaires pouvait être utilisée comme marqueur d'exposition à la piqûre de certains insectes hématophages comme les anophèles⁴ ou encore les phlébotomes⁵. De telles études menées à la fois chez la souris⁶ et chez l'homme⁷ montrent que l'analyse de la réponse IgG dirigée contre les antigènes salivaires de glossines peut également servir de marqueur d'exposition à la piqûre par ces insectes et constituer un outil pour l'évaluation du risque de transmission notamment de la trypanosomose humaine africaine. Il est maintenant important d'évaluer expérimentalement le rôle de cette salive dans le modèle animal naturel et économiquement important qu'est le bovin.

Ces pré-acquis sur le rôle de la salive des vecteurs en général et des glossines en particulier ont inspiré le CIRDES à réaliser un « Projet Anti-Salive de Tsé-tsé » (PAST). Ce projet se propose de mettre au point une nouvelle stratégie de surveillance et de contrôle des trypanosomoses animales africaines (TAA). Il s'agit concrètement de développer un outil séro-épidémiologique de mesure du contact bovins - glossines que le CIRDES compte mettre à la disposition de ses partenaires comme le Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC). Cet outil peu onéreux et facile à appliquer doit servir (i) à déterminer les zones prioritaires d'intervention pour la lutte anti-vectorielle (élaboration des cartes de risque glossinien) et (ii) à évaluer l'efficacité de cette lutte.

Matériel et méthodes

Echantillons provenant de bovins en milieu naturel

Nous avons utilisé 102 sérums de la sérothèque bovine du CIRDES pour la mise au point des différentes techniques du projet, 48 sérums de zébus et 54 sérums de métis. Les zébus provenaient du Nord du Burkina Faso, une zone à priori indemne de glossines et les métis du Sud-Ouest, une zone infestée de glossines et endémique pour les TAA. Pour ces bovins nous disposions également des informations concernant leurs statuts sérologiques et parasitologiques, la saison du prélèvement, ainsi que leur âge.

Exposition expérimentale des bovins à l'étable

Pour l'exposition expérimentale, dix (10) génisses âgées de 1 à 2 ans ont été exposées aux piqûres par différents insectes hématophages dans une étable sous-moustiquaire pendant 6 mois. Ces génisses ont été réparties en 6 groupes,

- 3 groupes exposés chacun aux piqûres par une des 3 espèces de glossines élevées au CIRDES (*G. palpalis gambiensis*, *G. tachinoides* et *G. morsitans submorsitans*) ;
- 2 aux vecteurs mécaniques (Tabanidés spp et Stomoxes spp) des trypanosomoses animales capturés dans la campagne par pose de pièges (N'ZI et monoconique) ;
- et le dernier groupe exposé aux nymphes saines d'*Amblyoma variegatum* (provenant de l'élevage de Tiques du CIRDES).

20 mouches (glossines, Tabanidés et Stomoxes) étaient exposées 2 fois par semaine au niveau des flancs rasés des bovins tandis les tiques étaient

fixées sur les oreilles des bovins (avec une alternance entre les oreilles pour une nouvelle exposition). Un prélèvement du sang jugulaire était fait une fois par semaine pour le suivi sérologique anti-salive de glossines.

Technique de salivation et dosage de la salive

Des glossines saines du CIRDES ont également été utilisées pour recueillir la salive par une méthode de salivation. Cette méthode utilise un dispositif constitué d'une plaque chauffante réglée à 37°C (pour mimer la température des animaux à sang chaud), sur laquelle est déposée une lame à microscopie contenant environ quelques microlitres du tampon de salivation (Hepes 10 mM - EDTA 5 mM - NaCl 150 mM). Afin de stimuler leur appétit, les glossines sont privées de repas sanguin pendant 2 à 3 jours. Elles sont ensuite réparties par lot de 4 à 6 dans des tubes Falcon de 50 mL fermés par de tissu moustiquaire (figure 1). Les tubes contenant les glossines affamées sont enfin déposés sur les lames où elles vont saliver dans le tampon. Après quelques minutes de salivation, le mélange tampon-salive est recueilli dans un tube Eppendorf et gardé à +4°C jusqu'au dosage des protéines salivaires.



Figure 1, Poste de salivation de glossines

Le stock de salive gardé à +4°C est ensuite dosée par un kit BCA (acide bicinchoninic) basé sur la méthode du biuret. Cette salive dosée est

utilisée par la suite pour sensibiliser des plaques à polystyrène pour des ELISA-indirect anti-salive de glossines.

Analyse statistique des résultats

Les données ont été analysées par le logiciel JMP 5.1. Les comparaisons entre les moyennes de densités optiques (DO) anti-salive des différents groupes ont été faites par le test t de Student. Le seuil de signification a été fixé à 0,05.

Résultats et discussion

Résultats préliminaires de PAST

La réponse humorale dirigée contre la salive de *G. palpalis gambiensis* (*G.p.g.*) sur la sérothèque bovine du CIRDES a été analysée par ELISA-indirect. Des réponses anti-salive plus élevées ont été détectées chez les animaux provenant des zones infestées (Sud-Ouest du Burkina Faso) par rapport à ceux de la zone indemne de glossines (figure 2). De plus les bovins des zones infestées avaient des réponses plus fortes pendant la saison sèche par rapport à la saison pluvieuse ($p=0,0004$) (figure 2). Cela peut s'expliquer par le fait qu'en saison sèche les bovins fréquentent régulièrement les cours d'eau pérennes dans les forêts galeries pour s'abreuver, où ils sont fortement exposés aux piqûres de glossines. Par ailleurs nous avons pu montrer une association significative entre les réponses anti-salive élevées et les statuts sérologiques et parasitologiques anti-trypanosomes chez les bovins des zones infestées de glossines ($p=0,004$). Les animaux qui ont une sérologie ou une parasitologie positive aux trypanosomes présentaient les plus fortes réponses anti-salive (figure 3). Ces résultats préliminaires indiquent que la réponse anti-salive chez les bovins semble être un bon marqueur d'exposition à la piqure par les glossines. Cependant des réponses anti-salive élevées ont également été détectées chez certains bovins de la zone indemne, ce qui suggère l'existence de réactions croisées entre la salive de glossines et celles d'autres arthropodes hématophages. Afin de valider l'utilisation de la salive totale comme marqueur d'exposition aux piqûres par les glossines, des expositions expérimentales de bovins à différents insectes hématophages ont été réalisées en conditions contrôlées dans une étable.

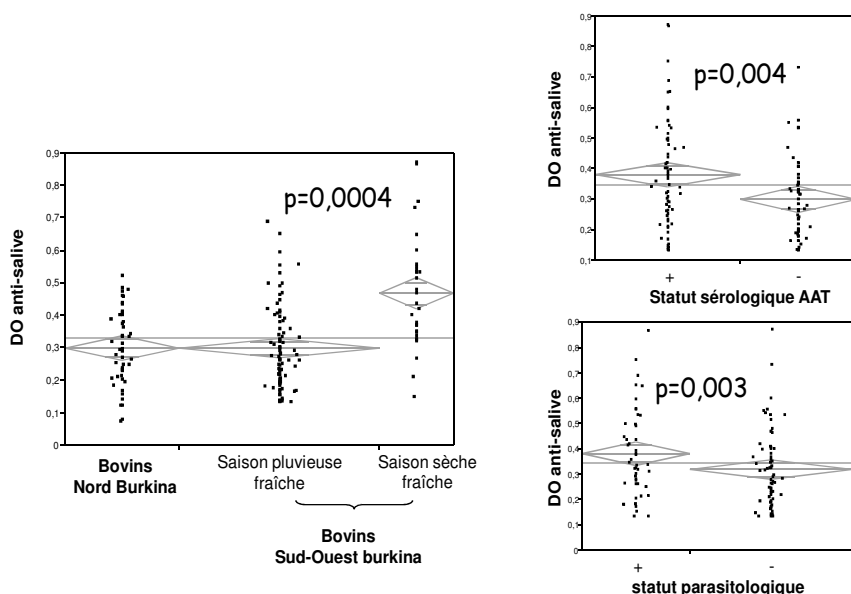


Figure 3: DO en fonction du statut

Figure 2: DO en fonction de la provenance

Les résultats de l'exposition expérimentale

Après les expositions des bovins aux piqûres par des insectes hématophages, leurs réponses humorales ont été analysées contre la salive de *G. palpalis gambiensis* (*G.p.g.*) et celle de *G. morsitans submorsitans* (*G.m.s.*).

La figure 4 montre les courbes de réponse anti-salive de *G.p.g.* au cours du temps.

Chez les bovins exposés aux piqûres de *G.p.g.*, un bovin a une bonne réponse anti-salive sur 2, ce qui nous permet d'évoquer l'existence d'une hétérogénéité inter individuelle en ce qui concerne la réponse anti-salive. Le bovin Gp1 exposé à *G.p.g.* présente une bonne réponse avec des anticorps anti-salive qui apparaissent à partir de la 7^{ème} semaine, avec un plateau qui est atteint à la 17^{ème} semaine. La réponse anti-salive décroît par ailleurs rapidement après arrêt de l'exposition expérimentale (4

semaines). La salive de *G.m.s.* n'induit pas réaction avec celle *G.p.g.*, attestée par la courbe en dents de scie de la réponse anti-salive du bovin exposé à *G.m.s.* Nous avons observé l'induction d'une réponse chez les animaux exposés à *Glossina tachinoides* (*G.t.*) et aux Tabanidés, ce qui suggèrent l'existence de réactions croisées entre la salive de *G.p.g.* et celle de *G.t.* et celle des Tabanidés. Par contre aucune réponse n'a été observée avec les autres insectes hématophages testés (stomoxes et tiques).

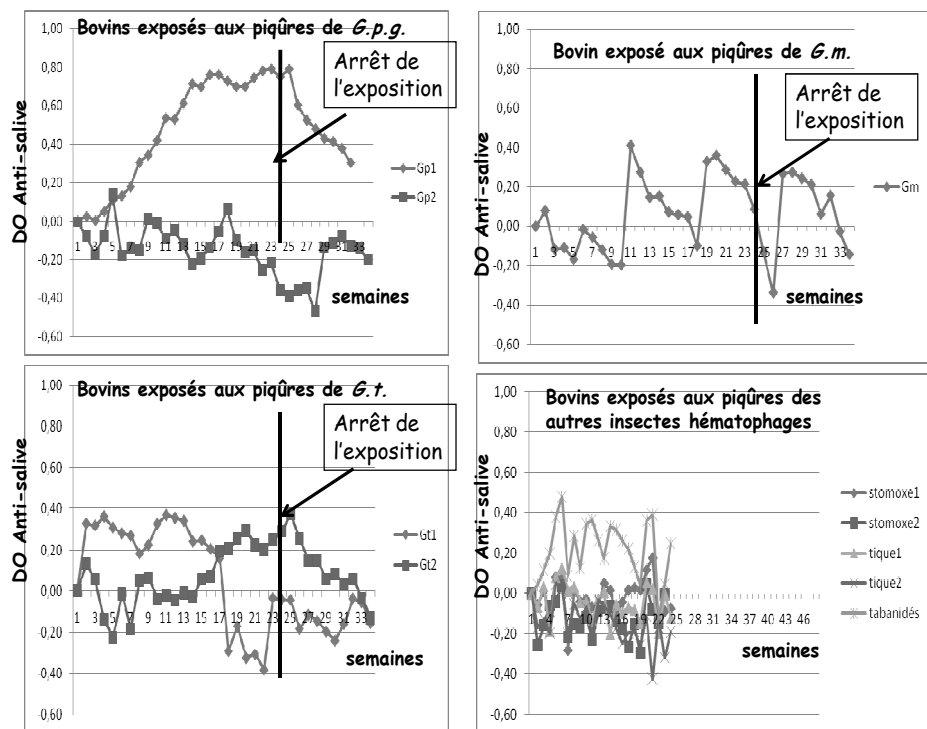


Figure 4, Courbes de suivi de la réponse dirigée contre les antigènes salivaires de *G. palpalis gambiensis* chez les bovins exposés expérimentalement à la piqûre par différents insectes hématophages

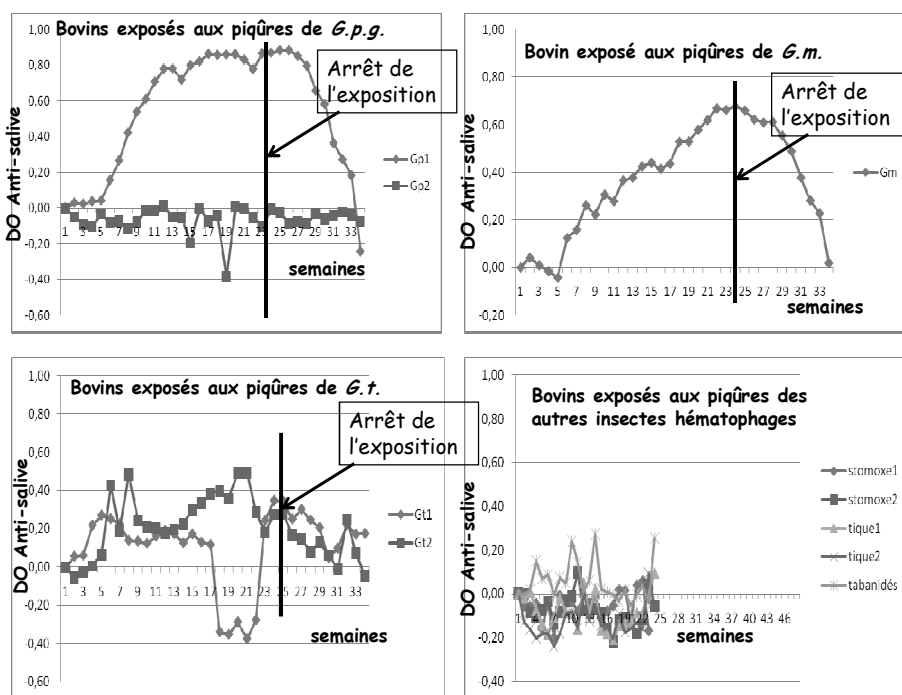


Figure 5, Courbes de suivi de la réponse dirigée contre les antigènes salivaires de *G. morsitans submorsitans* chez les bovins exposés expérimentalement à la piqûre par différents insectes hématophages

L'analyse de la réponse humorale anti-saliv de *G.m.s.* a donné les résultats suivants (figure 5).

Une forte réponse dirigée contre la salive de *G.m.s.* a été observée chez le bovin répondeur exposé à *G.p.g.* Chez ce bovin (Gp1), le plateau des taux d'anticorps est atteint au bout de la 9^{ème} semaine. Après l'arrêt de l'exposition à la 24^{ème} semaine, les titres d'anticorps anti-saliv décroissent jusqu'à s'annuler.

Le bovin exposé à la piqûre de *G.m.s.* présente également une bonne cinétique de croissance de la réponse anti-saliv, les anticorps apparaissent à la 7^{ème} semaine et un plateau fugace est lentement atteint à la 21^{ème} semaine. 4 semaines après l'arrêt de l'exposition, ces anticorps décroissent jusqu'à s'annuler.

La salive de *G.t.* induit peu de réactions avec la salive de *G.m.s.* Il n'existe pas de réactions croisées entre la salive *G.m.s.* et celles des autres insectes hématophages (stomoxes, tabanidés et tiques).

Le tableau ci-après fait un récapitulatif de ces différentes réactions croisées entre la salive de glossines et celles des autres arthropodes hématophages. Nous avons trouvé que i) la salive de *G.p.g.* croise avec celles de *G.t.* et des Tabanidés et ii) la salive de *G.m.s.* croise avec les salives de *G.t.* et *G.p.g.*

Tableau, Récapitulatif des réactions croisées entre les salives de glossines et des autres insectes hématophages

Animaux exposés aux piqûres de,	<i>G.p.g.</i>	<i>G.t.</i>	<i>G.m.s.</i>	Tabanidés	Stomoxes	Tiques
Réponse humorale contre la salive de <i>G.p.g.</i>	+++	+	-	+	-	-
Réponse humorale contre la salive de <i>G.m.s.</i>	+++	+	+++	-	-	-

Légende, + (présence d'une réponse anti-salive)

- (absence d'une réponse anti-salive)

Conclusion

Les DO anti-salive de *G.p.g.* élevées des bovins de la zone indemne (au niveau résultats préliminaires) étaient dues:

- aux réactions croisées avec la salive des Tabanidés ;
- ou à la transhumance des bovins de la zone indemne vers des zones infestées.

L'étude expérimentale des bovins exposés aux piqûres par différents insectes hématophages a montré que,

- la réponse anti-salive de glossines a une bonne dynamique d'apparition et de disparition. Par conséquent l'évaluation de cette réponse au sein d'un troupeau peut être utilisée pour évaluer l'efficacité d'une lutte anti-vectorielle ;
- la réponse anti-salive de glossines semble assez spécifique de la piqûre par les glossines, même si quelques réactions croisées ont été observées, notamment avec la salive des tabanidés. L'utilisation de la salive de *G.m.s.* semble donner les meilleurs résultats.

En conclusion, nous pouvons donc dire que la réponse anti-salive est un bon marqueur d'exposition à la piqûre par les glossines. Pour un diagnostic du risque glossinien, nous préconisons l'utilisation de la salive totale de *G.m.s.* car cette salive a l'avantage de croiser avec les salives de *G.t.* et *G.p.g.* et ne croise pas avec celles des autres insectes hématophages testés.

En perspectives, nous comptons caractériser parmi les antigènes salivaires totaux, ceux spécifiques de glossines en collaboration avec la plateforme IRD EPIVECT à Montpellier (spécialiste en salive des vecteurs). Pour ce faire, nous allons sélectionner des sérums à faire passer en immunoblots (2D-PAGE) et par la suite identifier les protéines salivaires spécifiques de glossines par spectrométrie de masse. L'objet visé étant de synthétiser des peptides pour un diagnostic plus spécifique et standardisé du risque glossinien.

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SEROEPIDEMIOLOGY OF DOURINE IN BALE HIGHLANDS OF OROMYIA REGION, ETHIOPIA/

SEROEPIDEMIOLOGIE DE LA DOURINE DANS LE MASSIF DU BALE, REGION D'OROMYIA, ETHIOPIE

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Résumé

Cet article présente les résultats de l'étude séroépidémiologique entreprise de September 2007 à Juin 2008 en vue de déterminer la prévalence de la dourine dans des échantillons de sérum collectés de façon aléatoires dans certains des districts qui pratiquent l'élevage des chevaux dans le Massif du Bale, en Ethiopie. La séroprévalence de la dourine a été de 173 (19,66 %) et de 140 (15,90 %) pour le test d'agglutination sur carte pour le dépistage de la Trypanosomiasse (CATT) / *T. evansi* et pour les tests d'agglutination indirecte au LATEX (*T. evansi*), respectivement. La forte séroprévalence de dourine observée dans tous les districts d'élevage de chevaux sélectionnés pour l'étude dans le Massif du Bale, en Ethiopie, sont des éléments en faveur de l'endémicité de la maladie dans la zone. Dans les anticorps détectés dans

les deux tests, on ne peut pas faire la différence pour savoir s'ils sont dus à l'infection au *T. equiperdum* (l'agent pathogène de la dourine) ou au *T. evansi* (l'agent pathogène du surra). Ceci reste une question ouverte. Une reproduction non contrôlée, le manque de contrôle des mouvements des animaux et l'absence de trypanocides efficaces officiellement approuvés contre les cas de dourine, sont les principaux facteurs qui jouent un rôle important dans la répartition et la transmission de la maladie. En outre, vu le nombre important de chevaux en Ethiopie et leurs déplacements non contrôlés, sur l'ensemble du territoire, il est probable que la dourine soit plus répandue en Ethiopie que ce qu'on pense. A partir des conclusions de la présente étude, l'on peut dire que les tests de dépistage sérologique sur le terrain comme le CATT/*T. evansi* et les tests LATEX/*T. evansi* sont utiles pour les études épidémiologiques et la lutte contre la trypanosomiose équine et peuvent être utilisés pour remplacer les tests en CFT recommandés par l'OIE.

Mots clés

CATT/ *T. evansi* – Dourine- Ethiopie – Chevaux, LATEX/ *T. evansi* – Séroépidémiologie

Summary

This paper presents the results of seroepidemiological study carried out between September 2007 and June 2008 to determine the prevalence of dourine in a randomly collected serum samples in selected horse breeding districts of Bale highlands of Ethiopia. Accordingly, the sero prevalence of dourine was found to be 173 (19.66 %) and 140 (15.90 %) for Card Agglutination Test for Trypanosomosis (CATT)/*T. evansi* and LATEX indirect agglutination (*T. evansi*) tests, respectively. The higher seroprevalence of dourine observed in all the selected horse breeding districts of the Bale highlands of Ethiopia strongly supports the endemicity of the disease in the area. From the antibodies detected in both tests one could not differentiate between the infections of *T. equiperdum* (the causative agent of dourine) and those of *T. evansi* (the causative agent of surra). This remains an open question. Uncontrolled breeding, unrestricted animal movement and absence of officially approved effective trypanocidal drugs against dourine cases are the main factors that play an important role in the distribution and transmission of the disease. Further more, in view of large numbers of horses in Ethiopia and the unrestricted movement of animals throughout the country it is likely that dourine may be more widespread than is currently realised.

From the findings of the present study it is proposed that field applicable and screening serological tests such as CATT/*T. evansi* and LATEX/*T. evansi* tests be used in epidemiological studies and control of equine trypanosomosis; it may also be used to replace laboratory-based tests, OIE recommended CFT.

Key words: CATT/ *T. evansi* – Dourine- Ethiopia - Horses, LATEX/ *T. evansi* - Seroepidemiology.

Introduction

Ethiopia has a very large equine population, approximately 5.2 million donkeys, 2.8 million horses and 0.65 million mules in Africa. Equines are extremely important in the Ethiopian agriculture and for the national economy. Nearly 90 % of agricultural operations depend on human muscle and because of rugged mountainous terrain of this country these animals are still the main method of transporting both people and agricultural products (9, 10).

In developing countries like Ethiopia the contribution of equines is much diversified. The provisions of transport through pack animals, drawing carts, as riding animals or taxi operations almost definitely contributes more to national economy (11). The Arsi and Bale people like many others in the remote areas of highland of Ethiopia, where communications are poor, use horses as pack animals and for work on farms (9).

Of the Non-Tsetse Transmitted African Trypanosomes (NTTAT), dourine is the only trypanosomosis that is not transmitted by an invertebrate vector. *T. equiperdum* differs from other trypanosomes in that it is primarily a tissue parasite that rarely invades the blood (16). The first official report of the disease in Ethiopia was made in 1980 when the Arsi Rural Development Unit (ARDU) requested the Tsetse and Trypanosomiasis Survey and Control Department to investigate a persistent disease problem in horses in the administrative regions of Arsi and Bale (22). Since then dourine was found to be prevalent in the highlands of Ethiopia particularly in Arsi and Bale zones (1). Similarly, multiple cases of serological Complement Fixation Test (CFT) and Enzyme Linked Immunosorbent Assay (ELISA) and Trypanozoon Polymerase Chain Reaction (PCR) positive, yet aparasitemic horses were reported in Arsi and Bale zones of Ethiopia (8). Diagnosis of *T. equiperdum*, the causative agent of dourine in horses, by standard

parasitological techniques is difficult, owing to the low numbers of parasites present in the blood or tissues fluids and the frequent absence of clinical signs of disease. Therefore, the demonstration of trypanosomal antibodies in the serum has become the most important parameter in determining the disease status of individual animals (4). The main reason for using serological tests for the diagnosis of trypanosomosis is to overcome the low sensitivity of parasitological tests in detecting chronic infection. The difficulty in diagnosis of *T. equiperdum* leads to difficulties in achieving reliable data on the prevalence, distribution, implementation, monitoring, treatment and control of the disease programs. On top of that there are other limitations such as trypanocidal drug shortage, absence of vaccines against dourine, which play an influencing role in controlling, and prevention of the disease in an endemic area (8). In view of the large number of horse population in Ethiopia and lack of adequate facilities for diagnosis and control of the disease in relation to breeding, dourine is potentially a very important disease. Moreover, some districts of Bale highlands has not been studied for the prevalence of dourine. Hence, the objectives of the present study was to determine the sero prevalence of dourine in selected horse breeding districts of Bale highlands of Ethiopia.

Materials and methods

Study area

The present study was conducted in the Oromyia Regional State in the Bale highlands mainly in four selected horse breeding districts namely Agarfa, Dinsho, Goba and Sinana. Bale zone is found in the Oromyia Regional state southeast of the country, located 430kms away from Addis Ababa. Topographically, the altitude ranges from 500 to 4377 m.a.s.l. Three climate zones, including arid (63.53 %), midland (21.54 %) and highland (14.93 %) forms are known to exist. The area experiences a bimodal rainfall occurring from March to May and July to October. Agriculture is the mainstay of livelihood of peoples and the leading economic activity of the area with a mixed farming system covering the highest percentage of the total agricultural activities with crop-livestock production (2).

Study design and sampling strategies

A cross sectional study design was employed using serological examination in four selected horse breeding districts of Bale highlands. Sampling points within the four study sites (Agarfa, Dinsho, Goba and Sinana) were selected in collaboration with respective district's animal health personnel. Thus a purposive sampling was performed on the basis of farmers' cooperation, logistics, share of communal grazing land and accessibility. The study area is located between 2800 to 4377 m.a.s.l. There was no evidence of *T. evansi* infection in the selected study area. However, adjacent districts in the Bale lowlands such as Barebre, Dello-Mena and Harena-Buluk were known to be endemic for surra (camel trypanosomosis due to *T. evansi*) (2). A total of 880 horses were sampled equally 25 % (220 animals) from each selected districts of Bale highlands. All horses (528 males and 352 females) used in this study were sexually matured and of both sexes living under a traditional management system of free grazing.

Serological survey

Blood samples were collected from jugular vein of 880 horses using plain vacutainer tubes and needles, after the site is wiped with cotton wool soaked in alcohol. The plain vacutainer tubes were labeled and the blood was allowed to clot overnight at room temperature and the serum was separated by centrifugation. Then sera were filled into serum storage (polypropylene sterile cryogenic vials and stored at -20°C until tested by card agglutination for trypanosomosis test (CATT/ *T. evansi*), and LATEX/ *T. evansi*. The test was conducted at Debre Zeit Faculty of Veterinary Medicine molecular parasitology laboratory, which established by Belgian - Ethiopia VLIR- UOS funded collaborative project. For both tests (CATT/ *T. evansi* and LATEX/ *T. evansi*) positive results were determined at cut-off point dilutions 1:4. The tests were checked with positive and negative controls before the whole samples are tested (3).

Statistical analysis

Difference in the estimated prevalence between the sex of animals and study districts was analysed statistically using the chi square test for independence (17).

Results

The results of the present serological study of dourine in four selected horse breeding districts of Bale highlands of Ethiopia disclosed an overall seroprevalence of 173 (19.66 %) and 140 (15.90 %) for CATT/ *T. evansi* and LATEX/ *T. evansi* tests, respectively (Table 1). The seroprevalence of dourine using the CATT/ *T. evansi* test on the basis of sex was found to be 98 (18.60 %) and 75 (21.03 %) for male and female horses, respectively. Similarly, the seroprevalence based LATEX/ *T. evansi* test revealed 88 (14.50 %) and 52 (19.10 %) for male and female horses, respectively (Table 1). The seroprevalence of dourine among the four selected study districts was found to be in the range of (18.64 % to 20.91 %) and (15 % to 16.82 %) for CATT/ *T. evansi* and LATEX/ *T. evansi* tests, respectively (Table 2).

Table 1: Details of the seroprevalence of dourine in horses in selected districts of Bale highlands of Ethiopia on the basis of CATT/ *T. evansi* and LATEX/ *T. evansi* tests.

Factors		No. tested	No. positive and seroprevalence	
			CATT/ <i>T. evansi</i> test	LATEX/ <i>T. evansi</i> test
Sex				
	M	528	98 (18.60 %) ^a	88 (14.50 %) ^a
	F	352	75 (21.30 %) ^a	52 (19.10 %) ^a
Overall		880	173 (19.70 %)	140 (15.90 %)

χ^2 between sex using CATT/ *T. evansi* test = 2.65 (d. f. 1) $p > 0.05$

χ^2 between sex using LATEX/ *T. evansi* test = 1.08 (d. f. 1) $p > 0.05$

a, seroprevalence values in the different sexes with the same superscript do not differ significantly ($p > 0.05$)

Table 2: Details of the seroprevalence of dourine in horses in selected districts of Bale highlands of Ethiopia on the basis of CATT/ *T. evansi* and LATEX / *T. evansi* tests.

Study districts	No. tested	No. positive and seroprevalence	
		CATT/ <i>T. evansi</i> test	LATEX / <i>T. evansi</i> test
Agarfa	220	42 (19.09 %) ^a	34 (15.45 %) ^a
Dinsho	220	41 (18.64 %) ^a	36 (16.36 %) ^a
Goba	220	44 (20 %) ^a	33 (15 %) ^a
Sinana	220	46 (20.91 %) ^a	37 (16.82 %) ^a
Overall	880	173 (19.70 %)	140 (15.90 %)

χ^2 CATT/ *T. evansi* seroprevalence between study districts = 2.13 (d. f. 3)
p>0.05

χ^2 LATEX/ *T. evansi* seroprevalence between study districts = 1.54 (d. f. 3)
p>0.05

a: seroprevalence values in the study districts with the same superscript do not differ significantly (p>0.05)

Discussion

The present study conducted in selected horse breeding districts of Bale highlands of Ethiopia disclosed a higher seroprevalence of dourine. The sero prevalence of dourine was found to be 15.90 % and 19.66 % by using CATT /*T. evansi* and LATEX /*T. evansi* tests, respectively. This sero prevalence result is almost consistent with the previous reports based on indirect methods (antibody and antigen detection) in the Arsi Bale highlands of Ethiopia (1, 22).

Statistically significant variation was not observed (p > 0.05) when the seroprevalence of dourine in both CATT/ *T. evansi* and LATEX/ *T. evansi* tests was compared across sex and study districts among the examined animals. This might be attributed to the fact that the animals in all sex group and study districts were equally exposed to the parasites and indicating uniform spread of the disease.

Though there was no statistically significant difference ($p > 0.05$) in the seropositivity between CATT/ *T. evansi* and LATEX/ *T. evansi* tests, relatively lower prevalence was observed in the later test. This might be due to the fact that LATEX/*T. evansi* test is a more specific compared to CATT/ *T. evansi* test (5).

Currently neither serological, parasitological nor DNA based tests allow a subspecies identification within the subgenus *Trypanozoon*, therefore no definitive diagnosis can be given for *T. equiperdum* (6, 7). In a recent study conducted in Ethiopia a very high concordance was observed between the serological results in both CATT/*T. evansi* and ELISA/RoTat 1.2 and the clinical (dourine) status of the examined animals (12). Hence it appears that these tests can be valuable for the detection of dourine although initially designed to detect surra infections in camel. For mass screening of the horse population for equine trypanosomosis, serological methods have been the method of choice since the early 20th century (15). The only diagnostic test for equine trypanosomosis at this moment, recommended by the World Animal Health Organization (OIE), is the Complement Fixation Test (CFT) (18). CFT detects antibodies against *T. equiperdum* in the serum of the host. Using this test, *T. equiperdum* has been eradicated in the USA, Canada and the EU. More recently, a Horse Complement Fixation Test (HCFT) has been developed for the diagnosis of dourine (Igekbayeva, 1994). In comparison to CFT, in HCFT antibodies are found earlier, in high titres and during a longer time (14). However, both these diagnostics tools are laboratory tests and thus samples need to be transported and processed in a serological laboratory.

On the other hand, a diagnostic antibody detection test for *T. evansi* based on the RoTat 1.2 VAT has been developed, namely CATT/*T. evansi*, a direct card agglutination test (3). This test is fast, uses a standardised antigen and can be performed in situ, i.e. without the need of a fully equipped laboratory. Recently, it has been proven that most so-called *T. equiperdum* strains also express isoVATs of *T. evansi* RoTat 1.2. Therefore, the CATT/*T. evansi* may prove to be a good test for equine trypanosomosis, regardless whether the causative agent is *T. evansi* (surra) or *T. equiperdum* (dourine) (5, 6). In previous studies done in South Africa it was examined and recommended that CATT can be an alternative for CFT in naturally infected horses. Accordingly, there was “almost perfect” agreement between the two tests. Interestingly all the tested horses showing clinical signs of dourine scored positive in CATT.

The authors stated that “CATT could be usefully employed especially as a field test in outlying districts, and should be further investigated” (20). A similar result was obtained in a previous study in Kazakhstan, where a high concordance was found between CFT and CATT/ *T. evansi* (6).

Thus, from our observations as well as the results of previous studies (Williamson et al., 1988) (8) it can be suggested that CATT may be proposed to replace CFT and HCFT as serological mass screening test (6, 12, 20). The CATT test is faster and easier to perform than CFT and HCFT. Moreover, anti-complement factors have no effect on the result. This may be particularly interesting in the testing of donkey sera where anti-complement effects are often observed (19). Finally, different reference centres usually use different antigens in CFT, often generated from trypanosome strains with an unknown history (21). This makes comparison of results difficult. CATT can overcome this problem, since this test uses a standardised antigen, a RoTat 1.2 cloned population.

Whether the examined animals in this study are infected with *T. equiperdum* (the causative agent of dourine) or with *T. evansi* (the causative agent of surra) remains an open question. However, the higher seroprevalence of dourine observed in all the selected horse breeding districts of the Bale highlands of Ethiopia strongly supports the endemicity of the disease in the area. Such seroprevalence report was established for the first time in those selected horse breeding districts of Bale highlands namely Agarfa, Dinsho and Sinana districts. The uniform and widespread distribution of the disease in the area could be attributed to the unrestricted animal movement from neighboring districts for the trade and transport purpose, uncontrolled animal breeding and absence of officially approved effective trypanocidal drugs against dourine. Moreover, as the distribution of dourine is not restricted by other environmental factors it can easily be established almost anywhere where there is large equine population (15).

Therefore, this study provides strong circumstantial evidence that dourine is highly prevalent endemic disease in the study area and a potential threat to equine population. In order to minimize the spread of the disease, there should be community awareness creation and extension specifically to stop using clinical dourine and recovered cases for breeding purpose, and apply strict animal movement control. Given the current difficulties in diagnosis particularly parasite isolation and differentiation with *T. evansi*, further detailed studies need to be

conducted to isolate new parasite strains and to explore the possibilities of molecular diagnosis of *T. equiperdum*.

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FIELD DETECTION OF CHEMORESISTANCE TO ISOMETAMIDIUM AND DIMINAZENE IN THE REGION OF BOUCLE DU MOUHOUN, BURKINA FASO/

DETECTION DE LA CHIMIORESISTANCE A L'ISOMETAMIDIUM ET AU DIMINAZENE DANS LA REGION DE LA BOUCLE MOUHOUN, BURKINA FASO

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Résumé

La présente étude a évalué la chimiorésistance au chlorure d'isoméтамidium (TRYPAMIDIUM[®]) et au diacéturate de diminazène (TRYPADIM[®]) dans la région de la Boucle du Mouhoun. Une enquête parasitologique transversale précédemment réalisée dans la région a permis d'identifier 10 villages où les prévalences parasitologiques étaient les plus élevées de la région (2,1% – 16,1%). Dans chaque village, il a été mis en place un troupeau sentinelle d'environ 100 bovins répartis en 2 groupes de 50 dont l'un a été traité à l'isoméтамidium (1mg/kg p.v.) et l'autre servant de témoin. Au total, 978 bovins ont été soumis à une enquête longitudinale. Un contrôle toutes les deux semaines de parasitémie a été effectué chez tous les animaux pendant 8 semaines selon la méthode de détection de la chimiorésistance sur le terrain d'Eisler. La résistance à l'isoméтамidium a été suspectée dans 4 des 10 villages à savoir Dèbé, Kangotenga, Mou et Laro. Cependant, l'isoméтамidium pourrait encore être utilisé dans 4 villages: Nokuy,

Bendougou, Déré et Boromissi, où la résistance n'a pas été suspectée et où la pression trypanosomienne est élevée. Dans les 2 autres villages à savoir St Michel et Soukoura, aucune nouvelle infection n'a été détectée dans le groupe traité, mais la pression de la maladie est faible, donc le traitement à l'isoméтамidium ne serait pas utile. Cette étude n'a pas identifié de résistance au diminazène dans les villages enquêtés. Cependant, dans 4 villages il y a eu des rechutes parmi les animaux traités au diminazène (3,5mg/kg p.v.), mais les taux de rechutes ont été faibles, avec toutefois un taux de 15,79% à Kangotenga. Au regard de ces résultats, une gestion rigoureuse des trypanocides s'avère nécessaire afin de retarder au mieux la généralisation de la chimiorésistance et la chimiorésistance multiple dans la région de la Boucle du Mouhoun.

Summary

The present study assessed the chemoresistance to isoméтамidium chloride (TRYPAMIDIUM®) and diminazene diaceturate (TRYPADIM®) in the Boucle de Mouhoun Region. A cross sectional parasitological survey previously carried out in the area allowed the identification of 10 villages where the parasitological prevalences were highest (2.1% - 16.1%). In each village, a sentinel herd of approximately 100 bovines was set up, divided into 2 groups of 50 of which one was treated with the isoméтамidium (1mg/kg b.w.) and the other left untreated as the control group. On the whole, 978 animals were subjected to a longitudinal study. A fortnightly control of parasitemia was carried out in all the animals during 8 weeks according to the method of detection of the field chemoresistance described by of Eisler. Resistance to isometamidium was suspected in 4 of the 10 villages; which were Débé, Kangotenga, Mou and Laro. However, isometamidium could still be used in 4 villages, of Nokuy, Bendougou, Déré and Boromissi, where the resistance was not suspected and where the trypanosomosis pressure was high. In 2 other villages i.e. St Michel and Soukoura, no new infection was detected in the treated group, and the pressure of the disease was low, therefore isometamidium treatment was not required. This study did not reveal any resistance to diminazene in the surveyed villages. However, in 4 villages there were relapses among the animals treated with diminazène (3.5 mg/kg b.w.), but the rate of relapses was generally low, although a slightly higher rate of 15.79% was reached at Kangotenga. Regarding these results, a rigorous management of the trypanocides is necessary in order to delay as much as possible the

widespread of the chemoresistance and the multi chemoresistance in the study area.

Introduction

The economy of Burkina Faso relied mainly on the rural sector which represents approximately 86% of the total population and contributes to a total value of 40% to the GDP (MEDEV, 2002). The livestock breeding contributes for more than 10% to the GDP and is the second provider of currencies in exportation after agriculture and before the mining sector (MEDEV, 2004; MRA, 2006). The Western and South-western parts of the country are the wetter with average annual rainfalls higher than 900mm. These zones have great agricultural and pastoral potentialities. Unfortunately the efficient development of livestock is confronted with the tsetse and animal trypanosomosis (T&T). Currently, more than 63% of burkinabese population cattle live in the zones at the risk of trypanosomosis (Kamuanga *et al.*, 2001a).

The economic impact of animal trypanosomosis is difficult to assess with accuracy. However, according to the Ministry for the Animal Resources of Burkina, 2.8 million preventive or curative doses of trypanocides are used annually for the maintenance of susceptible cattle to the disease in enzootic area of trypanosomosis. By estimating the cost of a dose of trypanocide at 1.5 US\$, the annual direct losses related to the purchase of the trypanocides are evaluated to 3.9 million US\$. The estimation of the other direct costs and the indirect costs of the disease require studies extended to the whole tsetse distribution area. The most illustrative socio-economic survey into the trypanosomosis in Burkina were carried out by Kamuanga *et al.* (2001a ; 2001b) in the pastoral zone of Yalé and in the province of Kénédougou by Ouédraogo (2002). The control of the trypanosomosis can induce a growth rate of 25% of the cattle population, an increase of the daily dairy production up to ten times (Kamuanga *et al.*, 2001b). In the province of Kénédougou, trypanosomosis high prevalent area, the cost of treatment of the disease by head of susceptible cattle was estimated at 2,500 FCA, about 6.25 US\$ (Ouédraogo, 2002). Because of the failure of the sustainability of the control campaigns against the T&T carried out in past (Bauer *et al.*, 1992, 1995, 1999, Cuisance *et al.*, 1990), the treatment remains the only means used by the stockbreeders to control the disease. The abusive use of the trypanocides often of doubtful quality led to the chemoresistance.

The chemoresistance was reported the first time in Burkina Faso at the beginning of the 1980's in the province of Kénédougou (Authié, 1984; Pinder and Authié, 1984), then it was confirmed on the field (Clausen *and al.*, 1992; McDermott *et al.*, 2003; Diarra, 2001; Talaki *et al.*, 2007). The resistance to trypanocides is since suspected in the other parts of the tsetse distribution area, especially the Region of the Boucle du Mouhoun, cotton zone and PATTEC (Pan African Tsetse and Trypanosomiasis Eradication Campaign) priority intervention area. However further field investigation are needed for the confirmation.

The goal of this study is to evaluate the degree of the resistance to isoméamidium chloride and/or to diminazene diaceturate in the Region of the Boucle du Mouhoun by the technique of the "block treatment" (Eisler *et al.*, 2000). This technique is the only one used for the detection of the chemoresistance to isoméamidium in the field. It was used successfully in Ethiopia by Tewelde *et al.* (2004), in Burkina Faso by McDermott *et al.* (2003), in the border area of Burkina - Mali and in Guinea by Talaki *et al.* (2007) and in Cameroon by Mamadou *et al.* (2006). This test is based on the comparison of the incidence rates of the trypanosomose in two groups of cattle followed under the field conditions, of which, one group is treated with isoméamidium and the other group is being used as control. The follow-up of the infections lasts 56 days.

This study was initiated following the baseline entomological and parasitological survey carried out by PATTEC. The study had set the hypothesis that the failures of trypanocide treatments in the Region of the Boucle du Mouhoun are due to the chemoresistance developed by the strains of trypanosomes against trypanocides. The results of this work should allow PATTEC to better set up its control strategies against animal trypanosomose in the Region of the Boucle du Mouhoun.

Materials and methods

Study area

The Region of the Boucle du Mouhoun, is located at the North-West of Burkina Faso between longitudes -2.4°; -4.6° and latitudes 11.23°; 13.7°, and it covers a surface of 34,497 km². The area is subdivided into six provinces and the provinces are subdivided in 47 districts and the districts themselves are composed of 1061 villages. The Region is located in the soudano-sahelian zone with average annual rainfalls

varying from 500 to 1400 mm of rain (MEDEV, 2005). Moreover, the area has a dense hydrographic network linked around the Mouhoun river which crosses the area on nearly 280 km. Around the Mouhoun river many permanent secondary rivers can be found (Figure 1). The socio-economic activities in the area are mainly agriculture and the livestock breeding. Agriculture is based mainly on the food crops and cash crop such as cotton. Livestock breeding are ubiquitous in the systems of production met in the area and are variable from one place to another because of the agro-climatic conditions. In the Region, there are 658,494 bovines and 1,455,622 small ruminants (MRA, 2006). The mode of livestock breeding remains mainly traditional profiting from the natural resources of which the availability of a significant biomass all along the rivers and the basin. The cattle raised in the area are mainly zebu, some taurine and their cross breeds.

Trypanocides

The isometamidium chloride (TRYPAMIDIUM[®] MERIAL SAS, Lyon, France lot n^oDG/20058) and the diminazène diaceturate (TRYPADIM[®] MERIAL SAS, Lyon, France lot n^oD53001A) were the trypanocides used in this study. The trypanocides were diluted in mineral water at the concentration of 2% and 7% for TRYPAMIDIUM[®] and TRYPADIM[®] respectively.

Cross sectional survey

Villages were randomly selected. Thus, a village was randomly selected by district. Hence, 47 villages were initially chosen. In order to increase the number of villages at higher risk of trypanosomosis, 7 other villages were randomly selected among those located at 5km or less of the Mouhoun river or its principal tributaries (41 villages), that made a total of 54 villages. The estimate of sampling envisaged to take by village 50 bovines. In each village, the number of animals resulting from the same herd is limited to 5 cattle in order to guarantee a good representativeness of the sample. Actually, the sampling was carried out in 53 villages and 2002 cattle were selected. The parasitological infections were assessed by the Buffy-Coat Technique (Murray *et al.*, 1977) while the serologic infections were diagnosed by ELISA. The pathogenic trypanosomes species were identified and their prevalences were recorded.

Baseline entomological survey

The PATTEC area was divided into grids of 10km on side or 100km² of surface. For the entomological survey, it was identified 3 sites per grid. The identification of these sites was made with satellite images using geographical information system (SIG) device such the ARCGIS® software. Challier-Laveissière biconical traps, on which sachets containing of olfactory bait were fixed, were used for the capture of the tsetse. Three traps distant of 100m one of the other, were displayed by site and they lasted for 72 hours. The traps were removed then flies identified by species and by sex and were counted. The apparent densities per trap (ADP) were reported by grid.

"Block treatment" study

Ten villages were selected regarding the results of the cross sectional survey. The parasitological prevalences of the 10 villages were the highest. The assessment of the resistance to isometamidium was carried out by the technique of "block treatment" of Eisler *et al.* (2000). In each of the 10 villages, 100 cattle of at least 1 year old, were tagged. Then they were divided randomly in two groups of 50 heads of which, one was the treated group and the other was the control group. The selected animals had not received any trypanocide treatment the three months preceding the beginning by this study. The first group was treated with isometamidium at the dose of 1mg/kg bodyweight in deep intramuscular. The weight of the bovines was estimated with weigh-band. The race, the age, the sex and other observations of the animals were recorded.

At the beginning of the study all the animals were subjected to parasitological analysis by the buffy coat technique (Murray *et al.*, 1977), then every two weeks during 8 weeks. Considering D0, the day of the treatment of isometamidium, 4 visits were carried out at D14, D28, D42 and D56 in the two groups according to the protocol of Eisler *et al.* (2000). During each visit, the PCV of each animal was measured and the positive diagnosed animals were treated with diminazene at 3.5mg/Kg bodyweight in deep intramuscular. This method also allowed assessing the resistance to diminazene by the systematic treatment of the positive animals of the control group.

The chemoresistance to isometamidium was estimated by comparing the parasitological incidence of trypanosomosis in the two groups. Hence, the relative risk (RR) or "ratio of Eisler" which is the ratio of the

incidence in the control group on that in the treated group, was calculated. The curative effect of isometamidium at the dose of 1mg/kg on the positive cases in the treated group at D0 was highlighted at D14 post treatment (Delespaux *et al.*, 2008).

Statistical analysis

The EXCEL software was used for the data processing and construction of the figures, and then the analysis was made with the software EPI INFO 6. The parasitological prevalence of the trypanosomosis was calculated by dividing the number of positive cases by the total number of cattle tested during the cross sectional survey. The incidence for the 8 weeks was calculated by making the ratio of the average number of new infections on the average number of cattle followed. Both, the incidence and the prevalence were expressed as a percentage.

The Ratio of Eisler (Eisler *et al.*, 2000) was calculated by dividing the incidence of the trypanosomosis in the control group by the incidence of the disease in the treated group. Resistance to isometamidium was suspected when the relative risk is lower than 2 (Eisler *et al.*, 2000); this is equivalent to a reduction of the risk of infection in the treated group of less than 50% compared to the control group. Resistance was also suspected when the rate of new infections in the treated group exceeds 25% (Eisler *et al.*, 2000). The proportion of the new infections is expressed as a ratio of the number of animals becoming positive during the 8 weeks of follow-up on the total number of followed animals. By analogy with the treatment of malaria due to *Plasmodium falciparum* in human health (OMS, 2004), the threshold of 25% of failure of treatment was set for suspicion of resistance to diminazene.

The difference between the proportions of new infections of trypanosomosis was compared with the test of χ^2 . In the same way, the test of Fisher (F) was used to assess the difference between the average PCVs of the 2 experimental groups.

Results

Cross sectional studies

Cross sectional parasitological survey

Positive animals were found in 13 villages, a quarter of the villages. The infections observed by microscopy were mainly *T vivax* in all the villages where positive cases were encountered and some cases of infections with *T congolense* found in only two villages (Table I). Neither mixed infection nor infection with *T brucei* was recorded. At the scale of the whole Region of the Boucle du Mouhoun, the parasitological prevalence was low, i.e. 1.25%. However the parasitological prevalence reached 7.4%, 9.6% and 16.1% at Bossé, Kangotenga and Soukoura respectively. The average PCV during this study was 28.37%, which was less than normal cattle PCV comprised between 30% and 40% (Troncy *et al.*, 1981).

The seropositive cases of trypanosomosis were reported in 50 of the 53 surveyed villages, with serologic prevalence up to 100% in some villages (Figure 2). These serological infections were mainly due to mixed infections to *T. congolense* / *T. vivax*, or simple one to *T. vivax* or *T. congolense* alone. Antibodies against *T. brucei* were detected in some cattle in 3 villages.

Entomological baseline survey

Two riverine species of tsetse i.e. *Glossina palpalis gambiensis* and *G. tachinoides* were captured at during the entomological basic investigation. The ADP given to the villages represent actually that of the grid in which the village is included. The ADP varied from 0.02 to 26.70 tsetses per trap and per day. The highest values were recorded in the grids of the villages of Nokuy, Bendougou and Mou (Table II). No entomological survey was carried out in the villages of Débé and Laro which were took into account in this longitudinal study.

Longitudinal study

Description of the sample cattle

Based on the results of the cross sectional study and with the aim of selection at least one village per province of the Region of the Boucle du Mouhoun, 10 villages whose 8 villages in which the cross sectional study noted a prevalence varying between 2.1% and 16.1% were selected for the longitudinal study. The two other villages were selected to replace two others of the list of the villages surveyed previously because of their proximity and of the availability of the stockbreeders to take part to the study. A total of 978 sentinel bovines whose 492 animals were treated group and 486 animals were the control group. The whole cattle belonged to 70 herds. The number of 100 animals was reached in all the villages except Déré and Nokuy. The sample was composed of 96.2% of zebus (*Bos indicus*), 3.6% of crossbreeds and 0.2% of taurines (*Bos taurus*). The average age of the animals was of 4.8 ± 2.9 years.

Parasitological follow-up

The parasitological diagnosis identified in addition to the pathogenic trypanosomes, *T. theleri* and microfilaria in the blood sample with incidences of 1.94% and 9.51% respectively. During all the follow-up, the infections of trypanosomosis were in majority due to *T. vivax* with 83.6% and 16.4% for *T. congolense*. There was only one mixed infection. The average PCV in the two experimental groups for all 10 village, were shown in figure 3. During the first 28 days of follow-up, there no was significant difference between the average PCV of the two groups ($p > 0.05$). However beyond D42, the average PCV of the treated group were significantly higher than those of the control group ($p < 0.05$). That could be explained by the effect of the double protection of the animals of the treated group. Indeed, in addition to the isometamidium treatment, all the positive cases detected in microscopy were treated with diminazene.

Chemoresistance to isometamidium

The proportions of the new infections of trypanosomosis during the 8 weeks by village in the two groups were shown in figure 4. The proportions of new infections were low at St Michel and Soukoura, the rates of new infections in the control group were 14% and 7.02% respectively. Whereas for the two villages, no new infection in the

isometamidium treated groups was recorded. For the eight other villages, the rates of new infections in the control groups varied from 24.3% to 59.52%. These rates were equal to or higher than 25% except to Débé and Laro where the rates were respectively 24.49% and 24.3%.

The statistical comparison of the proportions of new infection between the control groups and the treated groups, shows a strong suspicion of the chemoresistance in 4 villages (Débé, Laro, Mou and Kangotenga) according to the criterion of Eisler *et al.* (2000). In the same way, the test of χ^2 showed that there was no significant difference between the proportions of new infections in the two experimental groups at Débé, Laro and Kangotenga ($p > 0.05$) (Table III).

Curative effect of isometamidium in 2 weeks post treatment

No relapse was recorded at D14 among the positive cattle treated at D0 with isometamidium.

Assessment of the resistance to diminazene

Table IV shows the rates of relapse 14 days after the diminazene treatment of cattle of the control group. The cases of relapses were observed at Déré, Laro, Kangotenga and Nokuy. However all the rates of relapse were lower than 25%, therefore this study did not prove resistance to diminazene in the surveyed villages.

Discussions

According to the criteria of Eisler *et al.* (2000), this study confirmed a strong suspicion of resistance to isometamidium in 4 of the 10 villages surveyed in the Region of the Boucle du Mouhoun. In the village of Mou, the proportion of new infections were high, i.e. 59.52% and 37.5% in the control group and the treated group respectively; the ratio of Eisler was also lower than 2 there. However for the 3 other villages where resistance to isometamidium was suspected, namely Laro, Débé and Kangotenga, the proportions of new infections of the treated groups and control groups were around 20% and 25% respectively. In these 3 villages, there was no significant difference between the proportion of new infections of the 2 experimental groups ($p > 0.05$). The cattle treated with isometamidium at the dose of 1mg/kg did not have a protection against the trypanosomosis significantly compared to the control ones.

Whereas the local veterinary agents use the dose of 0.5mg/kg isometamidium as preventive treatment (results of investigation). If this study showed suspicions of resistance to this product in the area, all the more the half dose should also give at least the same results or worse. Indeed, the preventive effect of isometamidium is conferred by the slow diffusion of the product owing to the tissue binding at the intramuscular injection site (Eisler *et al.*, 1996). However, certain concentration of the product in blood is necessary to ensure this protection capacity (Eisler *et al.*, 1997).

Although the isometamidium treatment could always be recommended at Déré, Nokuy, Bendougou and Boromissi where the proportions of new infections in the control groups were higher than 25% and whose chemoresistance was not yet detected by the field detection technique of resistance to isometamidium. The tsetse challenge was high in Bendougou and Nokuy, 2 villages located at less than 5km to the Mouhoun River. For these villages, the ADPs by grid were 23.17 and 26.70 tsetse per trap per day for Bendougou and Nokuy respectively. This result then confirm a need for the preventive isometamidium treatment with the dose of 1 mg/kg instead of 0.5mg/kg. Indeed, even if the technique of Eisler *et al.* (2000) did not allow to highlight the chemoresistance in some of the villages, Rates of failures of protection conferred by isometamidium in 8 weeks were recorded in these villages with up to 21% at Boromissi and Nokuy, 22% and 16% at Bendougou and Déré respectively. It is necessary to manage well the isometamidium treatments in these localities in order to delay as maximum as possible the appearance of resistances.

For the villages of Soukoura and St Michel where no new infection of trypanosomose was found among the treated cattle and whose rates of new infections in the control groups were low, less than 15%, the use of isometamidium in preventive treatment is not be useful. In contradiction at Soukoura, the prevalence was the highest, i.e. 16.1%, during the cross sectional study. This paradox could be explained by a sampling bias. Indeed, during this study, the animals were selected in 5 herds in order to make a representativeness of the village. Moreover, the rate of parasitological infection at D0 was only 4% at Soukoura.

For the whole of the Region of the Boucle du Mouhoun, the average PCVs for the two experimental groups were, during the follow-up at an acceptable level, around 30%, the standard of the PCVs of the bovines is

ranging between 30% and 40% (Troncy *et al.*, 1981). The proportions of new infections during 8 weeks of follow-up reached 27.5% for the whole cattle followed with predominance for *T vivax* (83.6%). The mean PCV in the treated group was not significantly different from that of the control group during the first 4 weeks of follow-up ($p>0.05$). However from D42 the average PCVs of the treated group was significantly higher than those of the control group. In the long term, the preventive treatments based on isometamidium improve health statute of the treated animals (Camus, 1995).

The present study did not allow highlighting resistance to diminazène used at the dose of 3.5mg/kg, in the surveyed villages. The failures of diminazene treatment in the control groups were recorded in 4 villages, but the rates of failures were lower than 10% except in Kangotenga where this rate reached 15.79%. Although resistance to diminazene is not suspected in the surveyed villages, it requires a rigorous use of this product in the Region of Boucle du Mouhoun.

The study comforted the presence of the chemoresistance to isometamidium in Burkina Faso, especially in the cotton belt. Indeed, the chemoresistance was reported for the first time at the early 1980's in the province of Kéné Dougou and precisely in the agropastoral zone of Samorogouan (Authié, 1984). Resistance to isometamidium and diminazene was highlighted at strains of *T congolense* in mice (Pinder and Authié, 1984). Thereafter, the multi chemoresistance to isometamidium, diminazene and ethidium, was proven on in experiments infected sensitive animals (Clausen *et al.*, 1992). Resistance to the trypanocides was already described on the field in the same locality by longitudinal studies of naturally infested animals (McDermott *et al.*, 2003; Talaki *et al.*, 2007).

The chemoresistance to trypanocides was reported in 17 trypanosomosis enzootic countries of sub-Saharan Africa (Delespaulx *et al.*, 2008). One of the causes of the chemoresistance is the use of the same molecules for more than 40 years (Geerts *et al.*, 2001). In addition to the pharmacological factors, it is necessary to note the miss management of the veterinary drugs with trypanocides and anthelmintic representing 85 to 90% in Burkina Faso (MRA, 2006). Over the 2.8 million doses of trypanocides used annually in Burkina Faso, only 23% are official imports recorded from the veterinary services (MRA, 2006).

Recent studies on the quality of the trypanocides diminazene and isometamidium based sold in Sub-Saharan Africa, showed that a great majority of these products, in particular the generic ones did not respect the standards established by the original products (Tetty *et al.*, 1999; 2002; Schad *et al.*, 2008). Amongst preventive isometamidium based trypanocides in Burkina, only the TRYPAMIDIUM[®], drug introduced since 1958, is stable in its composition and its content (Schad *et al.*, 2008; Tetty *et al.*, 1999). More recently, the Ministry of Animal Resources of Burkina in collaboration with FAO financed an investigation on quality control of the veterinary drugs, as well the official circuit as smuggling. The study showed that about half of the officially recorded trypanocides were not in conformity (Têko, personal communication). It is also necessary to note an excessive use of the trypanocides by the farmers themselves, which contributes to the selection of the resistant even multi resistant strains of trypanosomes (Geerts *et al.*, 2001). Indeed, according to official report of the MRA (2006), from 2003 to 2006, the use of trypanocides in the area of the Boucle du Mouhoun decreased from 1,054,004 doses to 77,910 doses. This drastic drop of the official figures underlies the existence of parallel networks of provisioning of veterinary drugs. The improper and miss use of trypanocides by the farmers often without preliminary diagnosis of the disease increases also the cost of the disease at the farm level.

Conclusion

The chemoresistance to isometamidium in the Region of the Boucle du Mouhoun was shown in 4 of the 10 surveyed villages. This chemoresistance was due mainly to *T. vivax*, the rates of infections due to *T. congolense* was relatively low. Other techniques of detection of the chemoresistance such as the inoculation on ruminants and estimation of isometamidium concentration in sera by ELISA from the treated cattle should be used to corroborate our findings (Eisler *et al.*, 1997; 1993). For *T. congolense*, the molecular technique of detection of the chemoresistance should be carried out on the stabulats collected to establish and confirm the evidence of chemoresistance (Delespaux *et al.*, 2006; Delespaux *et al.*, 2005).

The isometamidium treatment is always useful in the bordering villages of the Mouhoun River where the ADPs are highest. Notwithstanding, the curative effect of isometamidium is still effective. The present study did not show resistance to diminazene. Therefore, diminazene could be used

alone in the localities where the chemoresistance to isometamidium is proven and in the villages where the trypanosomosis incidence is low. Diminazene could also and especially be used to clear up animals from trypanosomosis before the preventive treatments in the control measures in order to benefit from the sanative pair effect.

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Table I. Prevalence of bovine trypanosomosis in the Region of the Boucle du Mouhoun

Villages	Number	Positives	Prevalence (%)	<i>Trypanosoma sp.*</i>	
				<i>T. vivax</i>	<i>T. congolense</i>
Laro	47	1	2,1	1	0
Seyou	23	1	4,3	1	0
Boromissi	25	1	4,0	1	0
St Michel	44	2	4,6	2	0
Déré	51	2	3,9	2	0
Bendougou	21	1	4,8	1	0
Bamakoro	46	1	2,2	1	0
Nokuy	36	2	5,6	2	0
Saorokuy	50	1	2	2	0
Soukoura	31	5	16,1	3	2
Zelassé	44	1	2,3	1	0
Kangotenga	52	5	9,6	4	1
Bossé	27	2	7,4	2	0

* There was no case of infection to *T. brucei*, nor mixed infection

Table II:ADP of tsetse in the sites of the longitudinal study

Villages	DAP/espèce de glossine		Total
	<i>G. p. gambiensis</i>	<i>G. tachinodes</i>	
Déré	0	0.02	0.02
St Michel	0	0.4	0.40
Bendougou	16.28	6.89	23.17
Nokuy	25.67	1.03	26.70
Soukoura	0.02	0.09	0.11
Kangotenga	0	0.07	0.07
Mou	4.8	4	8.80
Boromissi	1.15	1.17	2.32

Table III.Proportions of new infection and the incidence in the two groups

Villages	Group	No.	Ni (%)	I(%)	RR	CI	T max (%)	T failures (%)	χ^2	p
Débé	Treated	50	20	5	1.22*	0.39-1.71	61	39 [§]	0.29	0.59 [#]
	Control	49	24.49	6.12						
Laro	Treated	37	21.62	5.41	1.12*	0.39-2.05	61	39 [§]	0.08	0.07 [#]
	Control	37	24.32	6.08						
Déré	Treated	43	18.6	4.65	2.89	0.17-0.66	84	16	14.18	0
	Control	39	42.86	13.46						
Bendougou	Treated	50	18	4.5	2.33	0.22-0.84	78	22	6.86	0
	Control	50	42	10.5						
Nokuy	Treated	45	20	5	2.44	0.21-0.79	79	21	8.14	0
	Control	43	48.84	12.2						
Kangotenga	Treated	45	24.44	6.11	1.02*	0.47-2.02	53	47 [§]	0	0.95 [#]
	Control	44	25	6.25						
Mou	Treated	40	37.5	9.38	1.59*	0.39-1.01	61	39 [§]	3.98	0.04
	Control	42	59.52	14.88						
Boromissi	Treated	48	14.58	3.65	2.24	0.21-1.06	79	21	4.38	0.03
	Control	49	32.65	8.16						

Ni: Proportion of new infections, I: Incidence, Ci: Confidence interval, T max: maximum rate of protection, T failure: rate of failures.

* $RR < 2$, suspicion of resistance to isometamidium (Eisler *et al.*, 2000) at Débé, Laro, Kangotenga and Mou.

§ The rate of relative failure is higher than 25%, which confirms the suspicion of resistance to isometamidium in these four villages

$p > 0.05$, the difference between the proportions of new infections in the two experimental groups is not statistically significant, the isometamidium treatment was not effective.

Table IV. Relapse 14 days after the diminazene treatment of the control group

Villages	Number treated	Number relapses	Rate of relapse (%)
Déré	23	2	8.70
Nokuy	34	2	5.88
kangotenga	19	3	15.79
Laro	17	1	5.88

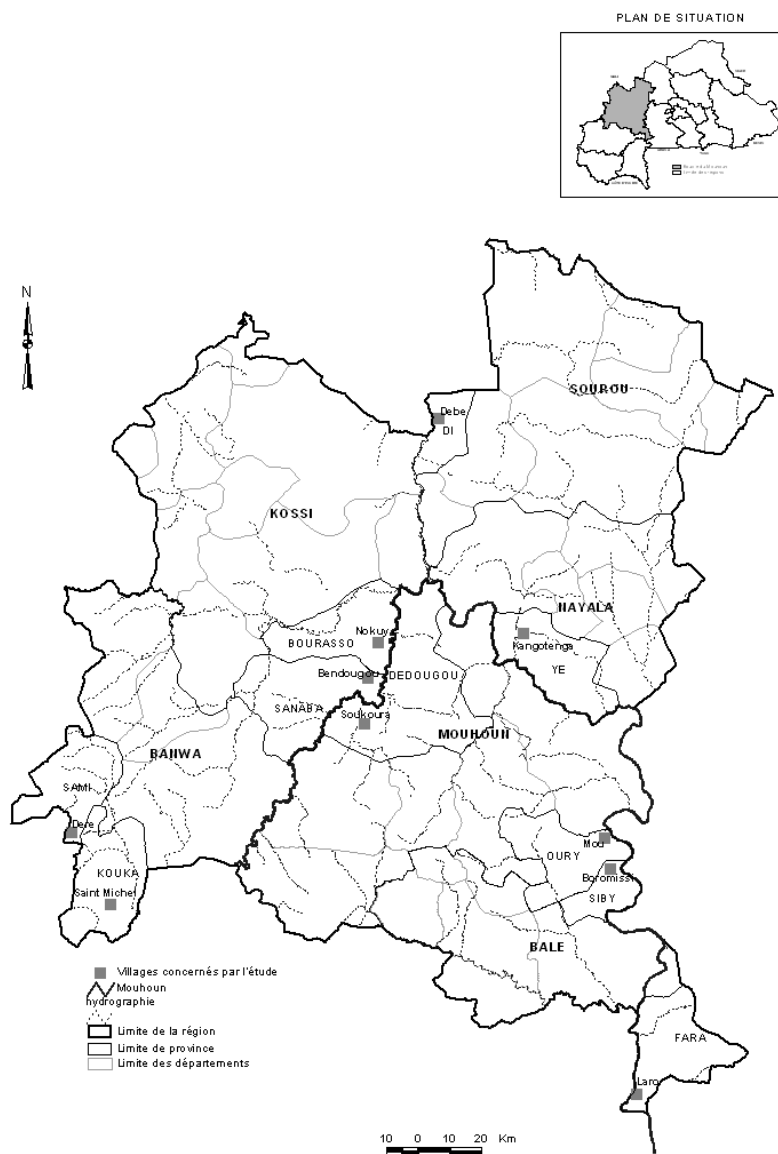


Figure 1: Localization of the study area

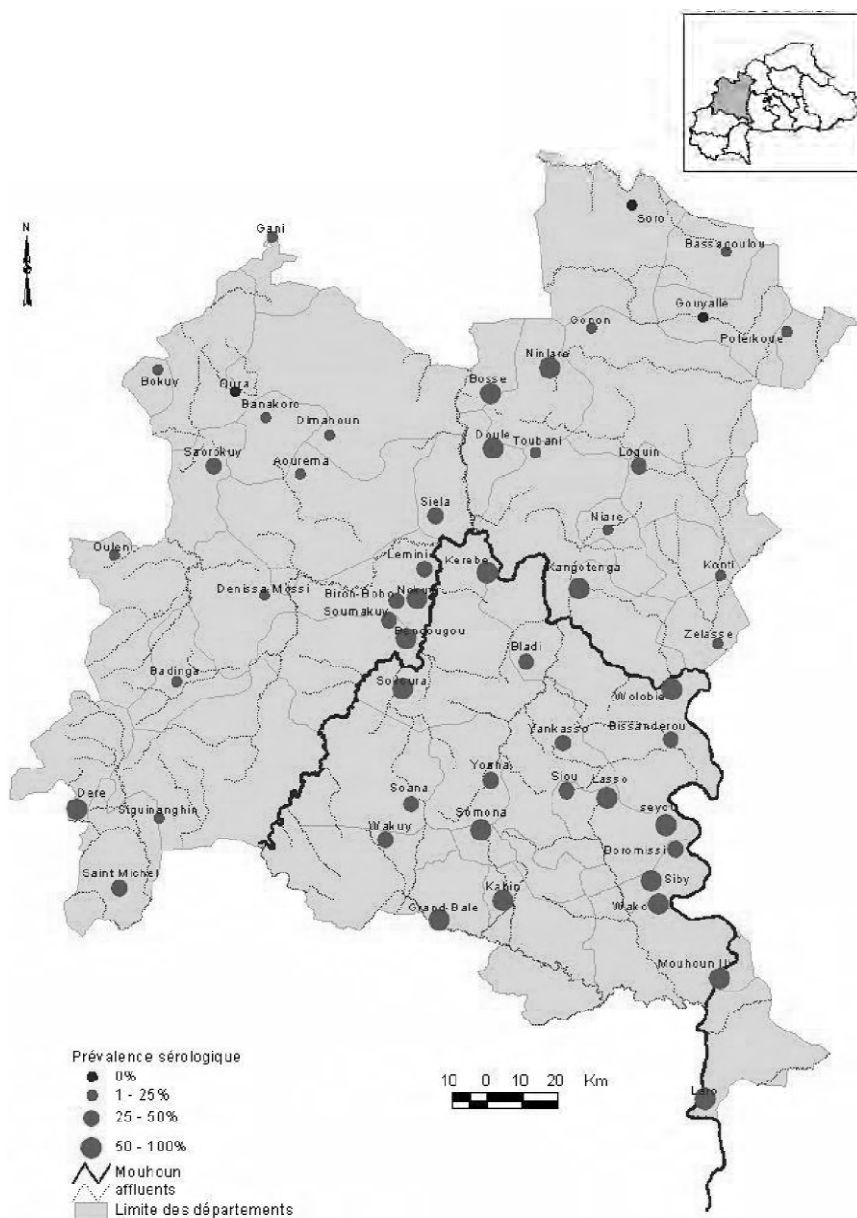


Figure 2: Serological Prevalence of the bovine trypanosomosis in the Region of Boucle du Mouhoun

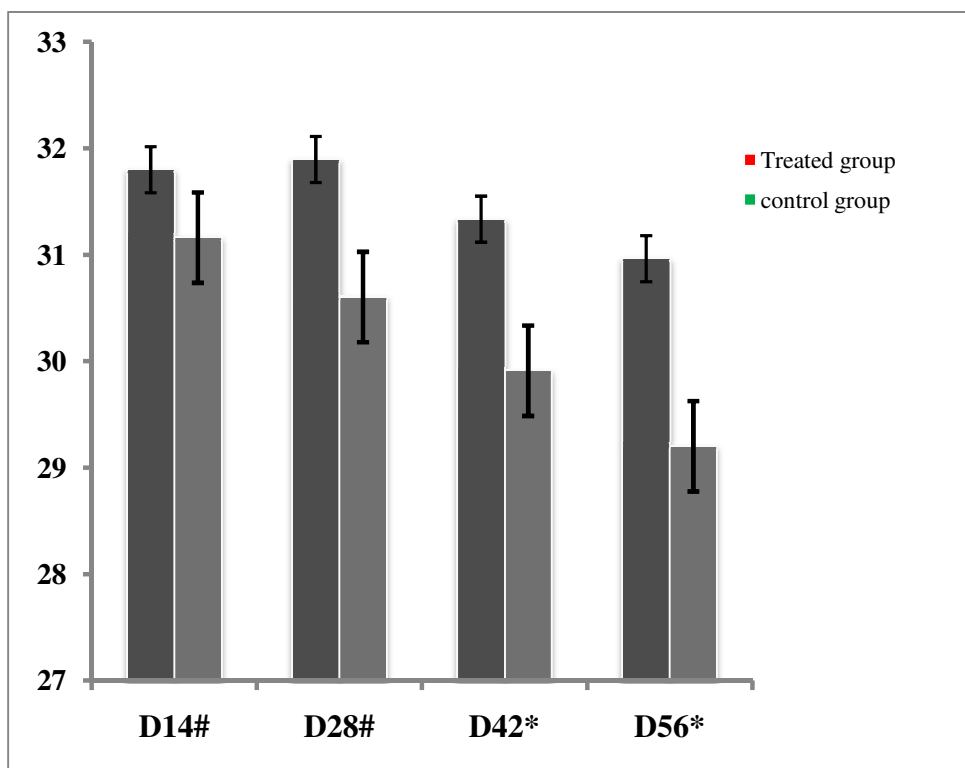


Figure 3: Means PCV in the 2 experimental groups according to dates of the follow-up

There was no significant difference between the average PCV of 2 experimental groups ($p > 0.05$) in D14 ($F = 1.09$; $p = 0.337$) and D28 ($F = 1.43$; $p = 0.063$)

* $p < 0.05$, there was a significant difference with D42 ($F = 1.91$; $p = 0.002$) and in D56 ($F = 1.78$; $p = 0.004$)

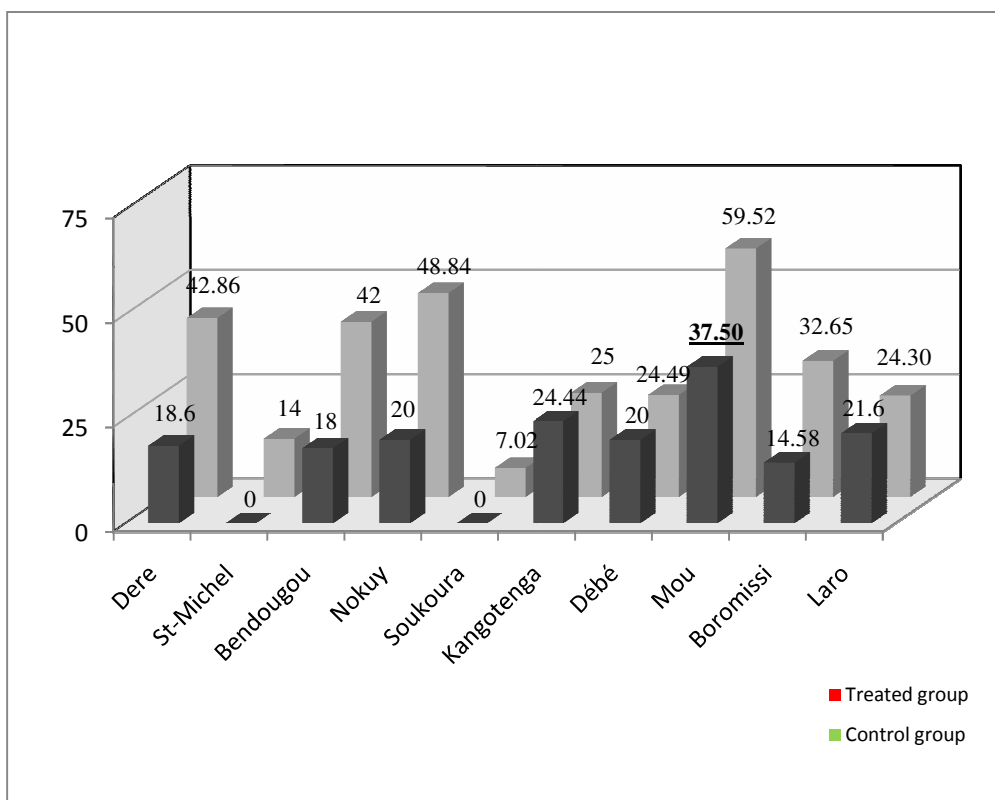


Figure 4. Proportions of new infections of trypanosomosis per village and according to the experimental group.

There is a strong correlation between the proportions of the new infections in the 2 experimental groups, $r=0.78$. According to the first criterion of Eisler et al. (2000), the chemoresistance is straightaway suspected in the village of Mou, where the proportion of new infections in the treated group was higher than 25%, i.e. 37.5%.

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**ANIMAL TRYPANOSOMIASIS SURVEILLANCE IN THE
NEW FOCUS OF SLEEPING SICKNESS IN DOKOLO AND
ALWA SUBCOUNTIES, NORTH EASTERN UGANDA/**

**SURVEILLANCE DE LA TRYPANOSOMIASE ANIMALE
DANS LE NOUVEAU FOYER DE LA MALADIE
DUSOMMEIL DANS LES SOUS COMTE DE DOKOLO ET
D'ALWA, AU NORD-EST DE L'OUGANDA**

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Résumé

Une étude de terrain sur trypanosomiase a été menée dans les sous-comtés de Dokolo et d'Alwa, des districts de Dokolo et de Kabaramaïdo, respectivement, au Nord-Est de l'Ouganda, où il y a eu des cas de trypanosomiase humaine, afin de déterminer la prévalence de la trypanosomiase animale chez les animaux domestiques et de caractériser les trypanosomes isolés des animaux domestiques, afin de déterminer les infections mixtes de trypanosomes. 435 animaux domestiques au total ont été examinés au plan parasitologique par microscopie et par hématoците pour détecter la présence de trypanosomes. 9 animaux avaient des trypanosomes, 3 avaient de la microfilariose and 1 avait le *T. theileria* dans les quatre paroisses du sous-comté de Dokolo et 4 animaux infectés ont été détectés dans le sous-comté d'Alwa. Les animaux testés positifs à la trypanosomiase ont été référés à l'agent vétérinaire du District pour traitement avec des trypanocides. Le taux d'infection à la trypanosomiase chez le bétail était de 3,0% à Dokolo et de 2,9% à Alwa, ce qui est très bas et pourrait être attribué au traitement de masse des animaux domestiques avec des trypanocides dans les districts de Dokolo de Kabaramaïdo, en avril 200, par une équipe de vétérinaires de la Faculté de Médecine vétérinaire, de l'Université de Makerere. Il a en outre été établi que ces animaux infectés avaient été achetés récemment dans les marchés à bestiaux du voisinage ou auprès d'agriculteurs qui

n'avaient pas présenté leurs animaux pour un traitement de masse avec des trypanocides en avril 2008. Ces résultats laissent supposer que le traitement de masse des animaux en avril 2008 dans les districts de Dokolo et de Kaberamaido a eut un effet marqué sur la trypanosomiase animale dans les paroisses étudiées, ce qui est positif pour les agriculteurs en vue d'une meilleure productivité du bétail.

Abstract

A trypanosomiasis field survey was carried out from 14th to 22nd July 2008 in purposely selected parishes in Dokolo and Alwa sub-counties in Dokolo and Kaberamaido districts, respectively, north eastern Uganda, where there are cases of sleeping sickness patients. The aim of the survey was to determine the prevalence of animal trypanosomiasis in domestic animals and to characterise trypanosomes isolated from domestic animals in order to estimate any mixed infections of trypanosomes. A total of 435 domestic animals were parasitologically examined by microscopy for the presence of trypanosomes and 9 animals had trypanosomes, 3 had microfilaria and 1 had *T. theileri* in the four parishes in Dokolo sub-county and 4 infected animals were detected in Alwa sub-county. The owners of all the animals found positive for trypanosomiasis were advised to seek assistance from the District Veterinary Officer for treatment of infected animals since the team did not have trypanocides. The trypanosomiasis infection rate in cattle was 3.0% in Dokolo and 2.9% in Alwa and the difference between trypanosomiasis infection rates in cattle from Dokolo and Alwa sub-counties was not statistically significant (with $P > 0.05$, by both Pearson Chi-square, Chi-square with Yate's correction). The low infection rates could be attributed to the previous mass treatment of domestic animals with trypanocides in Dokolo and Kaberamaido districts around April 2008 by a Veterinary team from the Faculty of Veterinary Medicine, Makerere University. It was further established that those infected cattle were recently bought from neighbouring cattle markets or from farmers who did not present their cattle for mass treatment with trypanocides in April 2008. These results suggest that the mass treatment of cattle in April 2008 in Dokolo and Kaberamaido districts has had a marked effect on animal trypanosomiasis in the screened parishes and this is good for the farmers for better livestock productivity.

Introduction

African trypanosomiasis is one the neglected tropical diseases which infect both animals and humans. This disease is often fatal to humans and livestock, if not treated, and is transmitted largely by tsetse flies. Besides other species of *Trypanosoma* transmitted by tsetse flies, *T. evansi* is transmitted mechanically beyond the tsetse belt by biting flies while *T. equiperdum* is by coitus (Hoare, 1973). In south east and north east Uganda, livestock production is hampered by trypanosomiasis. Previous reports in Uganda indicated that the prevalence of animal trypanosomiasis in cattle was 11.9% under intensive dairy system and 25% under communal grazing systems (Magona and Mayende, 2002). Tsetse flies are estimated to infest 106,400 square km and 50% of the entire landmass of Uganda and 2.2 out of 5.4 million estimated national cattle population are at a risk of trypanosomiasis (Chizyuka, 1998).

Transmission of both animal and human trypanosomiasis in south east Uganda has been attributed to a number of tsetse species which include *Glossina f. fuscipes*, *G. pallidipes* and *G. brevipalpis* (Moloo *et al* 1980, Magona *et al* 2005 and Waiswa *et al* 2006). However, studies in north east Uganda, the new focus of human trypanosomiasis, has been so far attributed to only *Glossina fuscipes fuscipes* (Abila *et al* 2008). The aim of the study was to determine the prevalence of animal trypanosomosis in domestic animals in the new focus of sleeping sickness in Dokolo and Kaberamaido districts

Materials and methods

Study area

This study was conducted in villages in Dokolo and Kaberamaido districts shown on the map of Kaberamaido (Fig 1). Villages in Dokolo and Alwa sub-counties referred to as administrative areas cover areas under cultivation, fallow land and communal grazing land extending to swamps and rivers. The study was conducted in 4 villages in Dokolo and 2 villages in Alwa sub-counties which had tsetse flies. These villages were located in the same agro-ecological zone and had similar vegetation, climate and farming system.

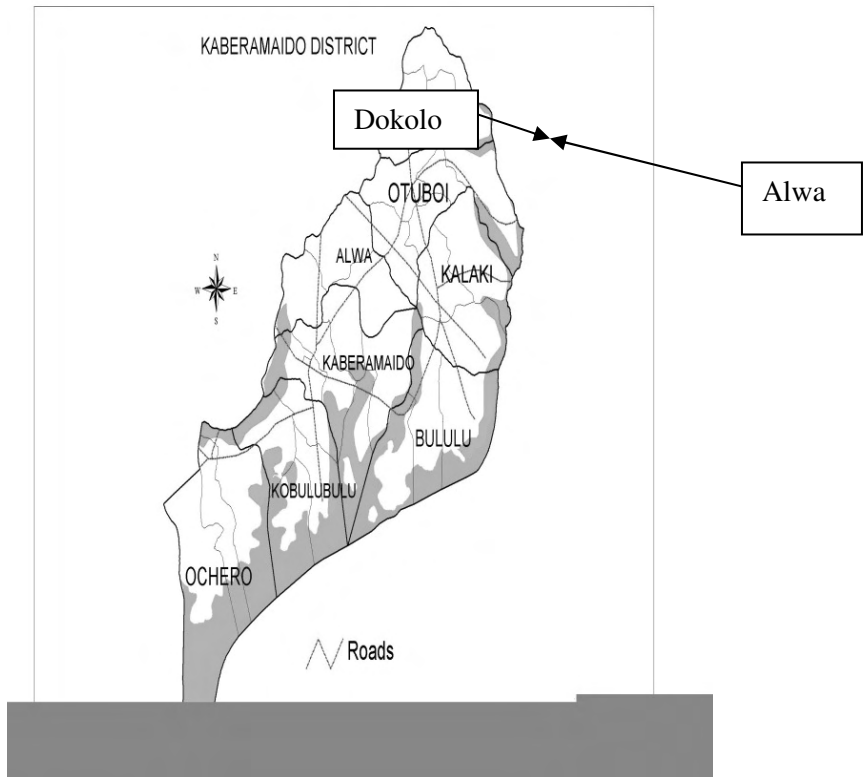


Fig.1: Map of Kaberamaido showing Dokolo and Alwa sub-counties where tsetse survey was done

Cattle management

In all the six villages, Zebu and Ankole cattle are kept under the traditional communal grazing management. The animals are either tethered or grazed under communal pasture during the day and tied up around homesteads or kept in kraals at night (Magona *et al* (2005)

Cattle survey

All cattle presented at designated screening centres were parasitologically examined for the presence or absence of trypanosomes. A total of 435 cattle (300 from Dokolo and 135 from Alwa) had their

blood samples taken from jugular vein and examined using thin wet blood smear under X 400 magnification for the presence of trypanosomes.

Data analysis

Data on trypanosome infection rates in cattle was analysed using the computer programme Graph pad Prism 3.0. The prevalence of animal trypanosomiasis in cattle in Dokolo and Alwa sub-counties was compared using Chi-square and Chi-square with Yates's correction. In addition, Pearson Chi-square test was performed to measure associations between trypanosomiasis infection and the two sub-counties of Dokolo and Alwa and p-values less than 0.05 was considered significant.

Results

Prevalence of trypanosomiasis

The overall prevalence of trypanosomiasis in Dokolo (3.0%) was not significantly higher than in Alwa sub-county (2.9%), Tables 1&2. (Pearson chi-square $X^2=$, d.f. =2, $P = 0.125$; Chi-square with Yate's correction $X^2= 0.08$, d.f. =1.0, $P = 0.77$ both of which are greater than 0.05).

Table 1: Summary of the screening results for the presence of trypanosomes in animals in Dokolo districts.

Date	Locality	Host	Total examined	Trypano-some positive	Micro-filaria positive	<i>T. theleria</i> positive
15-7-2008	Akongodyek Anwechbanyi	Cattle	88	0	1	0
16-7-2008	Atama -Village Atur-Parish	Cattle	50	0	0	0
17-7-2008	Otolomogo-Village Amwoma -Parish	Cattle	63	3	1	1
18-7-2008	Acan Onyomo – Village Aleng- Parish	Cattle	99	6	1	0
Total			300	9	3	1
Infection rates				3.0%	1.0%	0.3%

A total of 300 domestic animals were parasitologically examined by microscopy for the presence of trypanosomes and 9 animals had trypanosomes, 3 had microfilaria and 1 had *T. theileria* in four parishes in Dokolo sub-county (Table 1). The owners of all the animals found positive for trypanosomiasis were advised to seek assistance from the District Veterinary Officer for treatment of infected animals since the team did not have trypanocides. The trypanosomiasis infection rate of 3.0% obtained was low. Similarly, 135 domestic animals were parasitologically examined by microscopy for the presence of trypanosomes and 4 animals had trypanosomes, giving infection rate of 2.9% in two parishes in Alwa sub-county (Table 2).

Table 2: Summary of the screening results for the presence of trypanosomes in animals in Alwa subcounty, Kaberamido district.

Date	Locality	Host	Total examined	Trypanosome positive
22-7-2008	Olumai-Village Palatau-Parish	Cattle	55	2
23-7-2008	Awasi-Village Abalang-Parish	Cattle	80	2
Total			135	4
Infection rates				2.9%

Tables 1 & 2. (Pearson chi-square $X^2=$, d.f. =2, $P = 0.125$; Chi-square with Yate's correction $X^2= 0.08$, d.f. =1.0, $P = 0.77$ both p values are greater than 0.05)

Discussion

In this study, it was noted that the prevalence of animal trypanosomiasis in cattle was low (Dokolo 3.0% & Alwa 2.9%) in both sub-counties; and also the difference between trypanosomiasis infection rates in cattle from Dokolo and Alwa sub-counties was not statistically significant (with $P > 0.05$, by both Pearson Chi-square, Chi-square with Yate's correction). These low infection rates could be underestimation due to the low sensitivity of the parasitological test used (Picozzi *et al* 2002) and could also be attributed to the previous mass treatment of domestic animals with trypanocides in Dokolo district around April 2008 by a Veterinary team from the Faculty of Veterinary Medicine, Makerere University. It was further established that those infected cattle were

recently bought from neighbouring cattle markets or from farmers who did not present their cattle for mass treatment with trypanocides in April 2008. These results suggest that the mass treatment of cattle in April 2008 in Dokolo and Alwa sub-counties has had a marked effect on animal trypanosomiasis in the screened villages and this is good for the farmers for better livestock productivity.

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NOUVELLES DONNEES SUR LA TRYPANOSOMOSE CAMELINE A *T. EVANSI* EN MAURITANIE/

NEW INSIGHTS ON CAMEL TRYPANOSOMOSIS DUE TO *T. EVANSI* IN MAURITANIA

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Summary

There are two forms of camel trypanosomosis caused by *T. evansi*. An acute form characterised by general weakness, abortions and a high mortality within 10 days to 4 months. Yet, in 80% of the cases a more chronic form is observed, characterised by abortions, drop in milk production, gradual weight loss, cachexia, etc.

Previous work in Mauritania has shown that camel trypanosomosis is present and that farmers are aware of this problem. Depending on the season and the region, parasitological prevalence varies between 1.1 and 17.6%, while a seroprevalence of 13 to 58.8% was observed in the Card Agglutination Test for Trypanosomiasis (CATT). Trarza seems to be the most affected region. Potential vectors for *T. evansi* are Tabanidae (*A. tylotus agrestis*, *Tabanus taeniola*, and *T. sufis*), Stomoxynae (*Haematobia minuta* and *H. irritans*) and Hippoboscidae (*Hippobosca camelina*, *H. variegata*).

Given the importance of camels as livestock (source of meat and milk, we undertook a study to examine the current status of camel trypanosomosis in Mauritania. Camels brought to the slaughterhouse of the « Société des abattoirs de Nouakchott » were examined. On average 100 camels with a known clinical history were tested each day.

Of 254 dromedary camels examined, 14.2% were positive in CATT and 6.3% were doubtful. In contrast, no trypanosomes were found in Buffy coat examination. RealTime-PCR analysis on blood samples of CATT seropositive animals was also negative. These results will be discussed in relationship to camel husbandry practices, the various methods of

slaughtering and the intensive use of trypanocidal drugs by the smallholders.

Keywords: Mauritania, Dromedary camels, *T. evansi*, prevalence, Buffy coat, CATT, RT-PCR, trypanocidal drugs, slaughter.

Résumé

La trypanosomose cameline due à *Trypanosoma evansi* se présente sous deux formes. Dans sa forme aiguë, elle se traduit par une faiblesse généralisée, des avortements des femelles gestantes et une mortalité entre 10 jours et 4 mois. Mais dans 80 % des cas, elle se présente sous sa forme chronique qui se caractérise par des avortements, une diminution de la production laitière, un amaigrissement progressif, voire une cachexie, etc.

En Mauritanie, des travaux antérieurs ont montré que c'est une pathologie présente dans le pays et bien connue des éleveurs. Selon l'année et la région, la prévalence parasitologique varie de 1,1% à 17,6% contre une séroprévalence au CATT de 13% à 58,8%. Le Trarza semble la région la plus infectée. Les vecteurs potentiels de *T. evansi* sont des Tabanidae (*Atylotus agrestis*, *Tabanus taeniola*, et *T. sufis*), des Stomoxynae (*Haematobia minuta* et *H. irritans*) et des Hippoboscidae (*Hippobosca camelina*, *H. variegata*).

Les auteurs ont entrepris une étude sur la situation actuelle de la trypanosomose cameline en Mauritanie. Pour ce faire, ils ont entrepris des investigations à la Société des abattoirs de Nouakchott. Environ 100 dromadaires par jour, dont l'histoire clinique était connue, ont été examinés

Sur 254 dromadaires examinés, 14,2% étaient positifs au CATT et 6,3% douteux. En revanche, aucun trypanosome n'a été mis en évidence par la technique « Buffy Coat ». Parmi les animaux positifs au CATT, l'analyse par PRC-RT, s'est révélée négative. Ces résultats ont été discutés en fonction du mode d'élevage des dromadaires, des techniques d'abattage, de l'utilisation intensive de trypanocides de la part des petits éleveurs.

Mots clés. Mauritanie, Dromadaires, Mauritanie, *T. evansi*, prévalence, Buffy coat, CATT, PCR-RT, trypanocides, abattage.

Introduction

La Mauritanie est un pays quasi-désertique dont les conditions physiques, climatiques et socioculturelles font de lui un pays d'excellence pour l'élevage des dromadaires dont l'effectif est estimé à 1,3 millions (Direction de l'Élevage, 2000). Pendant longtemps, cet animal était le parent pauvre des projets et programmes de la Direction de l'élevage. Actuellement, il est devenu, une nécessité, un impératif de vivre et une source de rente. En effet, pour mieux exploiter les productions des dromadaires en particulier le lait frais très fortement prisé avec l'avènement des sociétés laitières, les dromadaires sont confiés à des bergers salariés extrêmement mobiles à la recherche de meilleurs pâturages et des points d'eau. Ce mode d'élevage expose ainsi ces animaux à certaines pathologies comme la trypanosomose.

Malgré leur sobriété, les dromadaires sont sensibles à différentes infections (Kane *et al.*, 2003). Mais selon de nombreux auteurs, la trypanosomose cameline est considérée comme pathologie dominante des dromadaires (Röttcher *et al.*, 1987, Mahmoud and Gray, 1980). La trypanosomose cameline bien connue par les chameliers mauritaniens est appelée localement *Tabourit*. Des études avaient montré qu'elle était bien présente en Mauritanie (Dia, 1997c, Jacquet *et al.* 1994 ; Christy, 1987) et listé un certain nombre de facteurs de risque et de variation pour connaître son épidémiologie. La région du Trarza était la plus infectée (Dia *et al.*, 1997b). Par la suite, d'autres études ont montré à partir d'un sondage réalisé à l'abattoir que la trypanosomose cameline est largement distribuée (Cnerv, 2001).

Avec la mutation qu'a connue l'élevage des dromadaires en Mauritanie due aux changements climatiques et le constat d'une multitude de trypanocides dans le marché, quelle est la situation présente de la trypanosomose cameline en Mauritanie, Pour ce faire, les auteurs ont entrepris des investigations à la Société des abattoirs de Nouakchott.

Matériel et méthodes

Lieu du sondage

Le travail a été conduit durant une semaine en juin 2008 à la Société des abattoirs de Nouakchott (SAN) qui est un abattoir moderne opérationnel depuis 2002 située à 12 Kms de la ville de Nouakchott. La SAN abat de

gros ruminants (bovins et dromadaires) à raison de l'ordre de 80 dromadaires par jour. Elle dispose d'un personnel qualifié et a mis en place un système de codification des bouchers permettant ainsi d'avoir la traçabilité de tout animal abattu. Elle est organisée en équipes chargées de la recette, du pointage et du marquage des animaux. Ces enregistrements effectués de 16h à 20h sont supervisés par un inspecteur de la Délégation du Ministère du Développement Rural chargé de l'examen sur pied des animaux à abattre. Au niveau post mortem qui débute de 4h à 7h, il existe un responsable des bouchers chargé de veiller au bon fonctionnement des opérations d'abattage et deux inspecteurs dont l'un pour l'inspection des carcasses des bovins et l'autre pour celles des dromadaires.

Echantillonnage

Les dromadaires sont prélevés au hasard selon le rythme et l'accessibilité des animaux contenus à abattre présents dans l'aire du sacrifice. Tout animal à prélever fait d'abord l'objet d'un examen ante mortem sommaire. Puis sur une fiche sont reportés, l'âge, le sexe, la couleur de la robe et l'origine géographique de l'animal. Ensuite, il fait l'objet de prélèvement au niveau de la jugulaire sur tube avec EDTA pour le sang total et sur tube sec avec activateur de coagulation pour la récolte de sérum.

Techniques de mise en évidence de l'infection

Une goutte de ce sang permet de remplir des tubes capillaires pour déterminer l'hématocrite de l'animal ainsi l'examen du buffy coat pour la mise en évidence éventuelle de la présence de *T. evansi* au microscope (Woo, 1970).

Le sang total de certains animaux ont été analysés aussi à l'aide de la PCR-RT (Macherey NAGel, Swift spectrum Real-time PCR cycler). L'extraction de l'ADN a été faite par le kit Quiagen. Comme contrôle positif c'est de l'ADN de *T. evansi* (6→991 d25) et contrôle négatif, de l'ADN humain. Le programme employé utilise 3 cycles, 1^{er} cycle, dénaturation, 95°C pendant 15 minutes ; 2^{ème} cycle, hybridation, 95°C /15 secondes, 55°C /20 secondes, 72°C/30 secondes pour 30 cycles ; 3^{ème} cycle, élongations finales à 72°C pendant 5 minutes.

Le sérum récolté a été testé sérologique par le CATT (Card Agglutination Test for Trypanosomosis) qui est un test d'agglutination rapide (Bajyana Songa and Hamers, 1988).

Résultats

Au total 254 dromadaires ont été sondés.

Aucun *T. evansi* n'a été mis en évidence à l'examen des buffy coat. La prévalence sérologique totale est de 14,2%. Le sérum de 16 dromadaires (6,3 %) était douteux. La moyenne des hématocrites des animaux sondés est de $28,5 \pm 4,5\%$. La moyenne des hématocrites des animaux négatifs est de $28,4 \pm 4,7\%$ contre $28,6 \pm 3,6\%$ pour les animaux à CATT+. Ces valeurs sont identiques. Le sang de 10 dromadaires dont 8 à CATT+ et 2 à CATT- a été analysé par PRC-RT; les résultats étaient négatifs.

Discussion

La trypanosomose cameline a toujours préoccupé les éleveurs des dromadaires en Mauritanie comme en témoigne leur engouement pour les trypanocides de tout venant. Il est connu que si certains trypanocides sont dotés d'une efficacité certaine vis à vis de *T. evansi* (Dia, 2001 ; Otsyula *et al.*, 1994 ; Lun *et al.*, 1991 ; Tager-Kagan *et al.*, 1982), d'autres le sont moins et mal supportés par les dromadaires (Balis *et al.*, 1977 ; Leach *et al.*, 1971). Cette situation est bien connue des éleveurs mauritaniens.

Des investigations à large échelle avaient révélé qu'il existait une corrélation positive entre la prévalence de l'infection et les régions boisées riches en cours d'eau où pâturent les dromadaires. D'autres facteurs de risque étaient liés à la conduite du troupeau, à l'âge des animaux (les animaux âgés de 5 à 10 ans étaient les plus infectés) et à la saison en particulier la saison sèche froide qui correspond à la période de pullulation des tabanides (Dia *et al.*, 1996). A l'abattoir de Nouakchott, la prévalence parasitologique était de 1,5% contre 26,7% de séroprévalence au CATT. En 2000, nos investigations avaient montré une recrudescence d'infections de *T. evansi*, 17,6% de prévalence parasitologique contre une séroprévalence de 58,8 % au CATT (Cnerv, 2001).

Notre présente enquête a révélé peu d'animaux positifs au CATT et aucun *T. evansi* n'a été détecté à l'examen parasitologique. Certes la méthode de détection directe des trypanosomes en général (Desquesnes, 1997) et *T. evansi* en particulier (Dia, *et al.*, 1997a) est peu sensible mais il est surprenant de ne pas en rencontrer dans tout cet effectif. Cette absence de *T. evansi* a d'ailleurs été confirmée par la PC-Real time, ce qui semble exclure qu'il s'agirait d'animaux en début de séroconversion.

Comme de nombreux trypanocides circulent en Mauritanie, le fait de constater un blanchissement des animaux pourrait être attribué à leur utilisation intensive par les éleveurs. Cet engouement pour les trypanocides s'expliquerait par le fait que les chameliers font beaucoup de transhumance intra régionale et dans les pays voisins. Si les trypanocides utilisés sont dotés de propriétés préventives reconnues, c'est à encourager car il semblerait que les cas de trypanosome cameline enregistrés aux Iles Canaries (Gutierrez *et al.*, 2005) seraient contaminés par des dromadaires mauritaniens. Car l'exemple des infections de *T. evansi* en France (Desquesnes *et al.*, 2008) montre l'intérêt de mettre en place un système de contrôle systématique de la détection de *T. evansi* chez les dromadaires à exporter ou transhumants hors du pays et de vulgariser le piégeage des vecteurs (Dia *et al.*, 2004). En effet, là où l'élevage des dromadaires est pratiqué, des foyers de trypanosomose cameline ne sont pas à écarter. La preuve est démontrée pour la première fois au Burkina Faso (Dia, 2006).

Conclusion

Des travaux anciens avaient montré que la trypanosomose cameline à *T. evansi* était bien présente en Mauritanie. Son importance serait probablement liée à la présence d'eaux de surface et le couvert végétal offrant ainsi des conditions favorables au développement d'insectes vecteurs de ce parasite.

Avec les changements climatiques occasionnant le déplacement permanent des dromadaires dans l'espace mauritanien et à l'étranger, et la mise en place de performants outils de diagnostic, les auteurs s'attendaient à détecter un nombre important de *T. evansi*. Force est de constater qu'ils n'en ont pas observé à l'examen direct, ce qui a été confirmé par PCR-RT. Ils ont conclu à la pression que cette situation serait liée à l'utilisation intensive de trypanocides.

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**EPIDEMIO-SURVEILLANCE NETWORK ON
CHEMORESISTANCE OF TRYPANOCIDES AND
ACARICIDES IN WEST AFRICA: CHALLENGES,
OBJECTIVES AND MISSIONS**

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**RESEAU D'EPIDEMIO-SURVEILLANCE DE LA
CHIMIORESISTANCE AUX TRYPANOCIDES ET AUX
ACARICIDES EN AFRIQUE DE L'OUEST (RESCAO):
ENJEUX, OBJECTIFS ET MISSIONS**

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Résumé

L'opérationnalisation en Avril 2009 du réseau d'épidémio-surveillance de la chimiorésistance aux trypanocides et aux acaricides en Afrique de l'Ouest (RESCAO) s'inscrit dans le cadre des activités du projet «Renforcement du CIRDES comme centre de référence pour le diagnostic et le contrôle de la trypanosomose et de la résistance aux trypanocides en Afrique de l'Ouest». Ce projet est réalisé au CIRDES en partenariat avec le département vétérinaire de l'Institut de Médecine Tropicale (IMT) d'Anvers, Belgique. Il vise à contribuer à l'amélioration des connaissances sur la chimiorésistance et des stratégies de lutte contre celle-ci en Afrique de l'Ouest. En effet, dans le contexte de la mise en œuvre généralisée du programme Panafricain d'Eradication de la mouche Tsé-tsé et des Trypanosomoses Animales Africaines (PATTEC en anglais), le développement progressif de la chimiorésistance constitue une menace sérieuse pour la réussite de ce programme. Or la situation épidémiologique réelle de la chimiorésistance est mal connue en Afrique de l'Ouest. Seuls le Burkina Faso, le Mali et la Guinée ont fait l'objet d'investigations. L'Objectif général de ce réseau est d'élargir ces investigations à tous les pays membres ou associés du CIRDES, puis à l'ensemble de tous les pays d'Afrique de l'Ouest. L'approche

méthodologique est la surveillance active ciblée sur les foyers suspects dans tous les pays engagés dans le réseau.

Summary

The epidemio-surveillance network on chemoresistance of trypanocides and acaricides in West Africa has been settled in April 2009 in CIRDES with the partnership of Institute of Tropical Medecine (IMT) in Antwerp, Belgium, within the program “Strephenning CIRDES as centre of reference for diagnosis and control of trypanosomosis and chemoresistance to trypanocides in West Africa”. The overall objective is the improvement of knowledge on the epidemiology of chemoresistance and the strategies to control it. Indeed, the development of chemoresistance in Africa represents a serious threat to the efficacy of the on-going Pan African Tsetse and Erypanosomosis Eradication Campaign (PATTEC) in many countries. In addition, the epidemiology of the chemoresistance is not known in many countries particularly in West Africa except Burkina Faso, Mali and Guinea. The specific objective of the network is to extend the investigations carried out in these three countries to all the member countries of CIRDES and later on in all West African countries. The methodology is an active surveillance in the main foci suspected by the different countries of the network to have the problem of chemoresistance.

Justifications

Les activités de recherche sur la détection et le contrôle de la résistance aux trypanocides réalisées par le CIRDES et ses partenaires au Burkina Faso depuis 1984, ont souligné l'importance croissante des résistances aux trypanocides dans la sous région d'Afrique de l'Ouest. Cependant, les données épidémiologiques récoltées jusqu'à présent sur cette problématique sont partielles comparativement aux autres sous régions d'Afrique. La situation épidémiologique sur la résistance aux acaricides est encore moins connue par rapport à celle relative aux trypanocides. Pour palier cette situation et dans le souci d'un contrôle efficient des résistances aux trypanocides et aux acaricides, le CIRDES en partenariat avec l'Institut de Médecine Tropicale, IMT d'Anvers en Belgique, ont organisé un atelier de réflexion tenu au CIRDES les 28 et 29 Avril 2009. Les participants à cet atelier ont fait l'état des lieux des résistances aux trypanocides et aux acaricides; la problématique du contrôle de la qualité des médicaments vétérinaires en Afrique en général et en Afrique de

l'Ouest en particulier, a été abordée. Ils ont recommandé, en particulier, la mise en place d'un réseau d'épidémio-surveillance de ces deux types de résistance en Afrique de l'ouest. Ce réseau est en cours de mise en place et finance en ce moment, grâce à l'aide de la coopération belge au développement, les actions de recherche prioritaires qui ont été définies et planifiées dans chaque pays en attendant de trouver d'autres sources de financements pérennes pour prendre en compte toutes les préoccupations liées à cette problématique dans les pays d'Afrique de l'Ouest.

Methodology

Pour atteindre les objectifs visés, la démarche adoptée se compose de deux étapes successives: l'élaboration d'une charte du réseau et la lettre d'engagement à celui-ci.

La charte du réseau

Cette charte comporte 9 articles dont les plus importants sont détaillés ci-après,

Article 1 - Objet

La présente charte a pour objet de définir le principe de fonctionnement du réseau et formalise les relations entre ses différents partenaires. Elle est proposée par la cellule de coordination du réseau du RESCAO basée au CIRDES.

Article 2 – Membres

L'adhésion au RESCAO est institutionnelle, volontaire et s'effectue au moyen d'une lettre d'engagement signée par la plus haute autorité de l'institution. Celle-ci désigne officiellement en son sein un point focal dont les activités doivent être en rapport avec les préoccupations du réseau. Il est en contact permanent avec la coordination du réseau, transmet un rapport trimestriel détaillé des activités du réseau réalisées par son institution.

Les membres du réseau peuvent être,

- les institutions de recherches en santé et productions animales ;

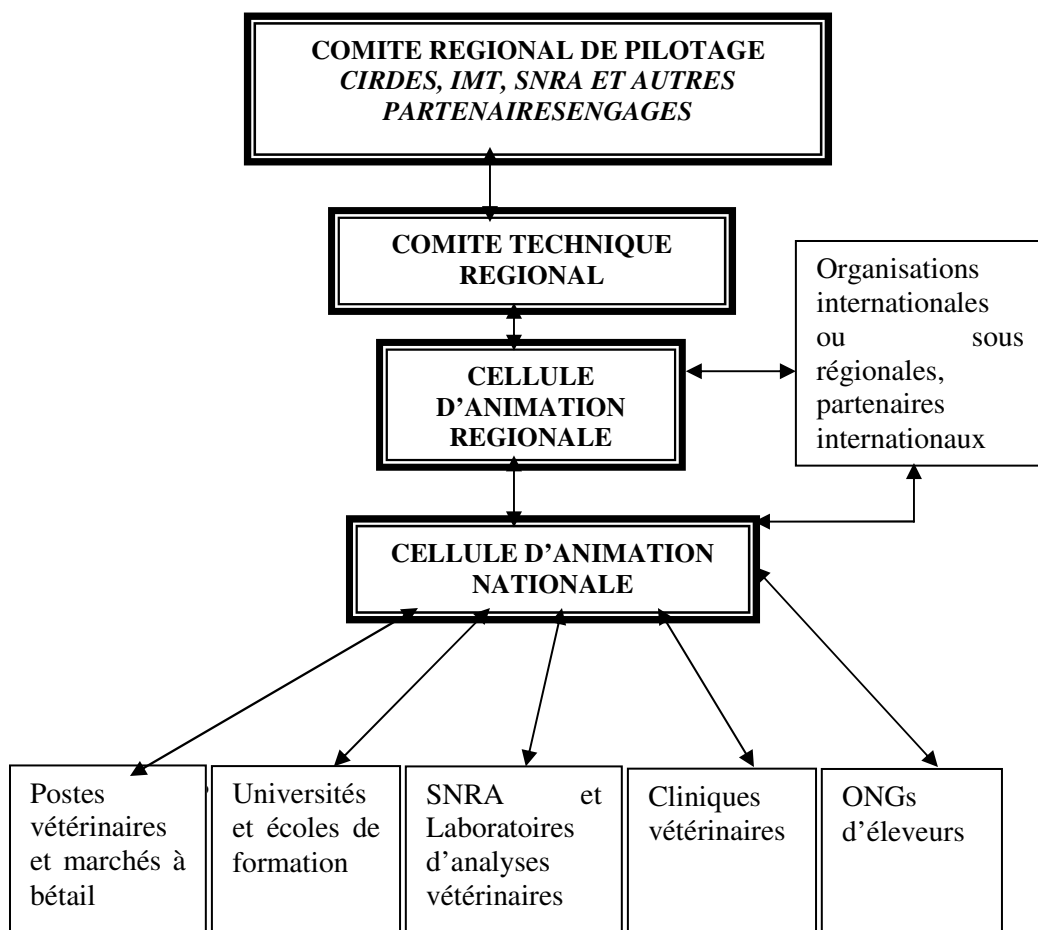
- les projets nationaux ou sous régionaux travaillant dans le domaine des recherches et de la lutte contre les résistances ciblées dans le réseau ;
- les institutions sous régionales ou internationales travaillant dans le domaine ciblé ;
- les universités ou écoles de formation sous régionales ou internationales en santé et productions animales ;
- les associations de producteurs;
- les ONG et OIG intéressées par la problématique;
- les sociétés savantes

Article 4- Organisation et fonctionnement du réseau

L'organigramme du réseau est présenté dans la figure 1 ci après. Il repose sur un modèle pyramidal (Figure 1) composé comme suit,

- **le comité régional de pilotage**, qui est constitué du responsable ou son délégué de chacun des organismes impliqués dans le fonctionnement du réseau. Ce comité décide des grandes orientations et fixe les objectifs généraux et spécifiques, valide les résultats obtenus, évalue l'état d'avancement et décide des mesures correctives si nécessaires. En outre, il s'assure que les objectifs sont atteints et arbitre les relations conflictuelles éventuelles entre les acteurs impliqués ;
- **le comité technique régional** composé d'un épidémiologiste animateur du réseau et de scientifiques connus pour leurs travaux dans le domaine ciblé. Ces personnes ressources sont identifiées et proposées par l'animateur du réseau et validées par le comité de pilotage. Il participe à la conception, l'élaboration technique et la critique des protocoles, et l'analyse des données récoltées et suivi des indicateurs de performance ;
- **la cellule d'animation régionale** regroupe un animateur et des assistants techniques spécialisés dans le traitement des données ou la diffusion des résultats obtenus. Celle-ci est en charge de coordonner l'ensemble des activités, d'animer la conception des différentes procédures, les réunions du comité technique et de suivre les indicateurs de performance. Elle est hébergée au CIRDES qui met les moyens à sa disposition pour assurer son fonctionnement harmonieux;

- **La cellule d'animation nationale** est constituée du point focal assisté de personnes ressources appartenant à la même institution partenaire du réseau et engagée dans ce sens. Elle est le relais national de la cellule d'animation régionale. Elle met en œuvre les procédures et les actions définies par le comité régional de pilotage.
- **les unités de terrain,** Ce sont les structures décentralisées du réseau à l'échelle nationale, les postes vétérinaires, les stations de recherches, les écoles de santé et de productions animales, les cliniques vétérinaires, les associations d'éleveurs, les ONG et OIG. Elles doivent être engagées comme acteurs dans les procédures et actions mises en œuvre par la cellule d'animation centrale.



- **Figure 1, Organigramme du RESCAO**

Article 6 – Moyens mis en œuvre

Ces moyens proviennent actuellement du projet cité ci-dessus.
Il est également prévu de rechercher des mécanismes de financement des activités du réseau pour assurer sa pérennité.

Article 7- Retour d'informations

Il se fera sous trois formes,

- Un bulletin d'informations courantes du réseau,

- Les publications scientifiques ;
- Le site web en cours d'élaboration.

Article 8- Suivi-Evaluation du réseau

L'évaluation du réseau portera sur,

- le niveau de réalisation des actions de collecte de données épidémiologiques ;
- le nombre de rapports techniques et publications spécifiques ;
- le respect des règles du réseau et des protocoles standardisés ;
- la satisfaction du comité des partenaires nationaux et sous régionaux.

Lettre d'engagement

La lettre d'engagement est formellement signée actuellement par le LNADA de Côte d'Ivoire, l'ITRA du Togo, l'INRAB du Bénin, le PATTEC du Mali, le TTCU du Ghana et l'ILRI. Les procédures sont en cours au Burkina Faso, en Guinée Bissau, en Guinée, au Niger et au Sénégal.

Actions en cours

La mise en place du réseau se poursuit pour couvrir tous les pays d'Afrique de l'Ouest. Parallèlement, une surveillance de la chimiorésistance aux trypanocides est mise en œuvre dans les pays membres du CIRDES et au Ghana. En outre, des projets de recherches sont en cours d'élaboration dans certains pays pour prendre en compte les préoccupations liées aux tiques et la qualité des médicaments vétérinaires.

Conclusion et perspectives

Le RESCAO est déjà opérationnel à travers une surveillance active en cours sur la chimiorésistance aux trypanocides. Il vise à étoffer les données épidémiologiques sur la chimiorésistance aux trypanocides et aux acaricides à l'échelle de toute l'Afrique de l'Ouest. Actuellement il s'étend progressivement sur tous les pays d'Afrique de l'Ouest et prendra en compte les actions prioritaires de recherches à impact ou envergures régionaux définies dans les pays membres. Il accueillera toutes les

bonnes volontés pour leur appui technique ou financier. Tous les bailleurs et organisations internationales intéressés sont les bienvenus. Les mécanismes de financements seront définis lors de la prochaine réunion du comité de pilotage prévu pour Janvier 2010.

**GLOSSINA BIOLOGY AND CONTROL/
BIOLOGIE DE LA GLOSSINE ET LUTTE CONTRE**

DOES THE SYMBIONT *SODALIS GLOSSINIDIUS* PLAY A ROLE IN THE VECTOR COMPETENCE OF TSETSE FLIES IN HUMAN AFRICAN TRYPANOSOMIASIS FOCI?

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LE SYMBIONTE *SODALIS GLOSSINIDIUS* A-T-IL UN EFFET SUR LA COMPETENCE VECTORIELLE DES GLOSSINES PRESENTES DANS DES FOYERS DE LA TRYPANOSOMIASE HUMAINE AFRICAINE?

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Résumé

Au cours d'études antérieures nous avons montré que la présence du symbionte, *S. glossinidius*, avait un effet favorable sur l'infection, par le trypanosome, de glossines élevées en insectarium, et donc sur leur compétence vectorielle. Le symbionte constituait donc potentiellement un facteur majeur dans la transmission de la maladie du sommeil. Cependant, il n'était pas certain que des résultats acquis sur des mouches d'insectarium soient directement transposables à des mouches présentes dans des foyers à THA, vu les considérables différences environnementales et nutritionnelles régnant entre un insectarium et un milieu naturel. Des prospections entomologiques ont donc été réalisées, à Bipindi et à Campo, deux foyers historiques, toujours actifs, de THA au Cameroun. La détection du symbionte et du parasite chez les mouches piégées dans ces foyers a été effectuée par PCR spécifiques.

Des différences statistiques hautement significatives ont été observées entre la prévalence des symbiontes et celle des trypanosomes, ceci à la fois au sein d'un même foyer et entre les deux foyers. Cependant le taux de mouches infectées possédant le symbionte - donc de mouches à la fois « symbionte positive » et « trypanosome positive » - était comparable dans les deux foyers. Ceci suggérait l'existence d'une relation directe entre présence du symbionte et présence du parasite, relation que l'analyse statistique a confirmée. D'après les données enregistrées, une

mouche possédant le symbionte aurait environ 3 fois plus de « chance » d'être infectée qu'une mouche dépourvue de symbionte. Cette étude montre donc que, chez la glossine présente en milieu naturel comme chez celle élevée en insectarium, la présence du symbionte *S. glossinidius* favorise l'infection par le trypanosome. Elle confirme surtout le rôle important que le symbionte joue dans l'acquisition du trypanosome par la glossine et donc la compétence vectorielle de la mouche tsé-tsé. Le symbionte pourrait donc constituer une cible majeure dans le cadre de recherches sur la lutte contre la maladie du sommeil.

Summary

In previous investigations we have demonstrated that the symbiont *Sodalis glossinidius*, could favour trypanosome infection of reared tsetse flies, and thus promote their vector competence. So the presence of *S. glossinidius* in the fly's midgut could possibly be a major factor in the transmission process of the disease. However, in Human African Trypanosomiasis (HAT) foci, environmental and nutritional conditions differ highly as compared with those occurring in an insectary. Thus, the possibility that *S. glossinidius* may not induce similar effects on field flies could not be excluded. Therefore, epidemiological surveys have been conducted in two historical and still active HAT foci, Bipindi and Campo, in Cameroon. Flies were trapped, dissected and the presence of both the symbiont and the trypanosome tested by specific PCR methods. Furthermore, the species of trypanosomes found in the fly were identified. Significant statistical differences were recorded between the prevalence of *Sodalis glossinidius* in the fly population and that of trypanosomes alone. The prevalence of, *S. glossinidius* and fly infection with trypanosomes, also differed significantly between the two foci. Of interest was that, despite significant difference in prevalence between the two foci, the rate of infected flies harbouring the symbiont was similar in both. This result suggests that trypanosome infection in the fly could be favoured by the presence of the symbiont. The data showed that the probability of tsetse harbouring the symbiont being infected by trypanosome was about 3 fold higher than that of those devoid of the symbiont. Conversely, all infected tsetse by *T. brucei gambiense* were symbiont -positive. This finding suggests that the symbiont could constitute an interesting target for controlling parasite transmission and thus the sleeping sickness disease.

Introduction

Tsetse flies are medically and agriculturally important vectors that transmit African trypanosomes, the causative agents of sleeping sickness in humans, and nagana in animals. Sleeping sickness, fatal if untreated, still affects a wide range of people in sub-Saharan Africa (World Health Organization, 2006), where more than 60 million people are at risk. Nagana is estimated to cost African agriculture US\$ 4.5 billion per year (Reinhardt, 2002).

The biological process leading to transmission of the trypanosomes from one mammalian host to another is complex. Prior to being transmitted, the parasite must first establish in the tsetse fly midgut following an infective blood meal and then mature either in the salivary glands or in the mouthparts, depending on the trypanosome species (Vickerman et al., 1988; Van Den Abbeele et al., 1999). Besides the parasite, tsetse flies may harbour three different symbiotic micro-organisms (Aksoy, 2000). Among them, *Sodalis glossinidius* (Cheng and Aksoy, 1999; Dale and Maudlin, 1999) is suspected of favouring the establishment of the parasite in the insect midgut through a complex biochemical mechanism (Maudlin and Ellis, 1985; Welburn and Maudlin, 1999; Dale and Welburn, 2001).

In previous studies, no exclusive relationship was found between the presence of *S. glossinidius* and the ability of the fly to acquire *Trypanosoma congolense* (Geiger et al., 2005a). We have also shown that *Glossina palpalis gambiensis* (*palpalis* group) and *Glossina morsitans morsitans* (*morsitans* group) harbour genetically distinct populations of *S. glossinidius* (Geiger et al., 2005b), suggesting that fly vector competence might be linked to given genotypes of *S. glossinidius* rather than to a mere presence/absence of the symbiont. We also demonstrated that the ability of *T. brucei gambiense* and *T. brucei brucei* to establish in the *G. palpalis gambiensis* midgut is statistically linked to the presence of *S. glossinidius*-specific genotypes (Geiger et al., 2007).

To gain clearer insight into the mechanisms involved, including the possibility that *S. glossinidius* controls the transmission of the parasites under natural field conditions, a large tsetse fly sampling campaign was conducted in two historical sleeping sickness foci, Bipindi and Campo in the south of Cameroon. For each fly, we have determined the species, the presence/absence of *S. glossinidius*, and have identified the trypanosome species and subspecies. The prevalence of each of them was calculated for the population of flies. Prevalence was also calculated separately within each sampling area (six villages).

Materials and Methods

Five entomological surveys were conducted in July 2007 and February 2008 in Bipindi (in the Memel, Ebiminbang and Lambi/Bidjouka villages) and in November 2007, March 2008 and October 2008 in Campo (in the Mabiogo, Akak and Campo Beach/Ipono villages).

The different *Glossina* species were first identified and then sorted into teneral (young flies that had never taken a blood meal) and nonteneral flies, according to morphological criteria (Grebaut et al., 2004). The nonteneral flies were dissected and their midgut was examined under the light microscope (magnification $\times 100$) for the presence of trypanosomes. Trypanosome-infected and -noninfected organs were then separately transferred into microfuge tubes containing ethanol (95°) for further symbiont and trypanosome PCR identification.

DNA was extracted from organs using the classical protocol (Navajas et al., 1998). The DNA samples were stored at -20°C until PCR amplification.

Specific trypanosome primers (Moser et al., 1989; Masiga et al., 1992; Majiwa et al., 1994; Herder et al., 2002) were tested on fly organs. To detect possible dissection and/or extraction contamination, controls were done both on noninfected midguts and on the salivary glands of flies showing *T. congolense* midgut infection. *S. glossinidius* primers (Darby et al., 2005) were tested on the midgut. Finally, specific primers amplifying mitochondrial DNA of tsetse flies were used to control the quality of the extracted DNA, as previously described (Cheng and Aksoy, 1999).

PCR amplification of parasites was performed as described by Herder et al. (2002). PCR amplification of tsetse fly mitochondrial DNA was performed as described by Cheng and Aksoy (1999).

Detection and identification of trypanosome species were confirmed using an alternative PCR method based on internal transcribed spacer 1 of rDNA as previously described (Desquesnes et al., 2001). The amplified products were separated on 2% agarose gel containing ethidium bromide and visualised under UV illumination.

Both negative and positive controls were included in each set of extraction and of PCR amplification experiment. PCR amplification giving a positive result was repeated to assess the result; we also verified randomly selected samples when the PCR amplification was negative. Finally, in order to assess whether the PCR product actually

corresponded to *S. glossinidius*, it was cloned into PGEM-T Easy (Promega, Charbonnières, France), as previously described (Geiger et al., 2005b), and several recombinant plasmids were sequenced and compared with the reference sequence of *S. glossinidius* isolated from *G. palpalis palpalis* (Darby et al., 2005) (GenBank accession number [AJ868435](#)).

Results

A total of 4717 flies were caught in the two foci during the five surveys. All the flies collected in Bipindi belonged to the *Glossina palpalis palpalis* subspecies, whereas in Campo, four species were identified: *G. p. palpalis* (93.6%), *G. pallicera* (4.3%), *G. caliginea* (1.6%) and *G. nigrofusca* (0.4%). The rate of teneral flies was 7.3% in Bipindi, 13% in Campo and 11.6% when the two foci were combined. The mean apparent fly density per trap per day (ADT) was 2.23 and varied from 0.63 to 4.64; the highest ADT was recorded in Akak belonging to the Campo focus that showed a mean ADT more than 2.4-fold higher than in Bipindi.

Further investigations (trypanosome and *S. glossinidius* prevalence) were conducted on the *G. p. palpalis* flies only.

Trypanosomes (and the symbiont, *S. glossinidius*) were detected on 450 randomly selected flies (75 per village) using specific molecular approaches that distinguished the different *Trypanosoma* species [*T. brucei* s.l. (Tbsl), *T. brucei gambiense* (Tbg), *T. congolense* forest (TcF) and savannah (TcS) types and *T. simiae* (Ts)].

Out of the 450 flies analysed, 247 (54.9%) harboured the symbiont, whereas 193 (42.9%) harboured trypanosome species. Out of the 193 infected flies, 145 (a total of 75.1%, with 76.7% and 72.6% for Bipindi and Campo, respectively) also harboured the symbiont (S+I+), while 48 (24.9%) were devoid of symbiont (S-I+). Interestingly, none of those flies (devoid of symbiont), was infected by *T. brucei gambiense* (responsible of HAT).

Both simple and mixed (two or three different *Trypanosoma* species) infections were recorded. Simple infections were predominant: 158 (81.9%) out of the 193 infected flies, most of them being due to *Nannomonas* (116/158 infections), with a majority (70/116) of *T. congolense* forest type. *T. brucei* s.l. simple infection prevalence was 9.3% (42/450). Twenty-nine flies were mixed-infected with two *Trypanosoma* species (including 18 *T. brucei* s.l. infections), and six with three species (including five *T. brucei* s.l. infections). Among *T.*

brucei s.l. simple or mixed infections, 15 were due to *T. brucei gambiense*.

In Bipindi, 53.3% of the flies were infected with at least one trypanosome specie, compared to only 32.4% in Campo; this difference was statistically significant ($\chi^2 = 20.04$, $p < 0.0001$). The rate of single infections in Bipindi (42.7%) and Campo (27.6%) were also statistically significantly different ($\chi^2 = 11.28$, $p < 0.001$). Finally, there were twice as many *T. brucei* s.l. (Tbsl) infections in Bipindi (28) as in Campo (14), and 1.8-fold more *T. congolense* forest type (TcF) infections in Bipindi (45) than in Campo (25). The mixed infection rates were relatively low (8% in Bipindi and 4.9% in Campo). In Campo, no mixed infections including *T. simiae* or three trypanosome species were detected and in Bipindi most of the mixed infections occurred in only one of the villages (Memel).

As for the trypanosomes, the symbiont prevalence differed significantly ($\chi^2 = 16.59$, $p < 0.0001$) between flies harvested in Bipindi (64.4%) and those harvested in Campo (45.3%). Prevalence in the villages of Bipindi and Campo foci were highly similar.

The statistical analysis carried out on the overall data shows a significant association between the presence of the symbiont and the tsetse fly infection whatever the trypanosome specie, except *T. simiae* (that caused a very low number of infections), and whatever the nature of infections, single or mixed. In particular, TcF, Tbsl and Tbg (the causative agent of sleeping sickness) infections were significantly related to the presence of the symbiont.

Discussion

Tsetse flies reproduce by adenotropic viviparity and *S. glossinidius* is vertically transmitted to the intra-uterine developing larva (Cheng and Aksoy, 1999). *S. glossinidius* is suspected to be involved in the vector competence of *Glossina* by favoring parasite installation in the insect midgut (Maudlin et al., 1985; Welburn et al., 1999). The results of experiments on insectary flies supported the idea that its presence favoured fly infection, but was not an absolute condition (Geiger et al., 2005a).

The present epidemiological investigation was conducted in two HAT foci in the south of Cameroon, in order to test, on field populations of

flies, the existence of interactions between the three “partners” (*Glossina*-trypanosome-symbiont) in disease transmission.

The results of fly trapping, the prevalence of *S. glossinidius* and trypanosomes, and the different symbiont / parasite associations (whether or not the infected flies harboured the symbiont) showed large differences between the fly populations in the two foci: (i) a much higher density of flies in Campo than in Bipindi (ADT = 3.01 versus 1.26) and a higher diversity of fly species, four in Campo and only one in Bipindi; (ii) conversely, a significantly ($p < 0.0001$) higher prevalence of *S. glossinidius* in Bipindi (64.4%) than in Campo (45.3%); and (iii) a significantly ($p < 0.0001$) higher prevalence of trypanosome infection in Bipindi (53.3%) than in Campo (32.4%). This means that populations of flies could show specific characteristics in each focus.

Despite these differences in symbiont and trypanosome infection prevalence between the two foci, the rates of infected flies carrying the symbiont were very similar: in Bipindi, 76.7% trypanosome-infected (I+) flies harboured the symbiont versus 72.6% in Campo. Finally, among the overall trypanosome-infected flies from Bipindi and Campo, 75.1% harboured the symbiont (S+I+), while 24.9% (48 flies) were devoid of symbiont (S-I+). This means that the presence of the symbiont should not be absolutely necessary for fly infection but would greatly favour it; this hypothesis fits with our previous results (Geiger et al., 2005a). In this respect, both in Bipindi and Campo, flies harbouring symbionts (S+) may have a threefold higher probability of being infected by trypanosomes than flies devoid of symbionts (S-).

Because the purpose of our work was investigating the possible relationship between the presence of *S. glossinidius* and fly infection by trypanosomes, the trypanosome species were identified in all infected tsetse flies. Four species of trypanosome, *T. brucei* s.l. [including both *T. b. brucei* (Tbb) and *T. b. gambiense* (Tbg)], *T. congolense* forest type (TcF), *T. congolense* savannah type (TcS) and *T. simiae* (Ts), were detected. Among the 48 (S-I+) flies, only seven (14.6%) were infected by *T. brucei* s.l., and interestingly, only by *T. b. brucei*. Among the 247 flies harbouring symbiont, 58 (23.5%) were infected by *T. brucei* s.l., among which 43 (74.1%) by *T. b. brucei*.

These results together provide findings of major importance: first, all flies (15) infected by *T. b. gambiense* were symbiont positive (S+); secondly, 89.2% of tsetse flies infected by *T. brucei* s.l. were symbiont-positive. This suggests that *S. glossinidius* strongly favours the establishment of *T. brucei* infections in *G. palpalis*.

Owing the apparent non-random distribution of the trypanosome infection between, respectively, the *S. glossinidius* positive and negative sub-populations of flies, the existence of an association between the presence of the symbiont and trypanosome infection was hypothesized. The hypothesis was statistically tested on different couples including, each, the symbiont and a given trypanosome species (in order to detect possible differences).

The results of the statistical analysis of the data from the overall 450 flies (Bipindi + Campo) showed a clear association between the presence of *S. glossinidius* and the infection by the different trypanosome species, except for *T. simiae* (probably due to the low number *T. simiae* infections – 9 out of 450 flies). Finally, owing: i) that there exist a statistical highly significant association between the presence of *S. glossinidius* and trypanosome infection, ii) that *S. glossinidius* establishes first in the fly's gut (it is vertically transmitted by the female fly to the intra-uterine developing larva), and thus that the symbiont is the only one, among the two organisms, that has the possibility to “influence” the subsequent installation of its statistically associated partner; iii) that, furthermore, the symbiont, by means of a complex biochemical mechanism, is able to suppress or at least to reduce the natural tsetse refractoriness towards trypanosome infections, all these results indicate that, although it is not an obligatory condition for the fly to get infected, the presence of *S. glossinidius* in the fly's gut actually favours the infection by diverse trypanosome species, especially *T. brucei* s.l. in field populations of tsetse flies.

So, such studies on tripartite interactions should be pursued and developed with the objective of controlling fly vectorial competence and possibly sleeping sickness.

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EFFECT OF STARVATION ON TSETSE FLIES' IMMUNE PEPTIDES EXPRESSION LEVELS/

EFFET DU STRESS NUTRITIONNEL SUR L'EXPRESSION DES PEPTIDES IMMUNITAIRES DE LA MOUCHE TSE-TSE

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Summary

Tsetse-transmitted trypanosomiasis is a vector-borne disease that poses a serious threat to human and animal health in sub-Saharan Africa. The majority of tsetse flies (*Glossina* spp.) in a natural population are refractory to mature *Trypanosoma congolense* and *T. brucei* spp. infections. This phenomenon is affected by different factors including the tsetse fly's immune defence. Starvation of the tsetse flies significantly increases the susceptibility of the fly to establish a trypanosome infection.

Studies were designed to determine whether starvation affects the tsetse's immune system, which has been shown to play a key role in determining the outcome of infection. We investigated the effect of nutritional stress (starvation) on i) the baseline gene expression level of the antimicrobial peptides attacin, defensin and cecropin in uninfected tsetse flies and ii) their induced expression level in response to bacterial (*E. coli*) or trypanosomal challenge using a quantitative Real Time PCR method.

Results showed that starvation of newly hatched, unfed tsetse flies significantly lowers their baseline antimicrobial peptide expression levels especially for attacin and cecropin. In response to trypanosome challenge, only non-starved older flies showed a significant increase of the antimicrobial peptide expression within 5 days after ingestion of a blood meal containing trypanosomes, particularly *T. brucei* bloodstream forms.

These results suggest that a decreased immune gene expression in newly hatched flies or a lack of immune responsiveness of older flies to trypanosomes, both as a result of fly starvation, could be one of the contributing factors to the increased susceptibility of nutritionally-stressed tsetse flies to trypanosome infections.

Résumé

La trypanosomiase transmise par la mouche tsé-tsé pose un sérieux problème de santé animale et humaine en Afrique sub-saharienne. Dans la nature, la majorité des glossines (*Glossina* spp.) ne développent pas d'infection mature à *Trypanosoma congolense* ou *T. brucei* spp à la suite d'un repas infectieux. Ce phénomène est probablement dû, entre autres, à la réponse immunitaire des mouches. Cependant, le jeûne augmente de façon significative la susceptibilité des mouches aux infections par les trypanosomes.

Des études ont donc été réalisées afin de déterminer l'impact du jeûne sur le système immunitaire de la mouche. La méthode de PCR quantitative en Temps Réel nous a permis d'étudier l'effet du stress nutritionnel i) sur l'expression de base des peptides antimicrobiens attacin, defensin et cecropin chez la mouche tsé-tsé et ii) sur la réponse immunitaire lors d'une infection par une bactérie (*E. coli*) ou un trypanosome.

Les résultats ont montré que le jeûne réduit significativement le niveau d'expression de peptides antimicrobiens, en particulier de l'attacin et de la cecropin, chez les très jeunes mouches. Seules les mouches âgées n'ayant pas subi de jeûne ont manifesté une augmentation significative du niveau d'expression de ces peptides antimicrobiens dans un intervalle de cinq jours suivant l'ingestion du repas sanguin contenant des trypanosomes, en particulier lorsqu'il s'agissait de formes sanguines de *T. b. brucei*.

Ces résultats indiquent donc qu'une diminution de l'expression des gènes immunitaires par les jeunes mouches ou l'absence de réponse immunitaire chez les mouches plus âgées seraient responsables de

l'augmentation de la réceptivité des mouches nutritionnellement stressées aux infections par les trypanosomes.

Introduction

The proportions of infected flies in a tsetse population as well as the age-specific susceptibility to a trypanosomal infection are important factors that affect the epidemiology of tsetse-transmitted trypanosomiasis. In principle, the large majority of infected tsetse flies are considered to have acquired their infection at their first blood meal as a teneral fly (newly hatched unfed fly). Older flies are reported to be refractory to trypanosome infection and to contribute little to the overall infection rate of a tsetse population. However, under specific physiological conditions, both teneral and older flies can become more susceptible to trypanosomal infections. Indeed, starving teneral and 20-day-old tsetse flies for several days resulted in significant increases in *T. congolense* as well as in *T. brucei* spp infections (Gingrich *et al.*, 1982; Kubi *et al.*, 2006). The underlying mechanism that causes this important change in susceptibility has not been clarified yet. There are indications in literature that the tsetse immune system plays an important role in determining the fate of a trypanosome infection (Hao *et al.*, 2001). Indeed, tsetse flies are able to synthesize a range of antimicrobial peptides (AMPs) such as attacin, defensin, cecropin and dipteracin, in response to a 'foreign' micro-organism. This tsetse innate immune response is suggested to affect the establishment and maturation of trypanosomes in the vector (Hao *et al.*, 2001; Boulanger *et al.*, 2002; Lehane *et al.*, 2004; Hu & Aksoy, 2006; Attardo *et al.*, 2006). Therefore, in this study we investigated whether starvation of the tsetse fly affects the uninduced and the pathogen-induced innate immune response in a way that it could contribute to the increased susceptibility to trypanosome infection in starved tsetse flies.

Materials and methods

Tsetse flies

Male *Glossina morsitans morsitans* tsetse flies from the colony maintained at the Institute of Tropical Medicine (Antwerp, Belgium) were used throughout the experiments. The origin of this tsetse fly colony and the rearing conditions were described by Elsen *et al.* (1993).

Nutritional stress by starvation

In all experiments, four groups of flies were compared. They differed in age and in their nutritional status: i) non- starved teneral flies (= newly emerged unfed flies) less than 32 hours old (TF0), ii) teneral flies that were starved for 4 days (TF4), iii) 20-days-old flies starved for 2 days after the last blood meal (AD2) and iv) 20- days-old flies starved for 7 days after the last blood meal (AD7).

Fat content determination

After removal of legs and wings, carcasses of 30 flies per group were dried to constant weight. Lipids were extracted for three days using chloroform (Langley *et al.*, 1990). After fat extraction and drying to constant weight, the residual dry weight of the flies was determined. The fat content of the flies was calculated as the difference between the dry weight and the residual weight.

Bacterial and trypanosomal challenge of tsetse flies

To evaluate the bacteria-induced immune response in each of the experimental groups, batches of 40 flies for each group were micro-injected either with 2µl PBS (control) or with 2µl of a suspension of live *Escherichia coli*. Four days after the bacterial injection, whole abdomens were removed and pooled by two for total RNA extraction.

For the trypanosomal challenge, batches of 40 flies for each group were given a blood meal on anesthetized mice (strain NMRI) infected with *Trypanosoma congolense* IL1180 or *T. b. brucei* ANTAR 1. Control flies were given a blood meal on uninfected mice. From the batches of infected flies, whole abdomens were removed on day 1 or day 5 following the initial blood meal and pooled by two for total RNA extraction.

Immune peptide genes expression analysis

To compare the baseline expression level of immune peptide gene in teneral and non-teneral unchallenged flies as well as the pathogen-induced immune response, total RNA was extracted from samples. RNA extraction was conducted in 1 ml Tripure[®] reagent (Roche) per sample

following the manufacturer's instructions. Afterwards, the mRNA in 400 ng of a total RNA extract was reverse transcribed using Oligo(dT)₁₅ primer (100 pmol) (Promega), dNTP-mix (10 mM each) and Transcriptor Reverse Transcriptase (10 units) (Roche). Quantitative real-time PCR was performed in a Bio-Rad iCycler iQ with Bio-Rad iQ SYBR green Supermix. PCR conditions comprised an initial 10 min polymerase activation at 95°C followed by 35 cycles each consisting of a denaturation step at 95°C /15 sec and an annealing/elongation step at 60°C. Used primers were: attacinFW: 5'-TTTTCACAGTCGCACCCATT-3' and attacinREV: 5'-AAACGCCTCCTGTCAAATCC-3', defensinFW: 5'-TAGTTTTGGCTTTTCTTACAC-3' and defensinREV: 5'-CGACTACAGTATCCGCTCTTT-3', and cecropinFW: 5'-ATACTC-GCTCTTTCAGTCAG-3' and cecropinREV: 5'-CTCTAACAGTAGCGGCAACA 3'; actinFW: 5'-CGCTTCTGGTCGTACTACT-3' and actinREV: 5'-CCGGACATC-ACAATGTTGG-3', tubulinFW: 5'-GATGGTCAAGTGCATCCT-3' and tubulinREV: 5'-TGAGAACTCGCCTTCTTCC-3'. Three replicates were performed for each sample and threshold cycles (Ct) were recorded and used to calculate gene expression levels. Target genes expression levels were normalized with housekeeping genes actin and tubulin. The normalization used was a modification of that proposed by Vandesompele *et al.* (2002):

$$response = \ln \left[(PCR_{pep})^{Ct_{pep}} \right] - \frac{\ln \left[(PCR_{act})^{Ct_{act}} \right] + \ln \left[(PCR_{tub})^{Ct_{tub}} \right]}{2}$$

with:

- $PCR_{pep/act/tub}$ = PCR efficiency of immune peptides, actin and tubulin respectively
- Ct_{pep} = number of cycles required for immune peptides genes to reach the threshold in each sample repetition

$Ct_{act/tub}$ = three repetition average number of cycles required for actin and tubulin genes respectively to reach the threshold in each sample.

Statistical data analysis

The loss of weight in individual flies was analysed using a linear regression in Stata software version 9.2 (Stata Corp, 2006). The weight difference was used as the response and fly groups (starved and non-

starved teneral and adult flies) as discrete explanatory variable. The effect of starvation on the expression levels of attacin, defensin and cecropin in teneral and adult flies was analysed separately using a robust linear model in Stata.

Results

Effect of starvation on fat body content and baseline expression levels of immune peptide in starved and non-starved flies

Starving teneral flies for four days resulted in a significant decrease in the fat body content compared to non-starved tenerals. Interestingly, starved teneral flies showed significant lower immune genes expression levels, especially for attacin and cecropin. In contrast, starvation of 20-day-old flies for seven consecutive days significantly reduced the fat body content but did not significantly compromise the baseline immune peptide expression levels (Table 1).

Immune gene response to bacterial/trypanosomal challenge and nutritional stress

Injection of bacteria (*E. coli*) resulted in a high induction of the expression of the genes in starved and non-starved teneral or adult flies (Table 2). The induced expression levels of attacin, defensin and cecropin were significantly increased in all experimental groups compared to control flies. No significant differences were observed in the non-starved versus starved flies challenged with the bacteria ($p > 0.5$).

For the trypanosomal challenge, immune gene expression level was monitored for the first 5 days following the bloodstream parasite uptake. Five days following the ingestion of the blood meal containing *T. congolense* or *T. b. brucei*, no increased expression levels of attacin, defensin and cecropin genes were observed in teneral tsetse flies compared to day 1 (Tables 3 & 4). On the contrary, in both non-starved and starved teneral flies challenged with *T. congolense* (Table 3), the gene expression levels of these immune peptides (except for defensin) at day 5 were significantly lower compared to day 1 ($p < 0.0001$). Non-starved 20-day-old tsetse challenged with *T. congolense* or *T. b. brucei* showed increased expression levels of attacin, defensin and cecropin 5 days after the infective blood meal compared to day 1 although this increase was demonstrated statistically significant only for the *T. brucei*

fly group. However, no significant changes in immune gene expression levels were observed in the starved 20-day-old tsetse fly group after a blood meal containing *T. congolense* or *T. b. brucei* (Tables 3 & 4).

Table 1: Fat content and normalized baseline expression levels of male teneral and 20-day-old *G. m. morsitans* after a period of starvation. The fat content was significantly reduced in four-day-starved teneral (TF4) and seven-day-starved 20-day-old flies (AD7) ($p < 0.05$) compared to respectively non-starved teneral (TF0) and 20-day-old flies (AD2). The immunepeptide gene expression values were normalized against the expression levels of the housekeeping genes actin and tubulin. The expression levels (mRNA) of attacin and cecropin were significantly reduced in four-day-starved teneral (TF4) compared to non-starved teneral flies (TF0) (* $p < 0.05$).

Fly groups	Attacin	Defensin	Cecropin	Fat body content (mg/fly)
TF0	1,11	0,22	0,31	2,48
TF4	0,25*	0,12	0,14*	0,76
AD2	0,48	2,46	0,36	6,87
AD7	0,57	1,94	0,16	2,02

Table 2: Normalized expression levels of attacin, defensin and cecropin in non- starved (TF0) and starved (TF4) teneral and in non-starved (AD2) and starved (AD7) 20-day-old male *G.m. morsitans* after bacterial (*E. coli*) challenge. The immunepeptide gene expression values were normalized against the expression levels of the housekeeping genes actin and tubulin. The expression levels of the three antimicrobial peptides were upregulated in the *E. coli* stimulated flies comparing to control flies (* $p < 0.0001$). The observed difference between starved and non-starved flies challenged with the bacteria was not statistical significant

Fly groups	Attacin	Defensin	Cecropin
TF0+PBS			
(Control)	1,157	2,643	2,393
TF0+ <i>E.coli</i>	124,138*	97,311*	346,638*
TF4+PBS			
(Control)	0,429	0,251	1,003
TF4+ <i>E.coli</i>	150,717*	134,813*	308,656*
AD2+PBS	1,324	2,697	2,397

(Control)			
AD2+E.coli	820,905*	986,808*	2410,927*
AD7+PBS			
(Control)	1,127	2,085	3,3028
AD7+E.coli	420,114*	878,262*	1891,149*

Table 3: Normalized gene expression levels of attacin, defensin and cecropin in non-starved (TF0) and starved (TF4) teneral or in non-starved (AD2) and starved (AD7) 20-day-old flies *G.m. morsitans* on day 1 (d1) or day 5 (d5) after the uptake of a blood meal containing *T. congolense* (Tc) bloodstream forms. The immunepeptide gene expression values were normalized against the expression levels of the housekeeping genes actin and tubulin. The expression levels of attacin and cecropin were significantly reduced in four-day-starved teneral (TF4) compared to non-starved teneral flies (TF0) ($p < 0.05$).

Fly groups	Attacin	Defensin	Cecropin
TF0Tcd1	0,129	0,087	0,132
TF0Tcd5	0,037	0,096	0,019
TF4Tcd1	0,083	0,075	0,082
TF4Tcd5	0,027	0,075	0,018
AD2Tcd1	0,245	1,556	0,127
AD2Tcd5	0,985	6,761	0,275
AD7Tcd1	1,893	5,649	0,520
AD7Tcd5	0,314	1,190	0,118

Table 4: Normalized gene expression levels of attacin, defensin and cecropin in non-starved (TF0) and starved (TF4) teneral or in non-starved (AD2) and starved (AD7) 20-day-old flies *G.m. morsitans* on day 1 (d1) or day 5 (d5) after the uptake of a blood meal containing *T. brucei* *brucei* (Tb) bloodstream forms. The immune peptide gene expression values were normalized against the expression levels of the housekeeping genes actin and tubulin. The expression levels of the immune peptide genes were significantly upregulated in non-starved adult flies after a blood meal with *T. brucei* bloodstream forms (*p < 0.05; **p = 0.06).

Fly groups	Attacin	Defensin	Cecropin
TF0Tbd1	0,164	0,131	0,209
TF0Tbd5	0,083	0,147	0,065
TF4Tbd1	0,037	0,042	0,034
TF4Tbd5	0,066	0,105	0,072
AD2Tbd1	0,117	0,553	0,251
AD2Tbd5	0,656**	3,444*	2,947*
AD7Tbd1	0,199	0,586	0,683
AD7Tbd5	0,230	1,255	0,249

Discussion

The result of this study demonstrated that both freshly-emerged flies as well as 20-day-old *G. morsitans morsitans* flies showed a significant baseline gene expression of all three antimicrobial peptides with a 10-fold increase in the defensin expression level observed in the 20-day-old flies. However, starving freshly-emerged flies for four days to a fat reserve level of less than 1 mg/fly significantly reduced this immune gene expression level. This suggests that the substantial nutritional stress in these young flies resulted in a significant reduction of the uninduced baseline immune gene expression (i.e. the innate expression without a pathogen challenge) which in turn could contribute to the increased susceptibility of these flies to trypanosomal infections. Indeed, the correlation between this baseline immune gene level and the fly's susceptibility to trypanosomal infections was recently suggested by Nayduch & Aksoy (2007) who showed that the uninduced baseline level of systemic attacin was significant higher in freshly emerged flies of

trypanosome refractory tsetse species than in susceptible species. For the 20-day-old flies, the nutritional stress as a result of starvation was less pronounced allowing the flies to maintain a baseline immune peptide expression level similar to that in the non-starved teneral flies.

Escherichia coli injection resulted in a high increase in expression of attacin, defensin and cecropin in all experimental fly groups confirming the strong immunogenic nature of bacteria in tsetse flies as was observed in previous experiments (Hao *et al.*, 2001; Boulanger *et al.*, 2002; Lehane *et al.*, 2004). Older flies showed a much higher responsiveness to the bacterial challenge than the freshly emerged flies. This clearly shows that the fat body, a major immune response organ in insect (Lehane *et al.* 2004), is operational in freshly emerged unfed flies but is not yet at its full capacity to respond to pathogens. Moreover, since no differences were found between the starved and non-starved groups it is clear that this immune responsiveness to the bacterial challenge is not affected by the nutritional status of the flies. The uptake of bloodstream trypanosomes (*T. congolense* or *T. brucei brucei*) by blood feeding did not affect the expression level of attacin, defensin and cecropin in the teneral fly groups, five days after the infective blood meal which confirms previous observations of Hao *et al.* (2001) that the tsetse immune system response to ingested bloodstream trypanosomes in young tsetse flies is low. For the adult fly group, only the non-starved flies significantly increased the gene expression levels of these immune genes in response to trypanosome infection (especially for *T.b.brucei*). Hence, this could be a contributing factor to the high refractoriness for trypanosome infection that is observed for these flies (Kubi *et al.*, 2006).

In conclusion, this study reports that a high nutritional stress decreases the baseline immune peptide gene expression level in newly hatched unfed tsetse flies and reduces the immune responsiveness of older flies in response to trypanosome challenge. This decreased immune gene expression as a result of starvation could be one of the factors contributing to the increased susceptibility of nutritionally-stressed tsetse flies to trypanosomal infections.

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ENTOMOLOGICAL BASELINE DATA COLLECTION IN PREPARATION FOR A TSETSE ELIMINATION CAMPAIGN IN THE NIAYES OF SENEGAL/

COLLECTE DES DONNÉES DE BASE EN ENTOMOLOGIE POUR UNE CAMPAGNE D'ÉLIMINATION DES TSETSE DANS LES NIAYES AU SÉNÉGAL

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Résumé

Le gouvernement du Sénégal s'est lancé dans un projet d'élimination de *Glossina palpalis gambiensis* Vandedrplank, et l'éradication de la Trypanosomiase Animale Africaine via une approche à grande échelle de gestion intégrée des insectes dans la région de Niayes. Les outils d'analyse spatiale et la modélisation mathématique ont été utilisés pour élaborer un protocole d'échantillonnage entomologique stratifié permettant une réduction de la zone à couvrir à 4% de la superficie totale (7350 km²) avec une meilleure précision. Un habitat adapté pourrait être différencié en utilisant une classification supervisée des images satellites LandSat 7 TM et être appelé « zones humaines ». A la fin du mois de Mars 2009, 683 pièges Vavoua non appâtés ont été posés dans la zone infestée observée de Niayes couvrant une superficie de 525 km². Dans la partie restante, un modèle mathématique a été utilisé pour évaluer le risque que les mouches soient présentes en dépit d'une séquence de

captures zéro. Cette procédure d'échantillonnage entomologique pourrait être utilisée chez d'autres vecteurs ou espèces parasites.

Summary

The Government of Senegal has embarked on a project to eliminate *Glossina palpalis gambiensis* Vanderplank, and the African Animal Trypanosomosis (AAT), from the Niayes area using an area-wide integrated pest management approach. Spatial analytical tools and mathematical modeling were used to develop a stratified entomological sampling protocol allowing a restriction of the area to be surveyed to 4% of the total surface area (7350 km²), with an improved accuracy and robustness. Suitable habitat could be discriminated using a supervised classification of LandSat 7 TM satellite images and called "wet areas". At the end of March 2009, 683 unbaited Vavoua traps had been deployed in the observed infested area in the Niayes which is about 525 km². In the remaining area, a mathematical model was used to assess the risk that flies were present despite a sequence of zero catches. This entomological sampling procedure might be used in other vectors or pest species.

Introduction

In Senegal like in most African countries infested by tsetse, AAT is a major obstacle to the development of more efficient and sustainable livestock production systems (Itard et al. 2003). The most north-western population of *G. p. gambiensis*, its main vector in this country, survives under very dry conditions, where annual precipitation is limited to 400-500 mm. This tsetse population is well adapted to specific habitats called the "Niayes" that owes its name to vestiges of Guinean forest in low-lying inter-dune depressions which are periodically or even permanently flooded (Morel and Touré 1967, Touré 1971, 1974).

The Niayes area harbours particular climatic conditions that allow intensive cropping and cattle breeding, including exotic cattle breeds for the production of milk. But their cost-effectiveness is continuously threatened by the exposure to trypanosomoses. Horses, that have a particular importance in Senegal, are also threatened by tsetse.

In 2005, the DIREL has thus initiated a tsetse control campaign called «Projet de lutte contre les glossines dans les Niayes» which was funded by Senegal and technically and financially supported by the International Atomic Energy Agency (IAEA). The project is being implemented in the context of the Pan African Tsetse and Trypanosomiasis Eradication

Campaign (PATTEC). The tsetse project in Senegal has adopted an area-wide integrated pest management (AW-IPM) approach that aims at integrating various control tactics (e.g. traps, insecticide-impregnated targets, live baits, the sterile insect technique (SIT)) (Dyck et al. 2005) to target an entire tsetse population within a circumscribed area (Klassen 2005). In order to develop an appropriate AW-IPM strategy, detailed and accurate data are required of the target population. The DIREL and ISRA in collaboration with the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), the FAO and the IAEA therefore developed a specific entomological sampling protocol to define as precisely as possible the present distribution of tsetse in the Niayes and La Petite Côte. These entomological surveys are part of a baseline data package (Vreysen et al. 2007) that include disease prevalence, tsetse population genetics, socio-economic and environmental impact data.

This paper presents the development of this entomological sampling process that was designed using modern tools to optimize efficiency and cost-effectiveness in this particular situation. This method might be useful for other tsetse control campaigns presently launched within the PATTEC initiative in West Africa.

Materials and Methods

The Study Area

The study area of 7350 km² is located along the Atlantic coast, at 13.5 - 15.5°N and 16.5°-17.5°W. The area is 180 km long and 30-35 km wide and can be divided into three main sections: the Niayes in the North, the Sine Saloum in the South, and La Petite Côte in between (fig. 1). Mean daily temperatures vary between 25 and 30°C and relative humidity between 60 and 80%. Annual precipitation is between 200 and 500 mm with a rainy season from July to September.

Preliminary Surveys

A 5 by 5 km grid overlaying the entire target area was developed with letters and numbers allocated to rows and columns, respectively, to facilitate the field sampling procedures (Leak et al. 2008). Phytosociological censuses were achieved to class the plant units in three classes of habitat suitability, based on the presence and abundance of

forest plant indicators described before (Bouyer et al. 2005): not suitable (NS), suitable (S) and suitable but degraded (SD).

Remote Sensing Analyses

These habitats could be discriminated using a supervised classification from a LandSat 7ETM+ image of April 2001 under ENVI 4.3 software. These areas corresponded to the presence of ground water at the end of the dry season, and are henceforth denoted “wet areas”. All S and SD sites were sampled. The sensitivity of this classification was then calculated.

Entomological Sampling Strategy

Within each grid cell of the study area, 1 to 43 unbaited Vavoua traps (Vestergaard Frandsen Aps, Kolding, Denmark) were deployed in the available using GPS (Map 75 GarminTM) that had the grid and polygons of the wet areas uploaded. Traps were deployed for three days and removed when at least one tsetse was captured during this period. When no tsetse was caught traps remained deployed for up to 30 days with traps checked every 1 to 5 days. In each trap site, ecological data were collected.

Each of the grid cells was thus assigned to three possible classes: « No wet area in dry season », where no trapping was needed; « No tsetse caught » if no tsetse flies were caught after sampling of the wet areas; « infested » if one tsetse fly was caught in at least one of the wet areas.

Probability Maps

Zero catch of tsetse flies does not mean absence of tsetse in the sampled area. Therefore, a probability model was used to evaluate the risk that tsetse are still present despite a sequence of zero catches (Barclay and Hargrove 2005):

$$p = \exp (- S t \sigma \lambda)$$

where S is the number of traps deployed in the total area, t the number of days for which each trap is operated, σ the trap efficiency and λ the population density (number of insects / area of suitable habitat). This probability was calculated for each grid cell. In the absence of any control effort, the minimal number of a resident tsetse fly population was

set at 10. The trap efficiency, defined as the probability that a trap catches a fly in an area of 1km^2 during one day, was determined at 0.01 using row data from the SidéréDougou elimination campaign in Burkina Faso (Cuisance et al. 1984, Politzar and Cuisance 1984).

Results

Suitable Habitats

Eight types of suitable habitats for *G. p. gambiensis* were identified: (1) natural Guinean forest galleries close to permanent springs; (2) semi-disturbed Guinean riverine tickets; (3) *Euphorbia spp.* fences; (4) swampy forests around permanent ponds; (5) riverine forests located inland adjacent to the mangroves; (6) palm-tree crops; (7) other tree-crops (citrus fruit, mango); and (8) lakes or swamps with reeds. In these habitats fresh water is available during the dry season, thanks to underground river networks, springs or human watering activities.

The sensitivity of the supervised classification was 0.96 (1 S and 3SD not detected on 110 validation sites) whereas its specificity was only 0.43.

Entomological Results

The total study area comprised 7150 km^2 which was overlaid by a grid with 286 cells. Wet areas were absent in 87 grid cells, the 199 remaining being retained to be surveyed, from December 2007 to March 2009: a total of 683 traps were set in 105 grid cells, corresponding to 3564 trapping events. *G. p. gambiensis* was the only tsetse species sampled and the total observed infested area was 525 km^2 .

Probability Maps

In 84 grid cells where no flies were trapped, the probability of tsetse presence was below 0.05 (the level of risk accepted) in 68 grid cells and above 0.05 in the remaining 16 grid cells. Figure 1 presents the probability of tsetse presence in those cells around the confirmed tsetse-infested area.

Although not all grid cells have been sampled, the survey methodology was adapted and adjusted as a result of this analysis during the whole sampling process. The grid cells where the probability of tsetse presence

is upon 0.05 despite a sequence of zero catches will be considered infested and treated as well, when in contact with infested cells. Additional sampling in these cells will be carried out during the control campaign.

Figure legends

Figure 1: Probability of presence of *Glossina palpalis gambiensis*. This probability was implemented in the grid cells where no flies were trapped and which are situated around the confirmed infested area.



Discussion

The collection of entomological baseline is a prerequisite for an AW-IPM programme to select appropriate control tactics, adapted to the characteristics of the target zone/population (Vreysen 2005, Leak et al. 2008). Especially when the targeted area is large (Alemu et al. 2007), logistics, time and economics will limit the amount of trapping that can be carried out. Therefore, a representative grid-based sampling approach is necessary (Leak et al. 2008), and has been fine tuned here using modern tools of spatial analysis (GIS/RS/GPS) (Cox and Vreysen 2005), mathematical modelling and ecological studies (Challier 1973). Since most other countries under the PATTEC initiative are in the phase of

data collection, the methodology described in this paper could assist in making the data collection more cost effective.

The remote sensing analysis allowed restricting the sampling area to only 4% of the original target area. It showed a very high sensitivity but a low specificity, thus increasing the probability that most of the sites inhabited by tsetse are detected. This method permitted detecting tsetse flies in unexpected sites like in a military camp, where sampling would never have been implemented without this wet area classification. The use of GPS guidance, thanks to the incorporation of the RS data in the GPS was also very much appreciated by field teams. Finally, the calculated probability of presence (Barclay and Hargrove 2005) provides a method to improve the cost-effectiveness and the reliability of the sampling process before the initiation of the operational phase of the actual control campaign in Senegal.

The first elimination effort in the 1970s most likely failed (Touré 1973) because not all ecological important areas were identified and treated. A population genetics study has been initiated to assess the relationship (and possible isolation) between the tsetse populations of the Niayes and those of the Sine Saloum (i.e. the nearest population of the main tsetse belt in southern Senegal).

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IMPROVING THE COST-EFFECTIVENESS OF VISUAL BAITS FOR *GLOSSINA FUSCIPES FUSCIPES* NEWSTEAD/

AMELIORATION DE LA RENTABILITE DES APPATS VISUELS POUR LES *GLOSSINA FUSCIPES FUSCIPES* NEWSTEAD

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Résumé:

Les appâts visuels artificiels tels que les pièges et les cibles sont couramment utilisés dans le cadre de la lutte contre les mouches tsé-tsé. Ces cibles, mesurant d'ordinaire entre 1,0m² et 1,7m², sont déployées à certaines densités au kilomètre carré, en fonction des espèces de tsé-tsé et de l'habitat. Le coût des cibles et des pièges dépend de la quantité de tissu utilisé, et peut constituer une partie substantielle du budget d'une opération de lutte contre la mouche tsé-tsé. Ces dernières années, peu d'enquêtes nouvelles ont été effectuées pour étudier la possibilité de réduction des coûts en termes de réduction de la taille de la cible.

Dans une étude réalisée au Kenya sur les *Glossina fuscipes fuscipes*, les enquêtes sur l'efficacité relative des pièges et d'une grande variété de cibles, ont démontré que les cibles peuvent être conçues pour capturer beaucoup plus de *G.f. fuscipes* que les pièges, et démontre qu'il y a un potentiel élevé dans l'utilisation de petites cibles pour les opérations de lutte. Les résultats montrent qu'une variété de cibles de petite taille (0,06 m²) avec un petit filet produisait des prises:

- (i) égales à environ la moitié de celles de la grande (1m²) cible noire,
- (ii) égales à environ celles du piège biconique pour les mâles, et

(iii) plusieurs fois supérieures au nombre de captures pour les femelles. Ceci donne à penser que les très petites cibles avec un très bon rapport coût-efficacité sont plus appropriées pour *les G. f. fuscipes*.

Des résultats prometteurs ont également été montrés par rapport aux *Glossina tachinoides* et aux *G.p. gambiensis*. Ces résultats ont des implications importantes sur la maîtrise de toutes les sous-espèces de *G. fuscipes* actuellement responsables de plus de 90% de nouveaux cas, et peuvent être bénéfiques pour les opérations à grande échelle prévues par la PATTEC.

Summary

Artificial visual baits such as traps and targets are commonly used for tsetse control. These targets, normally between 1.0m - 1.7-2 m, are deployed at certain densities per square kilometre, depending on the tsetse species and the habitat. The cost of targets and traps depends on the amount of cloth being used, and can constitute a substantial part of a control operation's budget. In recent years, few new investigations have been done to determine the possibility of cost reduction in terms of target size reduction. In a study in Kenya on *Glossina fuscipes fuscipes*, investigations into the relative effectiveness of traps and a wide variety of targets, showed that targets could be designed to catch several times more *G. f. fuscipes* than traps; and it demonstrated much potential for the use of small targets in control operations. Results show that a variety of small (0,06m²) targets with a small net gave catches that were: (i) about half of those from the large (1m²) black target, (ii) about the same as biconical trap catches for males, and (iii) several times greater than trap catches for females. This suggests that very small and therefore highly cost-efficient targets are suitable for *G. f. fuscipes*. Promising results have also been evident for *Glossina tachinoides* and *G.p. gambiensis*. These findings have important implications for controlling all subspecies of *G. fuscipes*, which are currently responsible for more than 90% of sleeping sickness cases, and can be of benefit for the large scale operations planned by PATTEC.

Introduction

The absence of a vaccine and problems with the availability, toxicity and resistance to drugs [1] mean that controlling the vector is a highly attractive means of tackling the diseases. One of the most important methods of tsetse control is the use of stationary artificial baits which

simulate host animals and consist either of three-dimensional traps, or cloth screens that are treated with insecticide and known as targets [2]. The recommended targets are black, blue or blue/black, about 1.0-1.7 m² and, for the savannah species of tsetse, they are baited with odour attractants and deployed at about 4 / km². For the riverine species of tsetse, traps rather than targets are commonly used and, since no effective odour attractants are known for these flies, the required density of baits is relatively great (>10 / km²). Hence, the cost of controlling riverine tsetse using artificial baits is at least twice that for the savannah flies [3]. Nevertheless, the use of artificial baits is favoured for controlling riverine tsetse, partly because it is cheaper than methods such as the sterile insect technique and aerial spraying [3], and also because it is suitable for community implementation [4]. Hence, any economies in the bait control of riverine species would be particularly welcome.

So far, attempts to improve bait control of the riverine tsetse have concentrated largely on traps, especially in the case of *G. fuscipes fuscipes* [5,6,7], which together with the other two subspecies of *G. fuscipes* are implicated in more than 90% of sleeping sickness cases [8,9]. Moreover, with all riverine species the refinement of targets has focused mainly on colour and materials [10,11,12], not size. Present work with *G. f. fuscipes* elucidated the relative effectiveness of traps and a wide variety of targets, with particular attention to size, and demonstrated much potential for the use of relatively tiny targets in control operations.

Materials and Methods

Studies were performed from August 2007 to December 2008 on the 0.5 km² of Chamaunga Island (0° 25' S, 34° 13' E), Lake Victoria, Kenya. Baits consisted of a blue biconical trap [13] and targets made from cotton cloth dyed black or Phthalogen blue

Electrocuting grids placed over fine black netting were also placed next to targets and traps where they intercepted flies in flight - these are the so called flanking nets. The fine black polyester net (Quality no. 166, Swisstulle, Nottingham, UK) and the electrocuting wires of the electric net used here are effectively invisible to tsetse [14,15].

Electrocuted flies fell into trays of soapy water below the grids. When no flanking nets were used the catches in the trap, and those made by grids on the target cloth, indicated the numbers of flies that would be killed in field campaigns to control tsetse by traps or insecticide-treated targets.

Results

Trap vs target. – Biconical traps typify the sorts of trap used to control *G. f. Fuscipes* and other riverine tsetse [5,11,12,18]. A 100 x 100 cm black target is the common benchmark for target performance with several species [19]. These two baits were compared in the presence and absence of flanking nets, 100 x 50 cm (all dimensions are reported as height x width). With the nets, the total catches suggested that the trap attracted 1.9 times as many males and 1.4 times as many females as the target, although the effect was significant only with the males (Table 1, Expt. A). However, comparison between the catches with and without the net showed that the trap efficiency was only 40% for males and 21% for females, as against efficiencies of 71% and 34% respectively for the target.

Table1: Summary of results showing detransformed means of catches

Expt.	Size	Males	Females
A 12 days, sed: males 0.068, females 0.096			
	~130 x 80	5.7a	4.8a
	~130 x 80	15.6b	23.9b
	100 x 100	5.6a	5.7a
	100 x 100	8.2c	16.8b
B 8 days, sed: males 0.076, females 0.093			
	100 x 100	12.6a	21.2a
	100 x 100	26.9b	40.3b
	100 x 100	20.0b	35.2ab
	100 x 100	18.0ab	26.1ab
C 8 days, sed: males 0.079, females 0.090			
	100 x 100	15.6a	21.9a
	100 x 100	21.5a	39.9a
	100 x 100	17.9a	25.0a
	100 x 100	21.2a	38.6a

Size reduction - The four experiments of Table 2 used square black targets to assess how much target size could be reduced. Sometimes the smaller targets were raised off the ground, so that their centres of visual conspicuousness were at the same height as that for the large target on the ground. The point was that reducing the target size to 25 x 25 cm, *i.e.*, to 1/16th of the area of the large target, gave catches that declined remarkable little, by a mere half on average, suggesting that the cost-effectiveness of per cm² of cloth would be enhanced about eight-fold by using tiny targets. Hence, further work concentrated on mostly the 1/16th- sized targets (0.0625 m²), although the biconical trap and/or the large (1 m²) black target were sometimes included to keep sight of the fact that an important criterion for any new target is its performance relative to more standard baits.

Colour. -- the catches with nets present were increased by about a third when the target was all-blue or blue/black instead of all-black. These effects were not significant, but they approximate to the effects of colour with large targets (Table 1, Expts. B & C). However, with the small targets in the absence of nets, the blue/black target caught several times more males and females than either the all-black or all-blue, and the effects were significant. The implication is that the percentage of flies alighting on the small blue/black target was 43% for males and 37% for females, compared with only 21-24% for males and 15-23% for females on the two small monochromes.

Table 2: Summary of results showing detransformed means of catches

Expt.	Size	Males	Females
A 12 days, sed: males 0.066, females 0.061			
	100 x 100	21.9a	26.9a
	50 x 50	20.2a	18.8b
	25 x 25	14.3b	15.9b
B 12 days, sed: males 0.057, females 0.048			
	100 x 100	26.9a	30.1a
	50 x 50	18.5b	21.2b
	25 x 25	15.8b	15.2c
C 12 days, sed: males 0.076, females 0.093			
	100 x 100	8.4a	5.4a
	50 x 50	9.3a	4.0a
	25 x 25	3.5b	2.9a
D 12 days, sed: males 0.057, females 0.075			
	100 x 100	15.7a	16.3a
	50 x 50	18.5a	13.1a
	25 x 25	9.0b	8.1b

Discussion

Present work shows that targets can be designed to catch several times more *G. f. fuscipes* than the traps; such targets are much cheaper and simpler than traps, and easier to maintain. These matters confirm the long-standing generalisation, based on studies with other tsetse species, that targets are much more cost-effective than traps [2]. Strikingly, the present work suggests very small and therefore highly cost-efficient targets are suitable for *G. f. fuscipes*.

More should be done to optimize target design for *G. f. fuscipes*, and to make fuller and more critical comparisons with other species, but it is

already clear that the cost-effectiveness of target operations against *G. f. fuscipes* could be improved substantially by using small targets with a little netting adjacent. The cost of materials, insecticide and transport would decline by about 90%, and the convenience of deploying each target would be enhanced. This would more than offset the fact that twice as many targets would be needed to maintain efficacy. Moreover, with such small cheap targets it might be acceptable to make them disposable and bio-degradable, giving further improvements in convenience.

Research in Burkina Faso showed similar promising results for *G. tachinoides* and *G.p.tachinoides*, and in DRC for *G.f.quanzensis*.

(For the full details and experiments, see Lindh *et al.*: PLoS NTD, July 2009)

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MANAGEMENT AND CONTROL OF VECTOR-BORNE DISEASES IN IMPROVED LIVESTOCK PRODUCTION SYSTEMS OF PERI-URBAN AREAS IN BURKINA FASO AND MALI/

GESTION ET CONTROLE DES MALADIES A TRANSMISSION VECTORIELLE DANS LES SYSTEMES AMELIORES D'ELEVAGE DES ZONES PERIURBAINES AU BURKINA FASO ET AU MALI

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Résumé:

La croissance démographique et l'urbanisation qui en découle exigent une réorientation des systèmes d'élevage extensif vers des systèmes intensifs. Les systèmes d'agriculture intensive périurbaine vont attirer une partie plus importante du marché. L'accès aux centres urbains peut être assuré au mieux dans la périphérie de ces agglomérations. L'intensification des systèmes d'élevage conduit à une augmentation de la nuisance des moustiques, des mouches domestiques et d'étable. Les maladies transmises par les arthropodes d'intérêt vétérinaire et médical (zoonoses) vont probablement accroître. La lutte chimique est principalement basée sur l'utilisation d'insecticides ou d'acaricides. Les pyrèthrinoides se révèlent particulièrement efficaces avec un avantage supplémentaire pour sa faible toxicité pour les mammifères. Leur efficacité est cependant sérieusement compromise par le développement de résistance aux insecticides, confirmée par l'apparition d'une résistance

croisée contre d'autres pyréthrinoïdes. La résistance chez les mouches domestiques (*Musca domestica*) peut déjà se développer en moins d'un an. La *M. domestica* transmet au moins 100 maladies bactériennes, virales ou parasitologiques affectant la santé humaine et animale.

Le contrôle des vecteurs arthropodes requiert donc un abandon de l'utilisation généralisée des insecticides au profit de leur application ciblée. Les clôtures à moustiquaire imprégnée d'insecticides se sont révélées efficaces dans la lutte contre une vaste gamme d'insectes nuisibles, y compris plusieurs espèces de mouches tsé-tsé. La TAA a été efficacement contrôlée dans plusieurs pays grâce à cette méthode à faible coût et à faible effet secondaire. Un contrôle approprié des vecteurs nécessite désormais une approche plus globale et plus stratégique, intégrant différentes méthodes de lutte.

Mots clés, Intensification de l'élevage, zoonoses, résistance aux insecticides, lutte stratégique contre les vecteurs, clôtures imprégnées d'insecticides contre les moustiques.

Summary

Steady population growth and an ensuing urbanisation require a shift from extensive to intensive livestock production systems. Peri-urban, intensified farming systems will pick up a larger part of the market share. Access to urban centres can be best assured in the periphery of these agglomerations. Intensification of livestock production systems leads to a rise in nuisance from mosquitoes, house and stable flies. Arthropod-borne diseases of veterinary and medical relevance (zoonoses) are likely to increase. Chemical control is mainly based on the use of insecticides/acaricides. Pyrethroids prove to be particularly effective and have a further advantage due to low mammalian toxicity. Their efficacy is seriously jeopardized by the development of insecticide resistance, which is confirmed by observations on the appearance of cross resistance against other pyrethroids. Resistance in house flies (*Musca domestica*) can develop in less than one year. *M. domestica* transmits at least 100 bacterial, parasitological or viral diseases of relevance for human and animal health. Controlling arthropod vectors thus requires a shift from the wide-spread use of insecticides to their targeted application. Insecticide-treated mosquito fences have proved efficacious in combating a wide range of insect pests including several tsetse species. AAT was effectively controlled in different countries by this low-cost,

user-friendly method. Appropriate vector control now warrants a more comprehensive and strategic approach, integrating various control methods.

Key words: livestock production intensification, zoonotic diseases, insecticide resistance, strategic vector control, insecticide-treated mosquito fences

Introduction

Previous work by Bauer et al. (2001 and 2006) has shown distinct improvements of health in cattle protected against tsetse-transmitted trypanosomosis (AAT) in western Kenya where insecticide-treated fences were used to protect the dairy cattle. Observations from the participating farmers had also indicated a reduction of nuisance insects in the vicinity of the protected pens. More recently, trials were conducted in the forest zone of Ghana near Kumasi showing significant reductions in trap catches of Muscinae and Stomoxyinae (Maia et al., 2010, accepted for publication) against the predominant species of nuisance insects (Muscinae and Stomoxyinae). In the trial, we are presenting here, it was tried to confirm the previous results in several currently existing animal husbandry management systems of West Africa.

Material and methods

Pre-treated (100mg/m² deltamethrin, UV-protection factor) polyester net (mesh width 1x2mm), was attached to existing timber poles of the unit containing animals.



Figure 1: Improved animal husbandry management system

Only occasionally the cattle are having the opportunity for grazing outside the paddock. Kept inside during much of the time they are fed with dried hay, crop residues such as maize stems and cotton seed cake. Improved genotypes are frequently introduced such as zebu Goudali or Azawak. Exotic breeds are often Holstein-Friesian or Swiss brown. Situated in peri-urban areas, the main purpose of this management system is to meet the increasing demand for beef, dairy products, particularly milk, cream, yoghurt and butter.



Figure 2: Intensified animal husbandry management system

The cattle are almost permanently stalled and fed as in the previous management system. Semen from Swiss brown and other exotic breeds such as Holstein Friesian are systematically introduced. The main purpose of this husbandry system is to provide dairy products.



Figure 3: Fence protection of a pen with concrete walls and geo-referenced mono-conical traps

Densities of nuisance insects outside each pen were evaluated with 5 – 7 geo-referenced (UTM GPS readings) mono conical “Vavoua” traps for 24h at fortnight intervals (Figure 4). Trap distances varied between 5 – 30m outside each protected pen and their exact position was retained throughout the trial which lasted for about six months. All insects were preserved in 70% ethanol and identified under a dissecting microscope. Mosquitoes were caught as well outside but nearer to each pen by using one battery-driven geo-referenced BG-Sentinel trap[®] (Figure 5) from dusk till dawn at the same fortnightly interval. This trap was found to be particularly effective in catching mosquitoes, notably *Aedes* spp., but also considerable amounts of *Culicoides* spp. Preservation and identification of collected insects was performed as described above. Baseline data for both protected and control pen were collected by catches before the start of the trial. Five mono-conical traps were deployed for 72 hours and the catches retrieved every 24h. At the same time the BG-Sentinel traps[®] were operated for one night from dusk till dawn (18.00h – 06.00h), which was consistent with the evaluation procedures throughout the trial period.



Figure 4: BG-Sentinel trap[®]

Presently, an improved version of the BG-Sentinel trap[®] with UV light is available and is used in Germany for monitoring of *Culicoides* spp.

Results

The impact of the protection with insecticide-treated polyester net is shown in figures 6 – 9 for Muscinae (*Musca domestica*, *M. autumnalis*, *M. sorbens*), *Stomoxys* spp., *Haematobia irritans*, *Anopheles* and *Culex* spp. The results of the baseline survey indicated only negligible differences in the numbers of all recorded insects between protected and control pen, confirming that the two pens were comparable in terms of densities of the target insects. At this stage in early July, after the rainy season had only started, the insect numbers rarely exceeded 70/trap/day and there were only slight differences between the protected and unprotected pen, which needs to be taken into account when statistically significant results are expected. As a consequence of the continuing precipitation the relative humidity and the humidity of the soil not only resulted in an increase of the vegetation cover but also provided optimal breeding conditions for all target insects. Therefore, the catches of flies and mosquitoes started to rise distinctly during the last period of the

rainy season from September onwards. This was reflected by an increase of all catches for nuisance and biting flies as well as mosquito species. This increase was particularly pronounced for catches in the vicinity of the control pen, while the outside catches near the protected pen either did not rise at all or to a much lower level (catches of Muscinae, Figure 6). These differences between protected pen and control were particularly pronounced in the case of biting flies (*Stomoxys* spp. and *Haematobia irritans*) as is shown in Figure 7.

Distinct differences were also observed in the case of mosquito catches. A single, six-fold increase, albeit from a lower level – initially 10 to more than 60 specimens of *Anopheles* spp. catches – was recorded at the beginning of September. These observations could have important implications for efforts to control malaria but clearly require the collection of more, statistically relevant data. The same conclusion can be drawn from the catches of *Culex* spp. A more than four-fold increase of catches outside the control pen was not matched with similar changes in the number of catches outside the protected pen. However, at this stage of experience and knowledge any claim about the impact of protection on mosquitoes appears to be premature even when taking into account concomitant observations from farmers in western Kenya.

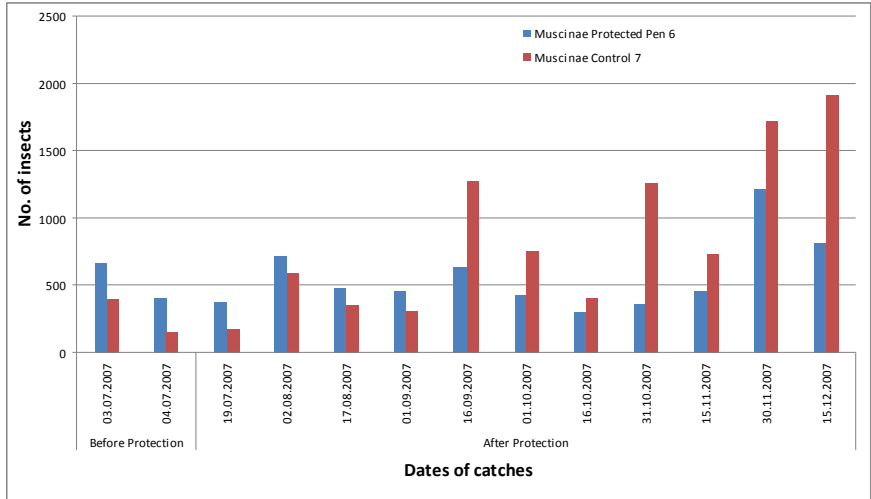


Figure 5: Impact of protection on outside catches of Muscinae with seven mono-conical traps, for 24h, in comparison to an unprotected control pen

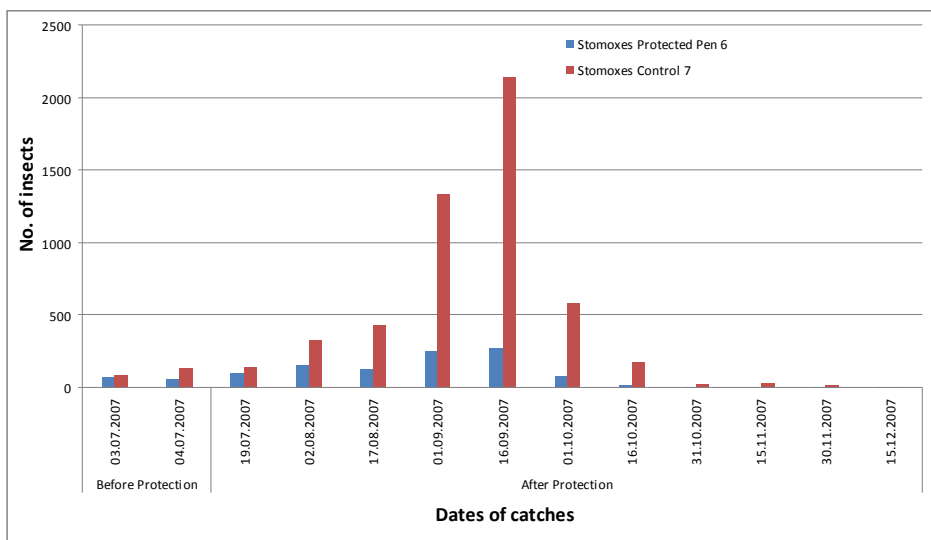


Figure 6: Impact of protection on outside catches of *Stomoxys nigra*, *S. calcitrans* and *Haematobia irritans* with seven mono-conical traps, for 24h, in comparison to an unprotected control pen

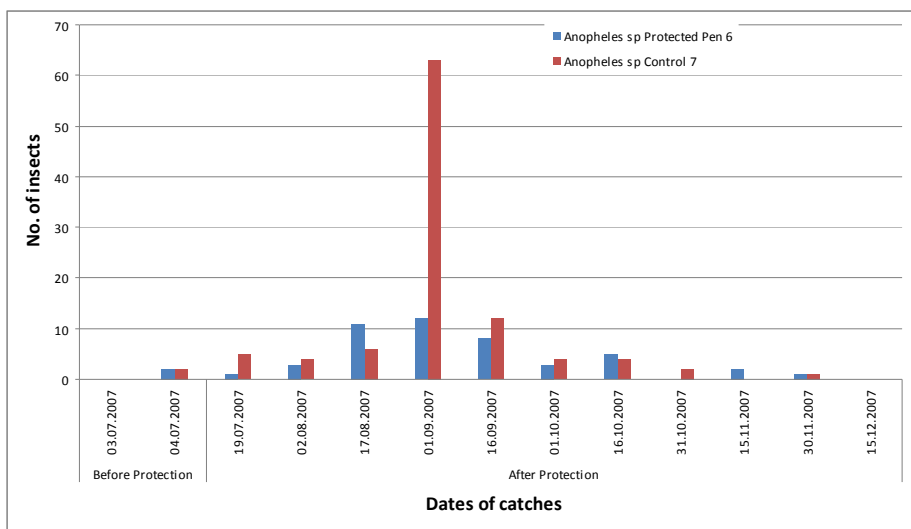


Figure 7: Impact of protection on outside catches of *Anopheles gambiae* s. l., *An. feroensis* and *An. r. rufipes* with one BG-Sentinel trap®, from 18.00h – 06.00h

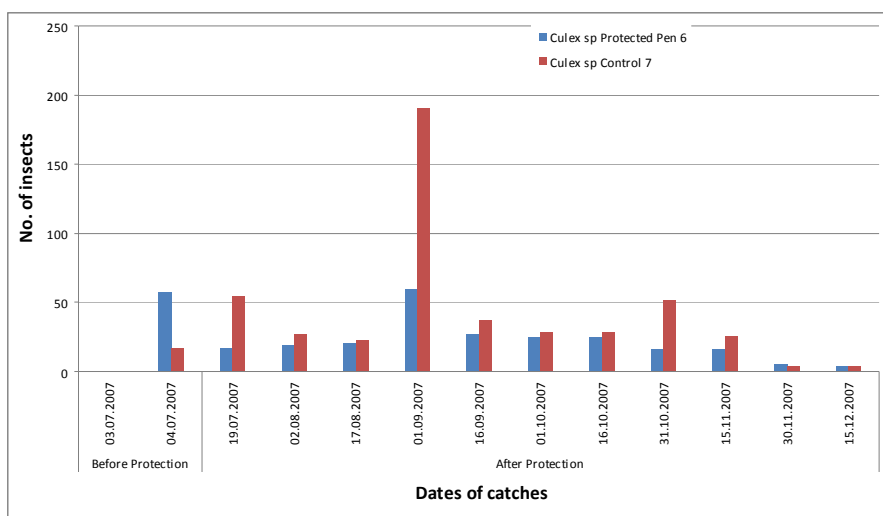


Figure 8: Impact of protection on outside catches of *Culex* sp. with one BG-Sentinel trap[®], from 18.00h – 06.00h

Discussion

A reduction of nuisance and biting flies in dairy production systems or feedlots will in all likelihood result in an increase in productivity. Catangui et al. (1993) reported losses of up to 0.16 kg/day in feedlots and (Morgan & Bailie 1980) observed a decrease in milk yields of up to 1.0 l/day/cow. The control of biting and nuisance insects should also reduce the risk of disease transmission. Mihok et al. (1995) were able to show the role of African Stomoxyinae in the mechanical transmission of *Trypanosoma* spp., also describing their impact on animal health and body condition with an estimated daily blood uptake of 13.7 ± 0.6 mg for a single *Stomoxys*. Under the prevailing climatic condition when several hundred or more than 1,000 haematophagous insects are feeding on one cow during a single day their negative impact cannot be overestimated (Mullens et al., 2006). Curtis (1998) described *Musca domestica* as a known vector of more than 100 pathogens of relevance for human and livestock health. Many of these pathogens are responsible for zoonotic diseases. Externalities are therefore expected to accrue in the case of diarrhoeal diseases and trachoma which are mainly transmitted by Muscinae. The results confirm earlier work by Maia et al. (2010) about an impact of protection – not only for confined livestock but also in the vicinity of a protected pen. Since these clear effects outside protected

pens on house and stable flies were as well recorded in the present trial, an assessment of the epidemiology of blinding trachoma with a particular focus on *Musca sorbens* is required in an experimental setup where mosquito fences are used on a wider scale.

Up to now there is only insufficient information with regard to the cost-efficiency of using insecticide-treated mosquito fences in sub-Saharan production systems. At present it is assumed that their use will result in considerable benefits for livestock keepers. Statistically quantifiable benefits can be best measured in feedlots. Statistically meaningful results are much more difficult to produce in dairy systems, given the need for comparability. The requirements for an identical management system and a genetically homogenous population are probably best met in a sufficiently large dairy plant. The protection has also indicated promising effects against potentially relevant mosquito species but further tests are needed before contemplating the large-scale application of this technique for the control of mosquitoes.

Despite the fact that distinct differences between the catches outside the protected and control pen were recorded, a meaningful comparison and statistical analysis turns out to be difficult if standard statistical methods are being applied. Maia et al. (2010) used generalized estimating equations (GEE) to estimate the effect of a treated net on the number of nuisance and biting flies collected outside the pens during their trial in Kumasi. GEE are particularly appropriate due to the nature of the data which had repeated measurements as was described by Zuur et al. (2009) and should also be applied in this case for ultimate statistical analysis and comparison. Seasonal variations – dry vs. rainy season and the ensuing climatic changes – need to be taken into account for the collection of conclusive information.

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**TRANSBOUNDARY PROGRAMME TO ELIMINATE
TSETSE FROM THE RIVER FRONTIERS OF BOTSWANA
AND NAMIBIA USING THESEQUENTIAL AERIAL
SPRAYING TECHNIQUE/**

**PROGRAMME TRANSFRONTALIERE POUR ELIMINER
LA MOUCHE TSE-TSE DES FLEUVES FRONTALIERS DU
BOTSWANA ET DE LA NAMIBIE A L'AIDE DE LA
TECHNIQUE DE PULVERISATION AERIENNE
SEQUENTIELLE**

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Résumé

En 2005, le Botswana et la Namibie ont rejoint la Campagne Panafricaine d'Eradication de la mouche Tsé-tsé et de la Trypanosomiase (PATTEC) sous l'égide de l'Union africaine (UA). D'autres membres de cette large coalition régionale étaient l'Angola et la Zambie. Ce qui a ouvert la voie à la participation du Botswana et de la Namibie à un programme transfrontalier de lutte contre la tsé-tsé basé sur la technique de pulvérisation aérienne séquentielle et mis en œuvre en 2006 sur une superficie totale de 10 000 km². La technique de pulvérisation aérienne séquentielle a été utilisée pour pulvériser de la deltaméthrine 0,35% sur 5 cycles avec 5 avions de type Ayres Turbo Thrush - tous équipés d'un système de guidage de précession permettant également de contrôler le débit des produits chimiques. Avant le début du programme, une barrière de cibles olfactifs appâtés a été déployée pour protéger des blocs ayant précédemment fait l'objet d'une pulvérisation aérienne au Botswana. A la fin, les captures de mouches tsé-tsé réalisées avant la pulvérisation ont été réduites, passant à près de 145 mouches par jour à zéro capture au bout de 5 semaines de pulvérisation sur une période de 3 mois. Le taux de dosage variait entre 0,26 et 0,3 g / ha. Les enquêtes d'après

pulvérisation se sont poursuivies 3 ans plus tard et n'ont pas révélé la présence de mouches tsé-tsé. Comme lors des précédentes opérations au Botswana, des études environnementales indépendantes ont été menées et aucun effet secondaire à long terme n'a été identifié. Le succès du programme démontre la faisabilité des programmes transfrontaliers SAT à titre de leçon pour de futures opérations de lutte contre la mouche tsé-tsé et la trypanosomiase à l'échelle régionale.

Summary

In 2005, Botswana and Namibia signed up to the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) under the umbrella of the African Union (AU). Other members of the broader regional coalition were Angola and Zambia. This paved way to collaboration by Botswana and Namibia in a transboundary SAT-based tsetse control programme implemented in 2006 over a total area of 10,000 km². The sequential aerial spraying technique was used to apply deltamethrin 0.35% (a.i) over 5 cycles using 5 Ayres turbo thrush aircraft – all fitted with precision guiding equipment that also controlled chemical flow rate. Prior to commencement of the programme, an odour baited target barrier was deployed to protect previously aerial sprayed blocks in Botswana. In the end, pre-spray tsetse catches were reduced from about 145 flies per day to zero catches at the end of 5 weeks of spraying over 3 months period. The dosage rate varied between 0.26 – 0.3 g/ha. Post-spray surveys continued 3 years later and have not found any tsetse flies. As in previous Botswana operations, independent environmental studies were conducted and no long-term ecological side effects were identified. The success of the programme demonstrates the feasibility of transboundary SAT programmes as blueprint for future area-wide tsetse and trypanosomiasis control operations.

Introduction

In northern Botswana tsetse occurs only in the Okavango delta and the Kwando and Linyanti Rivers bordering Caprivi Strip, Namibia. Aerial spraying for tsetse control was successfully reintroduced in the Okavango delta in 2001 and 2002. Thereafter, it was necessary to repeat the performance along the Kwando and Linyanti River systems to ensure that tsetse was removed completely from Botswana. In the event, a regional initiative to engage both Botswana and Namibia in a joint intervention programme was facilitated by the African Union's Pan-

African Tsetse and Trypanosomiasis Eradication Campaign (AU-PATTEC). The initiative would pave way to the first SAT-based transboundary campaign implemented by the two countries in 2006 under the auspices of AU-PATTEC.

The campaign's primary objective was to ensure that tsetse was eliminated completely from northern Botswana. The approach would also promote a start in Angola and Zambia, and ultimately kick-start a progressive 'roll-up' strategy for the entire southern Africa region. This ambitious plan would fit-in with the wider objectives of the PATTEC initiative aiming at complete elimination of tsetse from Africa (Kabayo 2002). In the event, Botswana funded the spraying operation (aircraft, insecticide, and environmental monitoring programme) while Namibia provided the essential operational airbase camp and additional ground support personnel. Independent assessment of the impact of the spray on non-target species was conducted alongside the spraying operation. One year later, a follow-up recovery assessment showed that almost all affected species had recovered to pre-spray levels (Bonyongo and Mazvimavi 2007, 2008).

Implementation

Study Area

Just north of the Okavango delta, the Kwando/Linyanti and Savuti river system provides the next suitable tsetse habitat outside the Okavango. The Kwando and Linyanti Rivers form the international boundary between northern Botswana and Caprivi Region of Namibia. West of the study area, the Kwando descends through Caprivi from upstream in Angola. The Linyanti River stretches eastwards at right angle to the Kwando. At due south of Linyanti, the Savuti Channel runs near parallel to Linyanti and drains into *mababe depression* which is located down east of the study area (fig. 1).

Riparian vegetation makes most of the tsetse habitat. Typically, the vegetation comprises mopane woodland (*colophospermum mopane* Fabaceae) and mixed communities of *Garcinia-Croton* and *Diospyros* woodland. The primary system of land use on Botswana side involves wildlife related tourism activities. On Namibian side, a few human settlements exist in addition to the tourism and Nature conservancies. Like the Okavango delta, the Kwando/Linyanti river system is subject to

seasonal flooding. It is also amassed by a variety of wildlife species that provide plentiful food source for tsetse flies. Tsetse distribution is largely confined to riverine vegetation with the highest density along the Kwando.

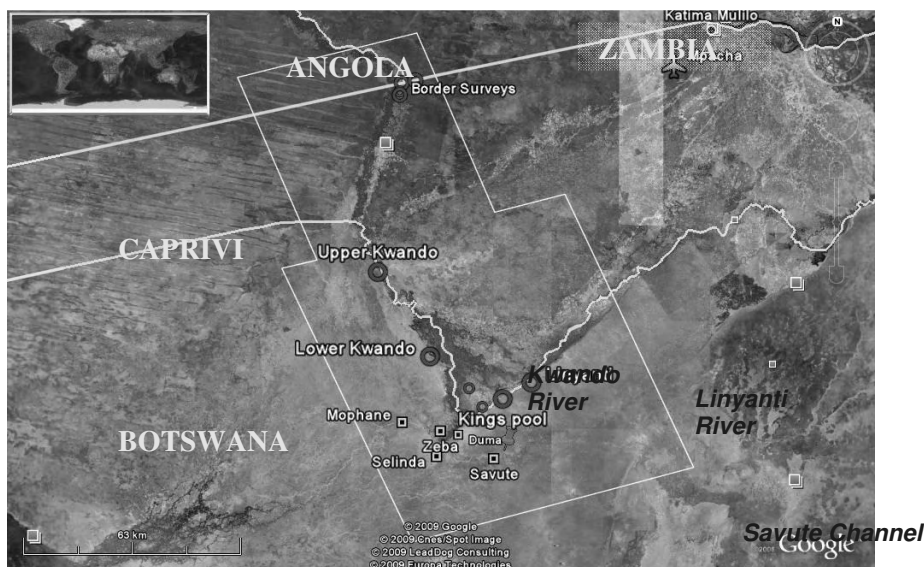


Figure 1: Overlay of 2006 transboundary Aerial Spray Block (10,000 km²) on Google Earth Image. Red circles denote tsetse monitoring survey sites. Placemark (orange) shows known tsetse distribution limits on Botswana side south of the Kwando and Linyanti Rivers. Squares (Botswana side of spray block) indicate location of some of tour-operator camps in the Kwando/Linyanti area.

Delineation of the Spray Block

The 10,000 km² spray block extended northwards from south of the Savuti Channel through the northern Caprivi border into Angola. Outside the Okavango delta, the Savuti channel marked the southern most limits of the remaining tsetse distribution in Botswana. The spray block was designed to include all known tsetse distribution north and south of the Kwando and Linyanti rivers. In effect, the riverine vegetation of the Kwando and Linyanti formed the epicenter of the fly distribution. The highest fly distribution occurred in Botswana and it was well known but relatively little was known in the area north of the Kwando and Linyanti

rivers. However, limited surveys carried out by Namibia along the border with Angola did help in defining the northern part of the spray block.

Respectively, the spray area covered 5,100 km², 4,700 km² and 200 km² in Botswana, Namibia and Angola. The entire operation would constitute the first largest single area ever treated by aerial spraying. It was also the first transboundary SAT campaign implemented under the auspices of AU-PATTEC.

Aerial Spraying

Spraying commenced 28 May through 18 August 2006. Five fixed-wing aircraft of Ayres

Turbo Thrush types 34 and 65 turbines (Orsmond Aviation, Bethlehem, South Africa) applied formulated "deltanex" insecticide 0.35% deltamethrin active ingredient (a.i) at dose rate between 0.26 and 0.30 gai/ha. Each aircraft was fitted with two boom-mounted micronair AU 4000 rotary atomisers (Micron Sprayers Ltd. Bromyard, UK) for dispersal of insecticide. Also fitted to the aircraft for improved navigation was the upgraded GPS-based SATLOC guidance equipment. The SATLOC upgrade *M3/Airstar* provided moving map display to guide the pilot along predefined flight paths (Kgori et al 2009).

To ensure constant application rate, the SATLOC guidance system (accurate to 1m) allows for automatic adjustment of the insecticide flow rate to match the aircraft's speed. In addition, the system has data logger that captures key operational data for subsequent analysis - including application rate, litres sprayed and area sprayed.

Five sequential nighttime treatments were applied involving the release of formulated insecticide aerosols at low altitude. The aerosol droplet size spectrum (Allsopp 1990) was in the range comparable to that used successfully in 2002. The Numerical Median Number and Volume Median Diameter were 25 and 41µ respectively. Spraying was carried out during suitable conditions of temperature inversion in order to optimize the efficiency of droplets settling through the habitat.

Timing of the intercycle period was as described in Kgori et al (2006). The aim being to remove newly emerged females before they can deposit their first larvae i.e. before the end of their first larval period – which is temperature depended (Hargrove 2003). Altogether, the cycles should

cover the entire estimated pupal period – which is also temperature dependant.

Monitoring surveys

Tsetse monitoring sites were established at strategic areas in the spray block. Two sites were selected along the Kwando River and another two along the Linyanti. Epsilon traps (Hargrove and Langley, 1990) baited with methyl ethyl ketone and 1-octen-3-ol as well as man-fly rounds were used to monitor fly populations prior to and during the spray operation. The Kwando sites were consistently productive whereas very low tsetse numbers were caught at Linyanti sites. Thus the Kwando became the primary survey sites with dissectors checking the age of all female catches. Fewer flies were being recorded at Mudumo in the Caprivi side of the Kwando River. A helicopter was used to position traps in areas that were inaccessible by ground means, including Nkasa Island in the Mamili National Park - also in Caprivi.

At each monitoring site, usually two permanent man fly-rounds each covering a distance of about 8km were patrolled regularly. In addition, about ten traps were operated at each of the four sites. During the spraying operation, both man fly-rounds and traps were operated daily and often supplemented by random man patrols (less coordinated form of man fly round). Operational surveys began about 13 May through September 2006.

Results

Within the spray block, daily tsetse catches from all man fly rounds and traps dropped from pre-spray peak of about 145 to zero post cycle 1. Thereafter, any signs of post-spray cycle recovery (which should be expected) were significantly small. No more catches were made in the latter part of cycle 4.

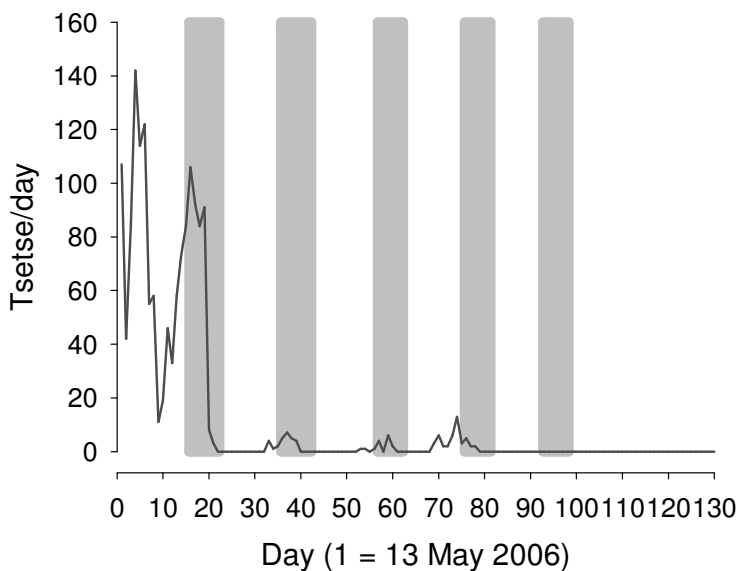


Figure 2: Operational tsetse survey results from the SAT monitoring sites at Kwando and Linyanti – showing pooled trap and fly-round catches. The vertical bars denote timing of the aerial spraying cycles i.e. cycle 1-5 beginning 28 May through August 18.

Discussion and Conclusions

Surveys following the 2006 aerial spraying operation between Botswana and Namibia suggest that the operation achieved two primary objectives: (1) to remove tsetse completely from Botswana by preemptive elimination of those factors and conditions that would potentially predispose to reinvasion of previously sprayed areas. (2) establish institutional capacity for effective implementation of transboundary operations within the region. The latter stands to promote continuity and therefore sustainable future benefits that are likely to accrue from the successful creation of area-wide tsetse free zones.

Preliminary results showed that the current operation was a success, reducing tsetse numbers from 145 flies caught per day at the beginning of spraying - to zero at the end of the 5 weeks spraying programme spread over 3 months. Coordinated post-operational surveys have not found tsetse in the sprayed area to date i.e. about 3 years since spraying ended. In retrospect, tsetse has still not been found in the Okavango delta

following the 2001 and 2002 Okavango campaign. Hence there can be reasonable optimism about the outcome of the 2006 campaign as well - both at local and regional level.

No adult tsetse females appeared to have survived the first spray cycle as it is often the case. The good overall results may be attributed to the presence of best and appropriate equipment, and of course a highly effective insecticide. The availability of SATLOC guidance system has introduced parallel swathe guiding system that applies GPS technology to position and guide aircraft accurately along predefined flight paths – and ‘precision’ is key. This precision factor could not be exploited by previous SAT operations in the 1970s and 80s. The insecticide formulation currently in use has also proved to be not only environmentally acceptable but also quite effective against tsetse flies. The same “Deltanex” formulation was used successfully in 2001 and 2002 in the Okavango delta with no significant environmental implications.

Future SAT operations will undoubtedly focus more around regional collaboration – with the current campaign being the first of such kind and scale implemented under the auspices of AU-PATTEC. Its successful implementation has significant bearing on the region’s capacity to follow through its obligation towards area-wide approach to tsetse and trypanosomiasis eradication on a progressive basis. From Botswana’s perspective, it was critical to extend the Okavango delta operations to the frontiers of neighbouring Caprivi Region, Namibia where tsetse flies remained. Only then would it be to achieve complete removal of tsetse in the country. For the plan to work however, a strategic collaboration with Namibia was also critical. Thus the collaboration approach needed to be agreed upon and implemented jointly without fail.

Clearly, the opportunity cost of failure to ensure continuity would have been considerable from Botswana’s stand point but also in the broader sense of area-wide approach to pest and disease control across the region. The overall success of recent operations involving modern SAT is testimony that the technique has undergone significant transformation over the years. Thus SAT should significantly improve on the requisite tools and methods that the continent so desperately needs in order to expedite the processes for creating tsetse-free zones in Africa. Challenges to the successful implementation of a transboundary collaboration programme could arise largely from lack of harmonization

among national priorities and policy frameworks that may be fundamental to the implementation of such programme. Hence, the role of AU-PATTEC will increasingly become critical in ensuring that the overriding principles of continuity and progressive engagement are upheld by tsetse affected countries.

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DEVELOPMENT OF ATTRACTANTS FOR *GLOSSINA PALPALIS GAMBIENSIS* AND *G. TACHINOIDES* IN WEST AFRICA/

RECHERCHE D'ATTRACTIFS POUR *GLOSSINA PALPALIS GAMBIENSIS* ET *GLOSSINA TACHINOIDES* EN AFRIQUE DE L'OUEST

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Résumé

Afin d'en améliorer l'efficacité sachant que des attractifs olfactifs existent pour les glossines du groupe morsiens mais pas pour celles du groupe palpalis, des études ont été menées à Solenzo sur le fleuve Mouhoun à l'Ouest du Burkina Faso, et au Sud à Folonzo le long de la rivière Comoé, en utilisant des odeurs naturelles ou de synthèse pour améliorer le piégeage de *G. p. gambiensis* et de *G. tachinoides*.

Aussi bien à Solenzo qu'à Folonzo, l'odeur brute de bovin a triplé les captures du piège biconique pour les *G. p. gambiensis* femelles, et les a multiplié par 6 chez les mâles ($p < 0.05$), pendant que celle de l'homme les a multipliées par 4 chez les femelles et par 2 chez les mâles. Pour *G. tachinoides* qui a été seulement capturée à Folonzo, l'augmentation de la capture due aux odeurs de bovins était de 5 fois pour les deux sexes, ($p < 0.01$) cependant non significative pour les odeurs humaines.

Le POCA, un mélange de 3-n-propylphenol, 1-octen-3-ol, et 4-methylphenol en des proportions de 1:4:8 en sachet, plus de l'acétone, a multiplié par deux les captures des mâles, en utilisant le piège ou la grille électrique ($p < 0.05$). Pour les femelles, l'augmentation était faiblement significative (1.6 fois). A l'opposé, *G. tachinoides* semble très sensible au POCA, avec parfois des index atteignant 10.

L'obtention d'attractifs efficaces contre ces espèces est particulièrement importante car il sera ainsi possible d'améliorer l'efficacité des pièges et des écrans de lutte contre cette espèce, ce qui permettra de réduire la densité de leurres à installer par km². Cela participera à n'en pas douter aux efforts actuels du PATTEC de lutte contre les espèces du groupe Palpalis.

Mots clés, *G. p. gambiensis* ; *G. tachinoides*, attractifs olfactifs ; hôtes naturels ; odeurs de synthèse

Summary

Olfactory attractants which are effective against *morsitans* group tsetse have proven ineffective against tsetse of the *palpalis* group. In order to improve vector control efficacy, especially for *G. p. gambiensis* and *G. tachinoides* which are the main vector of sleeping sickness in West Africa, experiments were undertaken (i) in Solenzo on the Mouhoun River in western Burkina Faso and (ii) in the South in Folonzo along the Comoe River, using natural and artificial odours. In Solenzo as well as in Folonzo, natural cattle odour increased *G.p.g.* catches in biconical traps up to 3 folds for females and 6 for males ($p < 0.05$), while human odour increased it up to 4 for females and 2 for males ($p < 0.01$). For *G. tachinoides* (only in Folonzo), the increase due to cattle odours was up to 5 folds for both sexes ($p < 0.01$) but was not significant for human odour. A synthetic blend named POCA which consists in a blend of 3-n-propylphenol, 1-octen-3-ol, and 4-methylphenol in a proportion of 1:4:8 in a sachet plus Acetone doubled the catch of males *G.p.gambiensis* from a trap or an electrocuting target ($P < 0.05$). For females, the increases with both POCA-baited traps and targets were significant but slightly smaller (1.6×). By contrast, using the POCA resulted in very significant catches of *G. tachinoides* (x 10 or more folds). Finding effective attractants for these species is particularly important and will improve control efficiency, and contribute to the current PATTEC efforts to monitor and control tsetse in West Africa.

Key words: *G. p. gambiensis*; *G. tachinoides*; attractants; natural hosts; synthetic odours

Introduction

Cost-effective methods of tsetse control exist for the Morsitans group tsetse that spread African Animal Trypanosomosis (AAT). Insecticide-treated cattle, or targets baited with attractants which mimic the odours of natural host, can be deployed at densities of just 4 targets/km² to eliminate population of *G. morsitans* [1]. However, far higher densities of traps or targets (e.g. 30-50/km²) are required to eliminate *G. palpalis* spp. [2]. One reason such high densities of artificial baits are required is that attractants effective against the major vectors of *T. brucei gambiense* in West Africa have not been identified so far.

Studies in Burkina Faso did show that at least one Palpalis group species, *G. tachinoides*, was responsive to host odours [3] and in Liberia, Cheke and Garms [4] found, albeit in a very preliminary study, that *G. p. palpalis* was responsive to some components of cattle odour. To date however, there has not been a comprehensive analysis of the olfactory responses of the Palpalis group species that spread HAT in West Africa, hence the need of further investigations. These studies aimed to assess the responses of *G. tachinoides* and *G.p. gambiense* to (i) whole natural odour from humans and cattle and (ii) synthetic host odours known to be effective against other species of tsetse. Identifying attractants effective for these two species is particularly timely since the African Union is currently initiating a major tsetse control operation in West Africa under the auspices of its Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC).

Materials and methods

Studies sites

For *G. tachinoides* the studies were undertaken along the Comoe river at Folonzo (~09° 54' N, 04 36'W), Fig. 1, in the Comoe province of southern Burkina Faso, in a protected area with a Sudanese gallery forest habitat. The area receives an annual rainfall of ~1100mm. For *G. p. gambiensis*, studies were performed at the same time and sites as for *G. tachinoides*, as the two species occur sympatrically along the southern Comoe river. However the Sudanese type gallery found on the Comoe is

more favourable for *G. tachinoides* which occurs at much higher densities than *G. p. gambiensis*. Additional studies were also conducted at Solenzo (~12°14' N, 04°23' W), in the Banwa province of western Burkina Faso along the Mouhoun river for *G.p. gambiensis*. Climatic conditions are similar to those along the Comoe river, with an annual rainfall of 1000mm. The habitat along the river, is classified as Sudano-Guinean gallery forest but is heavily degraded due to expansion of agricultural fields. Studies took place in the dry seasons between March to June 2007 and January to May 2008.

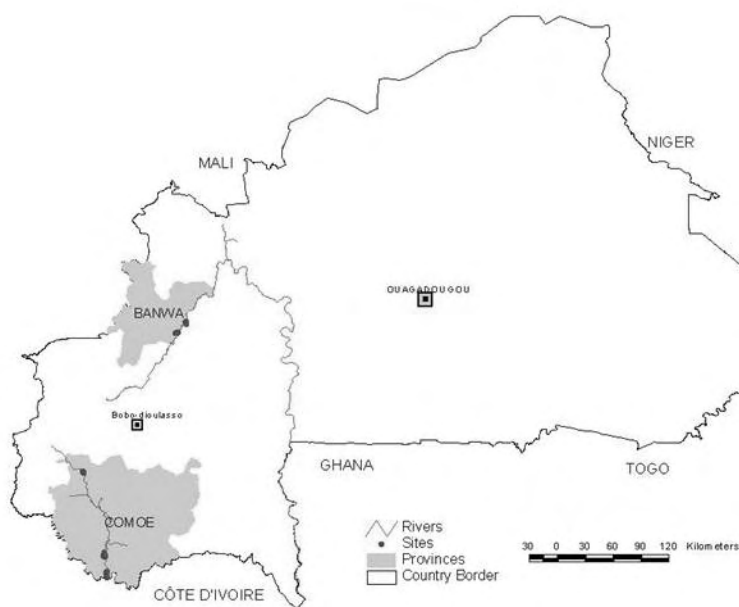


Figure 1. Studies sites

Natural host odours

At each study site, local cattle, or humans were used as sources of natural host odours. The baits were placed in PVC-coated tents (~3 × 2 × 2 m) from which the air was exhausted at ~2000 L/min using a 12 v co-axial fan connected to a flexible PVC-coated tube (0.1 m dia.) with the outlet placed at ground level, ~15 m away from the tent, where the Challier –

Laveissière biconical trap is placed. Studies with Morsitans group flies suggest that the effectiveness of odours from particular host species is related to the gross weight of animals used. Accordingly, to match the weights of different host species, tents normally contained a single ox or three men. Given the approximate weight of the cattle (~150 kg) and humans (~75 kg) used, the gross weight of baits within the tent was 150-200 kg. Experiments run from 8 to 12 am, i.e. for 4 hours

Synthetic host odours

Studies were made of the responses of tsetse to chemicals known to be present in cattle odour and known to attract some species of tsetse. Chemicals were dispensed following the methods of [5,6].

In some experiments chemicals were dispensed individually or as blends, at rates known to be effective for other species of tsetse. For these experiments, the doses of 1-octen-3-ol is ~0.2 mg/h, 4-Methylphenol, ~0.4 mg/h, 3-n-propylphenol, ~0.02 mg/h and acetone, ~500 mg/h. In experiments where 3-methylphenol was used, the release rate was ~0.4 mg/h. POCA is a blend consisted of P= 3-n-propylphenol + O = 1-octen-3-ol + C = 4-methylphenol (in a ratio of 1:4:8) + A = acetone. The dispenser containing 2ml of chemical is hung on the pole of the trap. Treatments are comparing following a Latin square $n \times n$ method, with n the number of treatments.

The experiments were carried out for 24 hours and the tsetse collect is made daily at 8 a.m.

Data analysis

The daily catches (n) were normalized and variances homogenized using a $\log_{10}(n+1)$ transformation and subjected to analysis of variance using GLIM4. To provide a common index of the effect of odours on catches, the detransformed mean catch of tsetse from an odour-baited device was expressed as the proportion of that from an unbaited one. The value is termed the catch index; odours which, say, double or halve the catch from a trap would have catch indices of 2 and 0.5, respectively.

Results

Natural odours

Cattle odour significantly increased the catch of *G. tachinoides* ~5× (Table 1) for both males and females whereas human odour had no significant effect. By contrast, both cattle and human odour significantly

Table 1. Detransformed mean daily catches (transformed mean $\pm$ SED in brackets) of *G. tachinoides* and *G. p. gambiensis* from odour-baited traps expressed as a proportion (Catch index) of that from an unbaited trap. Indices followed by * or ** are significant at the 0.05 or 0.01 levels respectively.

Species	Odour	Tsetse/day			Index	
		Reps	Males	Females	Males	Females
<i>G. tachinoides</i>	Cattle	8	14.9 (1.20 $\pm$ 0.106)	7.8 (0.95 $\pm$ 0.146)	4.8**	5.1**
	Human	12	10.3 (1.05 $\pm$ 0.092)	3.2 (0.62 $\pm$ 0.118)	1.4	1.1
<i>G. p. gambiensis</i>	Cattle	10	2.6 (0.56 $\pm$ 0.098)	3.6 (0.66 $\pm$ 0.083)	2.8*	6.2*
	Human	10	3.2 (0.62 $\pm$ 0.090)	2.3 (0.52 $\pm$ 0.059)	4.4**	2.2**

POCA consistently increased the trap catch of *G. tachinoides*, albeit the increases were not always statistically significant (Table 2). Pooling the results for the 31 replicates where a POCA-baited trap was compared with an unbaited one showed that POCA increased the catch of males four-fold, and the catch of females increased six fold, ($P<0.001$ for difference between means for both sexes). When the numbers of *G. p. gambiensis* caught were sufficient to allow robust statistical comparisons (>3 tsetse/trap/day for an unbaited trap), the blends which gave the best results were POCA and POC (i.e. POCA without acetone) (see Table 3). Pooling the results for the 78 replicates where a POCA-baited trap was compared with an unbaited one showed that POCA increased the catch significantly. The catch increased 2.2× for males, and by 1.8× for females.

Table 2: Detransformed mean daily catch (transformed means $\pm$ SED in brackets) of *G. tachinoides* from traps baited with synthetic host odours. Catch Index is the mean catch of an odour-baited trap expressed as a proportion of that from an unbaited trap. Asterisks indicate that the Catch Index differs from unity at the $P<0.05$ (*) or $P<0.01$ (**) levels of significance

Chemicals					Tsetse/day			Index	
A	O	4M	3nP	3M	Reps.	Males	Females	Males	Females
■	■	■	■	■	12	13.3 (1.16±0.198)	5.5 (0.82±0.209)	2.3	3.0
					12	15.2 (1.21±0.198)	8.8 (0.99±0.198)	2.6	4.8
					8	7.7 (0.94±0.191)	8.0 (0.96±0.191)	5.8*	14.2**
					8	3.8 (0.68±0.194)	5.9 (0.84±0.149)	5.4	7.4**
					3	9.7 (1.03±0.276)	9.8 (1.03±0.161)	16.6	2.8
					12	11.2 (1.16±0.198)	6.6 (0.88±0.198)	1.9	3.6
					8	1.7 (0.44±0.194)	5.6 (0.82±0.149)	2.4	7.0**
					3	5.9 (0.84±0.276)	7.3 (0.92±0.161)	10.1	2.1
					12	26.2 (1.43±0.194)	14.7 (1.20±0.183)	2.8*	3.2
					8	4.0 (0.70±0.194)	6.6 (0.88±0.149)	5.6	8.4**
■					12	21.2 (1.35±0.194)	15.7 (1.22±0.183)	2.2	3.5

Table 3: Detransformed mean daily catch (transformed means±SED in brackets) of *G.p. gambiensis* from traps baited with synthetic host odours. Catch Index is the mean catch of an odour-baited trap expressed as a proportion of that from an unbaited trap. Asterisks indicate that the Catch Index differs from unity at the $P<0.05$ (*) or $P<0.01$ (**) levels of significance

Chemicals				Tsetse/day			Index	
A	O	4M	3nP	Reps.	Males	Females	Males	Females
				8	8.8 (0.99±0.086)	2.5 (0.55±0.128)	4.7***	1.9
				10	5.9 (0.84±0.130)	7.9 (0.95±0.140)	1.9	1.4
				12	1.6 (0.41±0.107)	3.0 (0.60±0.155)	0.9	0.8
				16	6.4 (0.87±0.099)	5.8 (0.78±0.101)	2.9**	1.2
				12	4.8 (0.76±0.154)	9.4 (1.02±0.110)	1.9	2.5*
				20	5.8 (0.84±0.080)	8.9 (0.99±0.067)	2.5***	2.5***
				12	2.4 (0.53±0.107)	2.9 (0.60±0.155)	1.4	0.8
				12	8.5 (0.98±0.154)	8.0 (0.96±0.110)	3.4*	2.2*
				12	1.8 (0.44±0.107)	3.5 (0.66±0.155)	1.1	1.0
				12	1.5 (0.39±0.107)	3.4 (0.65±0.155)	0.9	1.0
				12	1.8 (0.45±0.107)	3.3 (0.63±0.155)	1.1	0.9
				12	1.3 (0.37±0.142)	1.0 (0.31±0.122)	1.3	1.6
				10	4.0 (0.70±0.130)	5.3 (0.80±0.140)	1.3	1.0
				10	3.4 (0.64±0.130)	6.5 (0.88±0.140)	1.1	1.2
				10	5.8 (0.83±0.130)	8.6 (0.98±0.140)	1.9	1.5

The large number of experiments done and the high numbers of flies caught provide firm evidence that *G. tachinoides* showed consistent increases in catch index of around 2× in response to natural cattle odour, confirming the previous findings of Mérot & Filledier [7]. Various combinations of acetone, 1-octen-3-ol, 3-*n*-propylphenol and 4-

methylphenol are used to increase the performance of traps and insecticide-treated targets to monitor and control various Morsitans- and Fusca-group species of tsetse. The results confirm those of earlier studies showing that the POCA blend, originally developed for use against *G. pallidipes* [8] is also effective against *G. tachinoides*. Our data suggests that the incorporation in the blend of 4-methylphenol is about twice as effective as 3-methylphenol. Our results combined with those of earlier studies suggest a blend of POC (i.e. without acetone) may be equally effective, producing increases comparable to natural cattle odour. This point is of practical importance as the large volumes of acetone required makes its use in long running control operations particularly difficult.

Natural odours from both cattle and humans increased the catch of *G. p. gambiensis* from traps but the low densities require very large numbers of replicates for robust statistical analysis to be possible. As a consequence, the absence of statistically robust effects has perhaps led to the erroneous conclusion that *G. palpalis* spp. are unresponsive to host odours. In the present study, experiments conducted at times or places where *G. p. gambiensis* were still low but more abundant than usual did show that baiting traps with natural odours and/or synthetic blends, particularly POCA and POC significantly increased the catches. This is to our knowledge the first published report of improvement in catches using olfactory attractants for this species. Further studies of the responses of *G. palpalis* spp. are clearly needed to confirm these findings and to identify cost-effective doses and blends.

Despite being lower than for Morsitans group flies, the increases in tsetse catches reported here promise improvements for Palpalis group tsetse control with respect to both human and animal trypanosomiasis. There are immediate applications of the use of POCA to improve trapping and control. Indeed, the AU-supported PATTEC program in Burkina Faso has already begun to use this blend for pre-control entomological surveys (I. Sidibe, PATTEC coordinator, Burkina Faso, pers. comm.). It is our intention to investigate in more detail the use of POCA blends and individual compounds to enhance control of Palpalis group flies.

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RESPONSE OF TABANIDS AND MUSCIDS TO NATURAL AND SYNTHETIC HOST ODOURS AT KHARTOUM, THE SUDAN/

REACTIONS DES TABANIDES ET DES MUSCIDES AUX ODEURS D'HOTES NATURELS ET SYNTHETIQUES A KHARTOUM, SOUDAN

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Resumé

Les réactions des tabanidés (Diptera, Tabanidae) et des Muscidés (Diptera, muscidae) aux attractifs naturels et synthétiques ont été étudiées pendant une année entière à partir d'octobre 2007 dans la zone agricole du Complexe industriel de El Yarmouk (15.29°N 32.30°E) situé dans la localité de Jabra, Khartoum, au Soudan. Une association de produits a été testée pour étudier leur effet sur la capacité de capture des pièges. Il s'agissait entre autres de l'urine fraîche et fermentée provenant de chèvres mâles et femelles, de moutons mâles du désert, d'une chamelle, d'un âne mâle, d'un cheval mâle et d'une vache (*Bos taurus*) ; la chamelle, l'âne, du sang, du fumier de bovin, des fleurs de Neem (*Azadirachta indica*) et de Sidir (*Zizyphus vulgaris*). Des attractifs synthétiques comprenant un mélange de 1-octenol-3-ol, 3-n-propylphenol et p-crésol dans les proportions 4:1:8, Octenol (1-octen-3-ol), de l'attrait d'octenol (l'octenolure de Dragonfly®), de l'ammoniaque et de l'acétone ont été également testés au cours de la période d'étude. Des mouches attirées ont été capturées avec des pièges NiTse. Seules des tabanides femelles ont été capturées par les pièges. Des *Atylotus Agrestis*, des *Tabanus sufis* et des *T. taeniola* ont été capturés par les pièges NiTse appâtés et de surveillance. Les *Atylotus agrestis* étaient les espèces les plus nombreuses 73.20%, ensuite les *Tabanus sufis* 6.30% et les *T. taeniola* 20.50%.

Des Muscids comprenant des *stomoxys calcitrans* (7.34%) et des Muscinae non piqueuses (92.66%) ont été capturés également en utilisant les pièges appâtés.

Les pièges appâtés avec de l'octenol et du mélange de phénols ont capturé un nombre de tabanidés sensiblement plus élevé que les autres pièges appâtés à l'aide des autres odeurs. La conclusion tirée est que les pièges NiTse appâtés peuvent être utilisés pour la surveillance des mouches piqueuses rattachées aux exploitations laitières au Soudan.

Summary

Response of tabanids (Diptera: Tabanidae) and Muscids (Diptera: muscidae) to natural and synthetic attractants were studied over one complete year starting in October 2007, at El Yarmouk Industrial Complex farming area (15.29°N 32.30°E) which is located in Jabra locality, Khartoum, Sudan. Combination of substances were tested to study their effect on trap catch including fresh and fermented urine from male and female goats, male desert sheep, a she-camel, male donkey, male horse and cow (*Bos taurus*); the she-camel, donkey, blood, cattle dung, Neem (*Azadirachta indica*) and Sidir flowers (*Zizyphus vulgaris*). Synthetic attractants including a mixture of 1-octenol-3-ol, 3-n-propylphenol and p-cresol in the proportions 4:1:8, Octenol (1-octen-3-ol), octenol lure (The Dragonfly® octenolure), ammonia and acetone were also tested during the study period. Attracted flies were caught by Nzi traps. Only females of tabanids were captured by the traps. *Atylotus agrestis*, *Tabanus suffis* and *T. taeniola* were collected by both the baited and control Nzi traps. *Atylotus agrestis* was the most abundant species 73.2%, *Tabanus suffis* 6.3% and *T. taeniola* 20.5%. The Muscids included *stomoxys calcitrans* (7.3%) and non biting Muscinae (92.66%) were captured also using baited traps. The traps baited with octenol and phenols blend caught significantly more tabanids than did other traps baited with the rest of odours. It is concluded that such baited Nzi traps can be used for controlling biting flies associated with dairy farms in the Sudan.

Introduction

Horseflies (Diptera: Tabanidae) and stable flies (Diptera: Muscidae) are blood-sucking flies that are pests of livestock and wildlife (Foil, 1989). During feeding horseflies transmit to hosts pathogenic viruses (rinderpest), bacteria (haemorrhagic septicaemia), Protozoa

(trypanosomosis) and helminthes (loaiosis) (Foil, 1989). The painful bites of tabanids also influence parameters of fitness in farm animals. These include reduction in: weight gain, milk production, feeding and feed conversion rates, blood, and traction power. Moreover, distraction and irritation by bites predispose animals to accidents and predation (Waage, 1979). Also *Stomoxys* spp. may play role in the mechanical transmission of Trypanosomosis. To attract biting flies and to increase the catch, natural and synthetic attractants were used around the world in the traps. Natural host odours were added in the past such as aged urine (Kremar 2005). Also chemical attractants were used to increase the efficiency of the traps like carbon dioxide (Mohammed-Ahmed and Mihok 1999).

The objective of this experiment was to study the effect of various attractants to use the most efficient one with Nzi traps in the control trial.

Materials and Methods

This study was conducted in 2007 and 2008 at El Yarmouk Industrial Complex farming area (15.29°N 32.30°E) which is located in Jabra Locality, Khartoum, Sudan.

Four Nzi traps made from phthalogen blue and black cotton and white polyester mosquito netting (Mihok, 2002) were deployed in the study site and randomized Latin squares (4X4) were used where the number of sites and days was equal to the number of traps tested (Perry *et al.*, 1980). Twelve experiments were performed and a combination of attractants was tested included natural and synthetic substances. Water as control and (octenol phenols mixture) were used throughout the whole course of study beside two other different attractants on each experiment. The baited traps were set up 500m apart to avoid the intervention between treatments. The flies were collected daily at 11.00 am for identification and calculation.

Natural attractants

Host odours such as fresh and fermented urine, dung, blood and plants were used in the study in different forms. Urine was collected from each of the following groups of animals: male and female Nubian/ Saanin goats, male desert sheep, a she-camel, male donkey, male horse and cows (*Bos taurus*). A plastic container (diameter 6cm; height 11cm) was used to dispense the fresh and aged urine. Cattle dung was tested with Nzi traps. The feces were collected directly from the animal rectum by

using plastic bags. Also Neem (*Azadirachta indica*) and Sidir flowers (*Zizyphus vulgaris*) were collected to use their nectar as odour bait.

Synthetic attractants

Traps baited with a mixture of 1-octenol-3-ol, 3-n-propylphenol and p-cresol in the proportions 4:1:8. The octenol blend was used all over the study due to their potential for attracting biting flies (IAEA, 2003), and to compare it with different olfactory attractants. The combination of octenol and phenols was dispensed in small vials (10ml), with an approximate release rate of 1mg/h. Octenol (1-octen-3-ol), octenol lure (The Dragonfly® octenolure), ammonia and acetone were tested as baits for horse flies. Octenol (1-octen-3-ol) was dispensed in 10-ml vials with a release rate 1mg/h. Octenol lure (a consumer formulation in a wax base) or The Dragonfly® octenolure which each lure contained 3.72 g octenol (1-octen-3-ol). Ammonia or Ammonium hydroxide solution (NH₄OH, 15M, 29.7% NH₃) was tested as attractant. The ammonia bait was delivered in a manner similar to that used by French & Kline (1989) for dispensing 1-octen-3-ol. The release rate for ammonia was calculated to be $1496 \pm 2\text{mg/h}$ at 37°C. Moreover, the traps were baited with Acetone with an evaporation rate 150mg/h. During the study acetone was used also with octenol phenols blend as complete bait, aged horse urine in separate containers and mixed with the aged horse urine in a combination 1:3 (10ml aged horse urine to 30ml acetone).

Results

Atylotus agrestis, *Tabanus sufis* and *T. taeniola* were collected by both the baited and control Nzi traps. *Atylotus agrestis* was the most abundant species 73.2%, *Tabanus sufis* and *T. taeniola* constituted 6.3% and 20.5% respectively. Only females of tabanids were captured by the traps. The traps also caught Muscids included *Stomoxys calcitrans* (7.3%) and non biting Muscinae (92.7%).

Table 1: Ratio of backtransformed mean catches in Nzi traps baited with natural attractants relative to the control.

Treatment	<i>A.agrestis</i>	<i>T.sufis</i>	<i>T.taeniola</i>	Total tabanids	Muscids
(Aged) Male Horse urine.	0.94	NS	0.81	0.93	NS
(Aged) Male Goat urine	1.01	1.77	0.88	1.0	0.87
(Aged) Male Donkey urine.	0.91	0.83	0.93	0.91	1.08
(Fresh) Male Horse urine	1.30	NS	NS	1.50	NS
Fresh) Female Goat urine.	1.62	NS	NS	1.61	NS
(Fresh) Female Cattle urine.	1.38	NS	NS	1.38	NS
(Fresh) Male Sheep urine	0.98	NS	NS	1.01	NS
(Aged) Female Cattle urine	0.99	1.27	1.60	1.07	NS
Sidir flowers	0.84	NS	NS	0.86	NS
Aged) Male Sheep urine	1.39	0.67	1.50	1.32	1.15
Cattle dung	1.09	0.68	1.11	1.07	NS
Neem flowers	1.07	0.70	0.58	0.94	NS
(Aged) Female Cattle urine*	1.35	1.13	1.62	1.39	1.37
(Aged) Male Camel urine	0.67	1.06	2.61	1.03	1.55

* Used for second time. *NS* not significant ($P > 0.05$)

Table 2: Ratio of backtransformed mean catches in Nzi traps baited with synthetic attractants relative to the control

Treatment	<i>A.agrestis</i>	<i>T.sufis</i>	<i>T.taeniola</i>	Total tabanids	Muscids
Octenol lure	1.45	0.96	0.88	1.26	NS
Ammonia	0.91	0.90	0.66	0.84	NS
(acetone+ octenol&phenols)	1.45	2.16	2.07	1.50	NS
Ammonia*	1.07	1.34	1.10	1.07	NS
Acetone	1.20	0.86	1.08	1.18	0.60
Acetone+Horse urine(combination)	1.05	0.70	1.69	1.34	NS
Acetone+Horse urine (separated)	1.19	1.00	1.96	1.55	NS
Octenol	2.46	1.94	1.02	1.93	NS
Octenol*	1.83	1.71	1.40	1.75	NS

*Used for second time. *NS* not significant ($P>.05$).

Table 3: Comparison between control and octenol phenols mixture mean catches

Exp	Control Water				Octenol phenols Mixture			
	Total Tabanids Mean	Total Tabanids Index	Muscids Mean	Muscids Index	Total Tabanids Mean	Total Tabanids Index	Muscids Mean	Muscids Index
1	275.4	1.00	1.9	1.00	607	2.2	2.6	1.32
2	104.0	1.00	11.3	1.00	274.8	2.64	15.3	1.36
3	95.9	1.00	15.1	1.00	280.4	2.92	29.3	1.94
4	2.3	1.00	3.9	1.00	13.5	5.9	4.8	1.24
5	10.2	1.00	4.6	1.00	34.2	3.34	5	1.3
6	69.5	1.00	5.1	1.00	137.4	1.98	7.9	1.54
7	148.6	1.00	1.5	1.00	308.4	2.08	3.8	3.47
8	72.6	1.00	1.0	1.00	206	2.84	2.3	2.35
9	57.0	1.00	0.6	1.00	225.7	3.96	1.1	1.9
10	46.4	1.00	0.5	1.00	180	3.88	1.5	2.91
11	63.9	1.00	2.7	1.00	164.1	2.57	4.5	1.7
12	11.4	1.00	2.5	1.00	37	3.24	5.2	2.08
Mean	79.8	1.00	4.23	1.00	205.71	3.13	6.94	1.93

It is clear that octenol phenols blend increased the catch of total tabanids in all experiments 2-7 times and similar happened in the case of *A. agrestis*. The blend was better than control water in catching *T. suffis* except in tow experiments. The octenol phenols mixture increased the catch of *T. taeniola* significantly more than control except in four experiments (Table 3). Having the twelve experiments the blend caught two times more muscids than the control traps did (Table 3). The mixture of octenol and phenols captured significantly more tabanids than any one of the natural attractants. But the blend significantly attracted *T. taeniola* more than control but not more than aged cattle urine by using Student-Newman-Keuls (SNK) test. The numbers of Muscids attracted to Nzi traps baited with natural odours and control traps didn't differ significantly. The chemicals significantly less effective against tabanids than octenol phenols blend, but they attract more horse flies than control and other traps baited with natural attractants (Table 2). The addition of acetone to octenol mixture didn't increase the flies' numbers experiment C (table 2).

Conclusions

Nzi trap is an effective for collecting tabanids and muscids (Mihok, 2002). In this study the traps caught three species of horse flies; *A. agrestis*, *T. sufis* and *T. taeniola*, but *T. gratus* wasn't showed in the study area. In this study the mixture of 1-octenol-3-ol, 3-n-propylphenol and p-cresol (4:1:8) is the most effective attractant for *A. agrestis* significantly which the most common horse flies in Sudan (Table 1). *T. sufis* are attracted to the octenol and phenols mixture but the numbers of flies were influenced by the low temperatures in the dry cool season in Khartoum area and that may affect the results at that period. The mixture also increased the numbers of *T. taeniola* but aged cow urine caught similar numbers too (Table 1). Kremer (2005) reported that the use of acetone and horse urine separately was more useful than use them as a combination to attract tabanids, but in this experiment significantly there is no difference. Acetone, ammonia and aged cow urine each one was used in two experiments for more confirmation. Overall the mixture was better than control water for attracting muscids while the other attractants were less effective. Acetone may act as repellent for muscids (Table 2). The Nzi traps baited with octenol and phenols in this study are the most efficient trapping method for horse flies and muscids. The control of biting flies with Nzi traps and octenol phenols mixture can be used for dairy farms in the Sudan in the future.

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ENTOMOLOGICAL SURVEYS ALONG THE SUDAN ETHIOPIAN BORDERS/

ENQUETES ENTOMOLOGIQUES LE LONG DES FRONTIERES ENTRE L'ETHIOPIE ET LE SOUDAN

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Résumé

Des études entomologiques ont été effectuées le long des frontières entre le Soudan et l’Ethiopie dans le cadre de la collecte des données de référence requises pour la lutte contre la mouche tsé-tsé autour de la ceinture de mouche tsé-tsé commune au Soudan et en Ethiopie. Les études ont démarré pendant les saisons sèches de 2007 et de 2008 et ont continué pendant la saison sèche de 2009. Elles ont été menées à l’aide de pièges et de patrouilles de capture de mouches. Les éleveurs de la localité ainsi que les communautés locales ont été interviewés au sujet de la présence de la mouche tsé-tsé dans les zones étudiées. La première phase de l’étude a couvert le parc national de Dindir qui borde l’Ethiopie le long de la région située au sud-est du centre du Soudan, le long des fleuves saisonniers de Dindir et de Rahad qui tirent leur source dans la région montagneuse de l’Ethiopie. En outre, deux marécages permanents situés au centre du parc ont été étudiés. Aucune mouche tsé-tsé n’a été capturée dans les endroits étudiés. La deuxième phase de l’étude concernait la province de Qissan qui comprend la zone du fleuve saisonnier de Tumad, kshankaro, Agro, Khor Dahab, Balwasho et Khartoum Billail. Aucune mouche tsé-tsé n’a été capturée dans ces endroits. La troisième étape de l’étude portait sur la province de Kurmuk où la plupart des localités sont limitrophes de l’Ethiopie. Les études réalisées dans ces endroits ont indiqué la présence de mouches tsé-tsé.

Des *Glossina fuscipes fuscipes* ont été détectés dans les ruisseaux saisonniers de Lili, d'Oos, du Muguf et d'Ora. Le fleuve Khor Yabus était également infesté de *Glossina fuscipes de fuscipes*, alors que des *Glossina morsitans Submorsitans* étaient détectés dans la région de Khor Jordan. Les études le long des frontières se poursuivent.

Summary

Entomological surveys were carried out along the Sudan/Ethiopian borders as part of the baseline data required for tsetse control at the common tsetse belt of Sudan and Ethiopia. The Surveys started during the dry seasons of 2007, 2008 and continued during the dry season of 2009. The surveys were conducted using traps and fly round patrols. Pastoralists in the area and the local communities were interviewed about the presence of tsetse in the areas surveyed. The first part of the survey covered the Dindir National park which borders Ethiopia along the south-eastern part of central Sudan, along Dindir and Rahad seasonal rivers which originate from the Ethiopian High lands. Two permanent swamps in the centre of the park were also surveyed. No tsetse was caught in all areas surveyed. The second phase of the survey was carried out in Qissan Province which includes the area of Tumad seasonal river, kshankaro, Agro, Khor Dahab, Balwasho and Khartoum Billail. Again, no tsetse was caught in those areas. The third phase of the survey was carried out in Kurmuk Province which mostly borders Ethiopia. Here, the surveyed areas were found to be tsetse infested. The seasonal streams of Lili, Oos, Muguf and Ora were found infested with *Glossina fuscipes fuscipes*. Khor Yabus River was also found infested with *Glossina fuscipes fuscipes*, while *Glossina morsitans Submorsitans* was found in Khor Jordan Area. The surveys along the borders are still going on.

Introduction:

The major review of tsetse fly problem in the Sudan was made by Lewis (1949) and also updated by Lewis (1950). Previous surveys of the Blue Nile area confirmed the presence of *Glossina fuscipes fuscipes* and *G.m.submorsitans* in Khor Yabus area, Blue Nile State, Mohamed Ahmed (1989) and Kheir *et al.* (1995). Considering the impact of tsetse & trypanosomosis on livestock in the bordering administrative regions of Sudan and Ethiopia, the Governments of Ethiopia and Sudan are taking appropriate steps for a joint tsetse & trypanosomosis (T & T) intervention and removal of the T&T from these areas. In the Sudan, this area

supports about 3 million head of cattle and four million head of sheep and goats, in the dry season. During their stay in this area the animals contacted the tsetse flies and contracted the disease which resulted in heavy losses. During the rainy season these animals move to the Blue Nile and the Gezeira agricultural schemes where they mix with the resident cattle in these localities. These migratory herds are the source of trypanosomosis infection to all cattle kept in the agricultural projects including dairy farms in Gezeira and Khartoum States.

Material and Methods

The following tsetse sampling methods were used during the entomological surveys.

- 1. Standard fly rounds by screen patrol, Hall *et al* (1984).
- 2. Biconical tsetse traps
- 3. NZI traps

Areas surveyed

1. Dindir National Park (Area 1, map of Sudan):

The park borders Ethiopia in the south eastern part of central Sudan. It lies following Rahad River at lat. 12° 55' N and Long. 35° 02' East and it continues in a north western direction up to Lat. 12° 42' N and Long. 34° 48' E at Dindir River. Then it continues up to Lat. 12° 32' N and Long. 34° 32' E. along Khor Kenana, then it diverts slowly towards the Sudan Ethiopian borders at Lat. 11° 55' N and Long. 34° 44' E (Wildlife Res. Centre, 2001)

2. Qissan Province (Leg 2, Map of Sudan)

3. Krmuk Province (Leg 3, Map of Sudan)

Results

The results indicated that the only tsetse infested Province is Kurmuk (leg 3) and the dominant species was *G. f. fuscipes* while *G. m. submorsitans* was found in low densities in limited patches of the woodland (Map2). Dindir national park is tsetse free though the densities of tabanids were very high especially around the large water bodies. The Dominant tabanids were *Tabanus taeniola* and *Atylotus* species. Qissan Province (Leg 2) was also tsetse free. Tabanids, mainly *Tabanus taeniola*

and *Atylotus spp.* *Tabanus bigutatus* and *Philoliche magretti* were caught in high densities.

Table 1: Entomological Surveys Results in Dindir National Park, Qissan and Kurmuk Provinces, Blue Nile State, Sudan

Leg	<i>G.m. Submorsitans</i>	<i>G.f.fuscipes</i> (Fly/Trap/Day)	Tabanids (Fly/Trap/Day)
Leg1 (Dindir Park)			
1/Karsh Al Feel	0.0	0.0	2.0
3/Galago	0.0	0.0	10
2/ Ras Ameer	0.0	0.0	454
3/Galago	0.0	0.0	110
4/El Abeyad	0.0	0.0	

Leg 2 (Qissan)			Not Done
1/Tumad seasonal river,	0.0	0.0	
-	-	-	
2/ Kshankaro, 3Agro	0.0	0.0	
4/ Khor Dahab,	0.0	0.0	
5/Balwasho,	0.0	0.0	
6/Khartoum Billail	0.0	0.0	
	0.0	0.0	
Leg3 (Kurmuk)			
1/ Khor Yabus	0.0	35	
2/ Lili	0.0	0.0	
3/ Oos	0.0	18	
4/ Muguf	2	0.0	
5/Ora	0.0	11	

Discussion:

The results of the present surveys indicated that the tsetse distribution in Khor Yabus locality is the same as it was reported before (Mohammed-Ahmed *et al* 1989 and *Kheir et al*, 1984). *G. f. fuscipes* is found in high

densities as the residents of this area were displaced due to war and most of their farming areas were abandoned and became thick gallery forests. *G. morsitans submorsitans* were found in low densities which may reflect their poor response to trapping. Qissan Province was surveyed extensively and in two consecutive seasons. The results of the surveys indicated that the province is tsetse free which confirms the finding of Kheir *et al* (1995). Reports from the Ethiopian teams indicated that the neighboring Benishangol/Gomoz Governorate is tsetse infested (Rahman, personal communications). Therefore joint tsetse surveys by the Sudanese and the Ethiopian teams are required to determine the tsetse limits in these areas.

Old records suspected the galleries of Rahad and Dindir seasonal rivers to be tsetse infested particularly at their upper parts in the Sudan/Ethiopian borders. The results indicated that these rivers and their tributaries are tsetse free. The tabanids were found in very high densities in all the areas surveyed in the park due to the presence of many permanent swamps which constitute suitable breeding sites of tabanids.

The Blue Nile Tsetse belt, (the project area), supports during the dry season 3 million heads of cattle and 4 million heads of sheep and goats. When these herds move to their original places they transmit the disease to the resident animals far away from the tsetse belt (Rahman 2005). These migratory herds are the source of trypanosomosis infection to all cattle kept in the agricultural projects including dairy farms in Gezeira and Khartoum. The results of the tsetse infestations along the Sudan Ethiopian borders indicated that the real infested areas are by far less than the predicted ones. This makes the eradication of this isolated belt feasible.

Conclusions

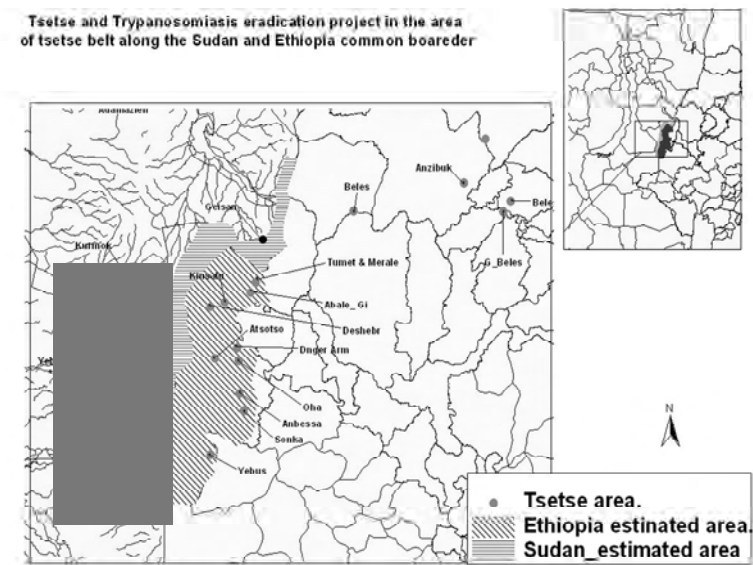
1. The results of this study indicated the presence of two species of tsetse flies, *G.f.fuscipes* and *G. morsitans submorsitans* in the Blue Nile tsetse belt.
2. The results were in line with the previous findings although the densities of the tsetse infestations are very high.
3. The total area infested is isolated and by far less than the predicted tsetse infested areas.
4. The eradication of this belt is possible and feasible.

Recommendations

- 1. The immediate start of the tsetse control operations
- 2. The use of traps and insecticides baited animal could be suitable methods of control.

Acknowledgment

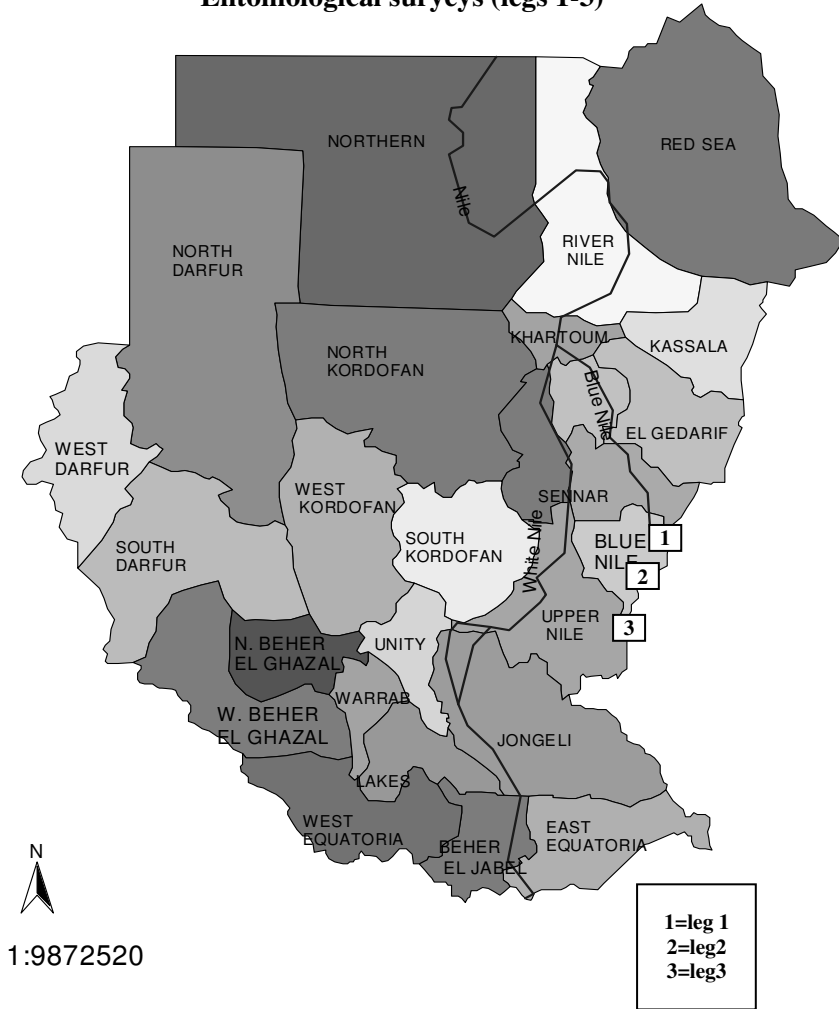
The financial support of the Director of the Central Veterinary Research Laboratories and the Director of Animal Resources Research Corporation is acknowledged.



Actual Infested area

Sudan States

Entomological surveys (legs 1-3)



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**SOCIO-ECONOMICS/
SOCIO - ECONOMIE**

DO SOCIAL NETWORKS INFLUENCE LIVESTOCK KEEPERS' KNOW-HOW ON ANIMAL TRYPANOSOMOSIS AND ITS CONTROL? /

LES RESEAUX SOCIAUX INFLUENCENT-ILS LE SAVOIR-FAIRE DES ELEVEURS SUR LA TRYPANOSOMOSE ANIMALE ET SON CONTROLE,

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Résumé

L'analyse des réseaux sociaux est devenue une question clé dans l'organisation communautaire et la littérature croissante commence à montrer de plus en plus l'importance des réseaux sociaux dans le développement économique et rural. Nous supposons que les différents agents pourraient établir un réseau pour échanger des informations et de cette façon renforcer leurs propres connaissances en matière de santé et de production animales. Le présent travail étudie l'impact des réseaux sociaux sur les connaissances des éleveurs par rapport à la trypanosomose animale et sa surveillance dans la zone de Solenzo, au Burkina Faso. Une enquête sur les connaissances, attitudes et pratiques (CAP) et une analyse des réseaux sociaux ont été effectuées dans deux villages où un total de 156 (54 à Montionkuy et 102 à Sanakuy) éleveurs issus des deux villages étaient concernés. L'analyse descriptive et un modèle de régression linéaire ont été utilisés pour l'analyse des données. Les résultats ont montré que la force d'un éleveur à capter toutes les informations qui circulent dans le réseau mesurée par son degré de centralité est négativement associée à sa connaissance sur la trypanosomose animale et son contrôle. Par contre la connexion stratégique d'un éleveur à une personne marginale mais exceptionnelle de

la communauté et qui dispose de connaissance spécifique, cette connection stratégique qui est mesurée par le degré d'intermédiation est positivement associée au niveau de connaissance.

Summary

The analysis of social networks has become a key issue in community organisation and a growing literature has started to show the importance of social networks in economic and rural development. We assume that individual agents could establish a network to exchange information and in this way increase their own knowledge in animal health and livestock production. The present paper investigates the impact of social networks on cattle farmers' knowledge on animal trypanosomosis and its control in Solenzo in Burkina Faso. A knowledge, attitude and practices (KAP) survey and a social network analysis were conducted in two villages where all 156 (54 in Montionkuy and 102 in Sanakuy) cattle farmers in both villages were involved. Descriptive analysis and a linear regression model were used to analyze the data. Results showed that the power of a cattle farmer for catching whatever is flowing through the network as information, measured as his degree centrality, is negatively associated with knowledge on animal trypanosomosis and its control. However, a person's strategic connection to the most marginal people, but exceptional in a specific knowledge in a community — a concept better reflected by a person's betweenness centrality — is positively associated with knowledge.

Introduction

Knowledge and information are elements that propel an increase in agricultural productivity and rural incomes (FAO, 1995). Farmers' capacity to control their livestock production environment is the result of the resources at their disposal; among these, knowledge and skills are key components. Indeed, the evolution of farming is influenced by the information that flows into the system. It is generally assumed that the knowledge and know-how available in a poor rural population is insufficient to be of much use in their training and development (Cabero and van Immerzeel 2007). This line of thought is understandable, as very few people within the population possess exceptional knowledge and know-how. According to van Immerzeel (2006), the knowledge and know-how of the rural population can be represented by the bell-curve of a normal distribution in a graph as shown in Figure 1.

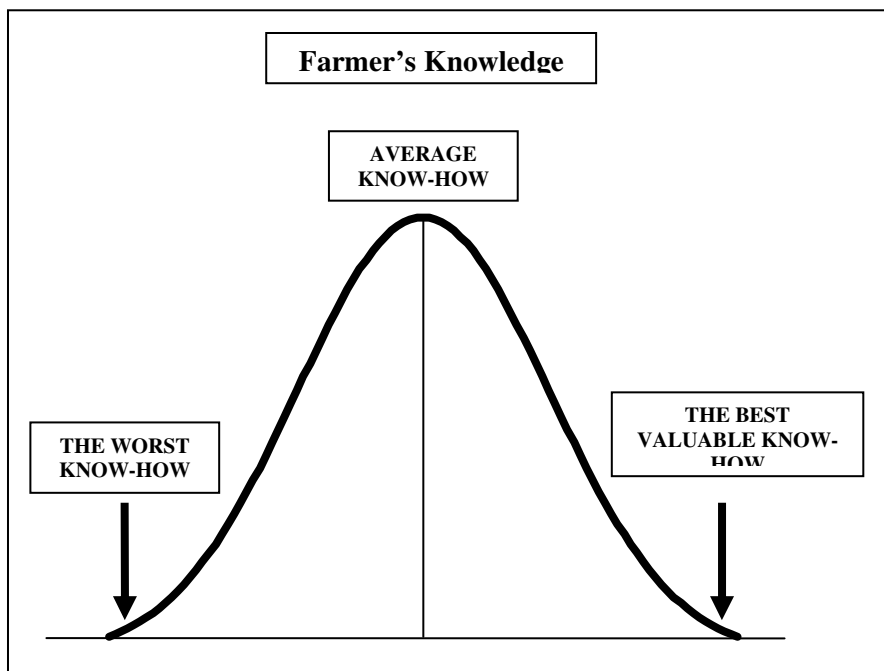


Figure 1: Normal distribution of knowledge in a large rural population
Source: Modified from Van Immerzeel (2006)

The bell-curve in Figure 1 is not static; people can learn from the “best” and the whole bell shaped curve can move towards the right. Shifting the curve to the right requires the contribution of those exceptional people that are on the extreme right of the curve, socializing their knowledge and know-how. Learning from the best implies the fact that knowledge is present in those people who know how. However, the great challenge is to find them and achieve a situation in which others can learn from them and with them. Knowledge needs to flow from the “best” to the rest of the community and one way to achieve this is through farmers’ social networks. Although there is increasing emphasis on farmer-led extension in rural development and the power of the word-of-mouth for the spread of knowledge and information (Oiana and Rasul, 2006), very few studies have been done at farmers’ level to understand the social processes involved and the impact a social network has on farmers’ knowledge on

a specific topic. The objective of the paper was to explore the relationship between a cattle farmer position in a community and his level of knowledge on animal trypanosomosis and its control in the commune of Solenzo in Burkina Faso.

Methodology

Study area and sampling

The study was carried out in the commune of Solenzo in the province of Banwa located in the North-West of Burkina Faso. Among the rural activities, rain fed agriculture is the most important, followed by livestock keeping. The main food crops are sorghum, maize, millet, cowpea and rice, while cotton is the major cash crop. The commune of Solenzo contains 2500 livestock keepers spread over 29 villages, and the majority of the households own oxen for animal traction, especially for cotton production. Four villages (Montionkuy, Sanakuy, Masso and Gnassoumadougou) were selected randomly from the 29 villages of the commune and all the cattle farmers in the four villages were included in the study in order to capture the whole picture of the social network in each village.

Data collection

We started the study by conducting a knowledge, attitude and practices (KAP) survey in the four randomly selected villages using a questionnaire. The questionnaire covered different aspects of trypanosomosis disease and its control: the cause, the signs of the disease, the prevention, and the treatment of the disease. A knowledge score was developed as a percentage of the total knowledge score. We then conducted a social network analysis in the four villages. Social network analysis is a diagnostic method for collecting and analyzing data on patterns of relationships among people and organizations (Wasserman and Faust, 1994; Scott, 1992). To understand how a social network is associated with farmers' knowledge, we constructed a social network at cattle farmers' level. Each node in the network represents a cattle farmer and each link between two nodes represents the exchange of information, including information about trypanosomosis and its control, between the two people. The information exchange can be in one or both directions. The software application Visualizer was used to derive the characteristics (the position in the community) of each node in the network.

Results

Cattle farmers' characteristics

All cattle farmers in the survey were male and are spread among eight different ethnic groups. Table 1 shows the repartition of cattle farmers in the study. Karaboro, Kado and Bozo represent less than 1% of the study population and were only found in Gnassoumadougou. The Samo which, account for almost 22%, were only represented in Sanakuy and Gnassoumadougou. The ethnic group Bobo was less represented in Masso and Sanakuy, while the Dafing were less represented in Sanakuy. However, Dafing and Mossi represent almost 50% of the study population. The Peulh or Fulani, known as livestock keepers, represent only 11% of the study population.

Table 1: Repartition of cattle farmers per ethnic groups per village

Villages	Ethnic groups						Total
	Bob o	Dafin g	Moss i	Peul h	Sam o	Other *	
Montionkuy	12	24	5	13	0	0	54
Sanakuy	5	1	22	17	62	0	107
Masso	1	62	64	7	0	0	134
Gnassoumadoug ou	53	19	16	11	32	4	135
Total	71	106	107	48	94	4	430

* Other include Karaboro, Kado and Bozo

Results from KAP survey in Solenzo, Burkina Faso, January to march 2009

The ages of cattle farmers ranged from 21 to 88 with an average of 47.5 (± 13.6) and the analysis of variance showed significant difference between villages ($p < 0.05$) (Table 2). Cattle farmers in the study owned an average of 13.3 (± 13.6) cattle per household, with a range of 1 to 200. There was significant difference ($p < 0.05$) between the average

herd size in Montionkuy compared to other villages (Table 2). Most cattle farmers (95.3%) owned oxen and there was no significant difference between villages for the average number of oxen. While only 8.8% of the study population participated in formal education, more than half (51.6%) attended Koran study. The average number of years of Koran study was 2.7 ($\pm$ 4.2) and there was significant difference ($p < 0.05$) between villages.

Table 2: Age of cattle farmers, herd size, number of oxen and number of years of Koran study

	Montionkuy (n = 54)	Sanakuy (n = 107)	Masso (n = 134)	Gnassoumadougou (n = 135)
Age (years) of cattle farmers	47.7 ^{ab}	44.6 ^a	47.6 ^a	49.7 ^b
Herd size (number of cattle)	25.0 ^b	10.9 ^a	11.6 ^a	12.1 ^a
Number of oxen	2.9 ^a	3.1 ^a	3.4 ^a	2.7 ^a
Number of years of Koran study	1.5 ^a	3.1 ^b	1.6 ^a	3.8 ^b

Values in the same row carrying different superscripts are significantly different ($p < 0.05$) Results from KAP survey in Solenzo, Burkina Faso, January to march 2009

Cattle farmers' knowledge of animal trypanosomosis and its control

Knowledge of the cause and signs of animal trypanosomosis

Nearly all cattle farmers (98.6%) said they were able to recognize the disease. However, only 26% of cattle farmers in the study knew that animal trypanosomosis followed from bites by tsetse fly or other biting insects, and there were significant differences between villages. Also

only a minority (7.2%) was able to recognize tsetse fly. Most cattle farmers (65%) in Montionkuy knew the cause of the disease, while in Masso and Gnassoumadougou few of them (14% and 10% respectively) knew that animal trypanosomosis followed from bites by insects. The proportion of cattle farmers in Montionkuy and Sanakuy that was able to recognize tsetse fly was significantly higher compared to Masso and Gnassoumadougou but there was no significant difference between Montionkuy and Sanakuy (Table 3).

Table 3: Percentage (%) of cattle farmers knowing the cause of the disease and able to recognize tsetse fly

	Montionkuy (n = 54)	Sanakuy (n = 107)	Masso (n = 134)	Gnassoumadougou (n = 135)
Knowing the cause of the disease	64.8 ^a	42.1 ^b	14.2 ^c	9.6 ^c
Recognizing tsetse fly	20.4 ^a	11.2 ^a	4.5 ^b	1.5 ^b

Values in the same row carrying different superscripts are significantly different ($p < 0.05$)

Results from KAP survey in Solenzo, Burkina Faso, January to March 2009

Cattle farmers cited 21 signs. However, only five signs were considered in the computation of the knowledge score: three signs that are always present (emaciation, enlarged lymph node and fever) and two signs that are important but not always present (pica and lacrimation). Cattle farmers reported knowing an average of 2.2 of those five signs and most (98%) were able to cite at least one sign. Most (87.7%) of cattle farmers were able to cite at least three important signs of the disease but considerably fewer (1.2%) were able to cite the five signs considered in the study for the computation of the knowledge score.

Knowledge of animal trypanosomosis prevention and control

Cattle farmers in the study cited in total 14 different strategies for the prevention of animal trypanosomosis. The strategies cited can be categorized as follows: modern medicines, traditional medicines, good husbandry, nutritional complements, avoidance of tsetse fly and control of tsetse fly by using insecticides.

Treatment methods known by cattle farmers in the study consisted of the use of modern or traditional medicines. Most cattle farmers (93.7%) used trypanocidal drugs: isometamidium chloride (ISMM) and diminazene aceturate (DIM). Only 13.5% used traditional medicine to treat the disease. Many cattle farmers (67.2%) believed that non-trypanocidal drugs (anthelmintics, vaccines and antibiotics) can also be used to treat animal trypanosomosis. Table 4 shows that there was no difference between the proportions of cattle farmers who used modern medicines (ISMM and DIM) to treat the disease in the four villages respectively. However, the proportion of livestock keepers in Montionkuy who believed that non-trypanocidal drugs can be used to treat animal trypanosomosis was significantly less than in the other three villages.

Table 4: Percentage (%) of cattle farmers using modern medicines for treatment per village

	Montionkuy (n = 54)	Sanakuy (n = 107)	Masso (n = 134)	Gnassoumadougou (n = 135)
Isometamidium chloride	3.7 ^a	3.7 ^a	1.5 ^a	3.0 ^a
Diminazene aceturate	94.4 ^a	91.6 ^a	94.0 ^a	88.1 ^a
Non- trypanocidal drugs	42.6 ^a	63.5 ^b	74.6 ^b	74.8 ^b

Values in the same row carrying different superscripts are significantly different ($p < 0.05$)

Results from KAP survey in Solenzo, Burkina Faso, January to march 2009

Impact of social networks on knowledge

We first compared cattle farmers' knowledge measured as a percentage of the total knowledge score of the villages. Table 5 shows that cattle farmers in Montionkuy know more about the disease and its control than their neighbors in other villages. However, there was no significant difference between the knowledge of cattle farmers in Sanakuy, Masso and Gnassoumadougou.

Table 5: Average knowledge score (% of total knowledge point) per village

	Montionkuy (n = 54)	Sanakuy (n = 107)	Masso (n = 134)	Gnassoumadougou (n = 135)
Knowledge score	31.8 ^a (0.8)	23.9 ^b (1.1)	24.0 ^b (0.7)	23.2 ^b (0.6)

Values in the same row carrying different superscripts are significantly different ($p < 0.05$). Values in brackets are standard errors

Results from KAP survey and Social Network Analysis in Solenzo, Burkina Faso, January to march 2009

Empirical model and estimation procedure

We employed a linear regression model to explore the relationship between a cattle farmer's position in a community and the level of knowledge on animal trypanosomosis and its control. The dependent variable is knowledge score; two individual network characteristics were used in the regression analysis as explanatory variables: the degree centrality and the betweenness centrality. Degree centrality is defined as the number of ties that a node has. Degree is often interpreted in terms of the immediate power of a node for catching whatever is flowing through the network as information (Hawe and Ghali, 2008). If the network is directed (meaning that ties have direction) then we define two separate measures of degree centrality, namely in-degree and out-degree. In-degree is a count of the number of ties directed to the node, and out-degree is the number of ties that the node directs to others. In this study we used the degree centrality, which is the sum of in-degree and out-degree ties. Betweenness centrality is a measure based on the idea that an actor who lies on the paths connecting many other actors exerts control

over the flow of information and may be in a position to filter and acquire the best information or know-how. We assume that individual actors could establish a network to exchange information and in this way increase their own knowledge in animal health and livestock production. However, being nominated by lots of others as a person to turn to for information, a concept reflected in a person's degree centrality, is not always the best guide in selecting the best and valuable knowledge champion. Indeed, a person's strategic connection to the most marginal people, but exceptional in a specific knowledge in a community, could be the most important criterion for socializing the best know-how — a concept better reflected by a person's betweenness centrality score (Hawe and Ghali, 2008).

The model to be estimated is represented by the following equation:

Knowledge score_i = α + β_1 Degree centrality_i + β_2 Betweenness centrality_i +

$$\sum_{k=3}^n \beta_k X_k + \varepsilon$$

Where α is the intercept, X_k represents a vector of individual cattle farmer characteristics apart from network characteristics, β_1 , β_2 , and β_k represent coefficients to be estimated and ε the error term. We expect the coefficient of betweenness centrality to be positive, while the coefficient of degree centrality may be either positive or negative.

Statistical methods for estimating population parameters and their associated variances are based on assumptions about the characteristics and underlying distribution of the observations. Among these assumptions are that the observations were selected independently and that each observation had the same probability of being selected. Data collected through surveys often have sampling schemes that deviate from these assumptions. For our study, cattle farmers are clustered geographically within villages and each observation had a different probability of being in the total sample. Hence, to estimate unbiased parameters and to compute an approximation of the true variance of estimates, we used the Taylor series linearization technique (Carlson et al., 1993; Rust, 1985; Kish and Frankel, 1974).

Estimation results

Results of the model in Table 5 show that being alphabetized, or having cattle farming as first activity; has a positive but not significant impact on cattle farmers' knowledge of trypanosomosis and its control. However, the age of a cattle farmer is negatively associated with the knowledge, meaning that young cattle farmers know more about the disease and its control. Receiving information about the disease from technical services, and the number of years of Koran study, are both significantly and positively associated with knowledge of trypanosomosis and its control. Cattle farmers who exchanged knowledge on the disease and its control acquired better knowledge. The number of cattle owned and the number of oxen in the herd are also significantly and positively associated with cattle farmers' knowledge. The model shows that the power of a cattle farmer for catching whatever is flowing through the network as information, measured as his degree centrality, does not have a positive association with knowledge on animal trypanosomosis and its control. As expected, betweenness centrality is significantly and positively associated with knowledge.

Table 6: Results of the empirical model

Knowledge score	Coefficients	Linearized Std. Err.
Being alphabetized	1.49	1.039
Having cattle farming as first activity	0.27	1.584
Exchange knowledge on trypanosomosis	1.85*	0.975
Receiving information on trypanosomosis from technical services	3.25**	1.536
Betweenness centrality	0.01***	0.001
Degree centrality	- 0.02***	0.004
Age of cattle farmer	- 0.05*	0.029
Number of years of Koran study	0.23**	0.089
Number of oxen owned	0.35***	0.074
Cattle herd size	0.03**	0.016
Constant	26.42***	1.659
N = 414		
F(10, 401) = 13.07		
R-squared = 0.1761		

* = $p < 0.1$, ** = $p < 0.05$, *** = $p < 0.001$

Results from KAP survey and Social Network Analysis in Solenzo, Burkina Faso, January to March 2009

Conclusion

This study shows that social networks are key components of human resource development. Social networks play an important role in helping cattle farmers to access valuable information through their contacts, and to improve their knowledge on animal trypanosomosis and its control. However, the study shows that what is important in the cattle farmer's position in the social network is not the degree centrality measured as the number of people an individual cattle farmer is linked to in terms of information exchange. Instead, it is the ability of farmers to detect marginal people within the population who possess exceptional knowledge and know-how. Cattle farmers who establish strategic relationships with these people know more about animal trypanosomosis and its control.

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LIVELIHOOD STRATEGIES IN ENDEMIC LIVESTOCK PRODUCTION SYSTEMS: TRENDS, TRADEOFFS AND IMPLICATIONS^{1/}

LES STRATEGIES DE SURVIE DANS L'ELEVAGE DES RACES ENDEMIQUES BABE SUR LES SYSTEMES DE PRODUCTION: TENDANCES, OPTIONS ET IMPLICATIONS

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Résumé

Les moyens de subsistance en Afrique de l'Ouest dépendent en grande partie de l'élevage. Les zones sub-humides et humides de la région sont toutefois fortement infestées par les mouches tsé-tsé, vecteur de la trypanosomose qui affecte aussi bien les êtres humains ainsi que leur bétail et leurs moyens de subsistance. Les races endémiques de bétail sont trypanotolérantes, mais leurs populations sont en baisse suite aux croisements accrus avec les zébus et les moutons du Sahel et de la dégradation de l'habitat à cause de la déforestation et des feux de brousse. Ces tendances laissent supposer des options entre les moyens de subsistance (accouplement croisé et culture du coton) et la conservation de l'écosystème (les ressources génétiques des ruminants endémiques et leur habitat). Toutefois, en mettant au point des stratégies appropriées favorables aux populations pauvres pour réduire au minimum de telles options, nous devons mieux comprendre les facteurs socioéconomiques dictant les choix de races au sein des ménages agricoles et des communautés ainsi que les contraintes et limites liées aux races endémiques telles que perçues par les communautés et les institutions influençant les décisions au niveau de la sous-région. L'étude se fonde

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sur l'évaluation rurale participative dans les communautés choisies pour examiner les tendances, les options et les implications actuelles des stratégies observées en matière de reproduction et de gestion des ressources naturelles en Afrique de l'Ouest. L'étude vise spécifiquement à: (1) caractériser les moyens de subsistance ou conditions de vie des populations vivant dans les zones d'étude, (2) évaluer la contribution et la valeur perçue des ruminants endémiques aux ménages agricoles et d'éleveurs, (3) identifier les principaux facteurs socioéconomiques qui dictent les choix des races, (4) déterminer les contraintes qui entravent le développement de la reproduction du bétail en général et du bétail endémique en particulier, et (5) comprendre les options et les impacts possibles des ressources naturelles alternatives et des initiatives de reproduction sur les tendances des moyens de subsistance dans la région couverte par l'étude.

Summary

Rural livelihoods in West Africa depend largely on livestock. The sub-humid and humid zones of the region however are highly affected by the tsetse flies, vector of trypanosomosis, which affects humans and their livestock. Endemic ruminant livestock breeds are trypanotolerant, however their relative population is decreasing as a result of both increased crossbreeding largely with zebu cattle and Sahelian sheep/goats, and degraded habitat due to forest conversion and increased cropping activities and bushfires. As habitat degradation drives crossbreeding, livelihoods of local communities are affected as well. To assess the tradeoffs between livelihood strategies and endemic ruminant and habitat conservation, we need a better understanding of the socio-economic conditions and factors driving breed choices within households and communities. Livelihood analysis is an important step in understanding varying responses to development projects and to ensure that the poorest categories are not excluded from development interventions.

Key words: Endemic ruminants, Habitat, livelihoods, tradeoffs

Introduction

Livestock contribute significantly to livelihoods of rural populations in West Africa. A significant area of the region (humid and sub-humid zones) however is highly infested by the tsetse flies, vector of trypanosomosis, which affects humans as well as their livestock and livelihoods. Besides indirect impacts, in terms of productivity deterioration, the disease engenders losses in meat and milk that amount to \$1.3 billion per year in Africa (Kamunaga, 2004). Being the major constraint to socio-economic development of infested areas (Kristjanson et al. 1999), trypanosomosis disease is also an important factor shaping livelihood strategies in these regions. Efforts to control the disease such as using trypanocides to treat infected animals or clearing tsetse habitats, although successful for some time, often didn't reach the expected success. In addition to the uncertain efficacy of such practices, they engender high costs both financially and ecologically, the latter because they contribute to the pollution and degradation of the environment.

Endemic ruminants, such as Ndama cattle, Djallonké breeds of sheep and West African Dwarf goats, are sought as an alternative to overcome the trypanosomosis problem (Agyemang et al, 1991). They are well adapted and productive in tsetse infested areas, tolerant to heat and resistant/resilient to helminths ticks and tick-borne diseases (Grace, 2005). They also have low nutritional and husbandry requirements, which along the previous features could be thought of as being preferred pro-poor options.

Endemic breeds, despite their multiples attributes, are often perceived as inferior in term of productivity and marketing (Agyemang, 2005). But, real threats to endemic livestock arise from observed trends of degradation and destruction of their natural habitat. Moreover, the rising crop cultivation and migration following drought seasons have increased the inclusion of zebu and sahelian breeds for crossbreeding, seen as more productive when they can be supported (tsetse treatment and additional inputs available). These changes are not without impacts on livelihoods strategies.

A number of research projects have addressed different dimensions of endemic ruminants. A BMZ/GTZ funded project (ILRI, 2007), examined the use of trypanocides as a component of integrated control of

trypanosomosis as part of a strategy to reduce the risk of trypanocide resistance in the cotton zone of West Africa. The International Trypanosomosis Centre (ITC) which operated in the region for many years has also made significant contributions to scientific research on endemic ruminants with a particular focus on N'dama cattle breeding. Other projects in the region have looked into ways to improve farming systems, crop-livestock integration, and natural resources management. These projects, however, at least in their implementation, remain sectoral (improvement of the cultivation methods, crop-livestock integration) and limited to the biophysical aspects and practices (IER-Mali, 2008). Our aim is to build on the interactions between the three components of the system: livelihoods of livestock keepers, development activities and the ecosystem.

This paper uses Participatory Rural Appraisal in selected communities in the Gambia to understand the linkages between livelihoods, livestock production objectives and habitat quality. The paper attempts to ascertain the potential tradeoffs and implications based on the observed trends. Specific objectives of this paper are to:

- Characterise the livelihood of the people living in the study sites
- Assess the contribution and the perceived value of endemic ruminants to farm and herding households.
- Characterise the Habitat in which the endemic breeds live;
- Understand the trends in livelihoods, habitat and breed composition, and the tradeoffs between ecosystem and breeding conservation and livelihood strategies in the study region.

Conceptual framework and model

According to the sustainable livelihoods framework, household livelihood strategies—defined as the activities that they engage in to make a living—are a function of the assets that the household has access to, given the broader socio-political and agro-ecological context. Household assets, or capitals, can be divided into five categories: human, social, physical, natural and financial (DfID, 2001 in Adato et al., 2007).

Human capital consists of the quantity and quality—increased by education, reduced by ill health—of labor that a household has available. Social capital is defined as the relationships, networks and the trust to which a household has access. Social capital can influence livelihood

strategy by providing information or access to market or other shared resources, or reducing transactions costs. Physical capital consists of tools and machinery, and financial capital includes both savings as well as access to credit. Natural capital is generally defined to include land and other resources such as forests, water or wildlife. Natural capital can be owned outright or can be accessed through different type of use right regime. Livestock do not fit easily into one asset category; they can be considered natural, physical, or financial depending on which aspect is being considered.

Different livelihood strategies require different endowments of the different assets. Household asset endowments, along with their preferences, determine the strategies in which they can engage. According to microeconomic theory, households choose strategies and decide how to allocate their resources across them in order to maximize utility subject to prices, technology, and full income and credit constraints.

On the basis of the returns it expects to obtain from a particular strategy, a household decide whether or not, and how much, of its assets to invest in it, subject to the household having the asset endowment necessary to undertake the strategy. Over time, in addition to livelihood strategies, households can also invest in accumulating assets, which can then broaden the set of possible strategies from which they can choose.

In the mixed crop livestock systems of the Gambia, the major livelihood strategies in which households engage are crop production, livestock rearing, harvesting forest products, and off-farm employment. The major assets are expected to be labor, land, livestock, tools, forest, and cash. The assets required for crop production are land, labor, tools and, possibly, cash if purchased inputs are used. Harvesting forest products requires forest, labor and possibly tools. It might also require social capital depending on forest governance.

Livestock keeping requires animals (species and breed) and labor, as well as land or capital². While forest may or may not be a direct input into livestock production, the presence or absence of forest is a major determinant of tsetse pressure and therefore will affect the set of breeds from which households can choose.

² Animals can graze, fodder can be collected, or purchased.

Finally, off farm employment is a function of the quantity and quality of labor available, of physical and/or financial capital in the case of trading or running a small business, and possibly also social capital if it is used to obtain jobs or access to markets.

Several factors complicate the solution to this problem, mainly relating to the use of forests and forest products. Household can generate regular returns from sustainable harvesting of non-timber forest products (NTFPs), or they can generate high short term returns through logging and extraction of other valuable species followed by burning the forest to open new land. The returns to clearing forest would then consist not only of the benefits from the forest products in the short term but also the benefits that could be obtained from cropping or livestock production in the longer term. It would be difficult for the returns from NTFPs alone to compete with that, and in fact the optimal solution is unlikely to be complete destruction of the forest. In the absence of clear property rights to the forest, people have even more incentive to act quickly to clear it before someone else does. The fact that insecure tenure causes people to be influenced by what they think their neighbors will do.

Another way that people are influenced by the behavior of others with regard to the forest is that depending on how much total forest is cleared by all community members combined, the tsetse pressure could be affected which in turn would affect the breed choices available to households. Further, presence of tsetse can affect human, reduce labor productivity and reduce welfare directly. Key issues will be relative returns to NTFPs versus clearing, to the household and to other household. This project is attempting to increase household welfare and at the same time maintain forest and the natural resource base and ERL populations. The way it expected to do this is via interventions designed to simultaneously

- 1) increase the returns to households from maintaining the forest by improving markets for NTFPs or through some type of payment for environmental services scheme that would internalize the externalities associated with deforestation;
- 2) increase the returns to households of maintaining ERL through increased productivity and markets for ERL and their products and services (including breeding), and, as above, through an incentive

- scheme based on PES for conservation of ERL genetic resources;
and
- 3) increase costs to households of clearing forests through strengthening of NRM institutions.

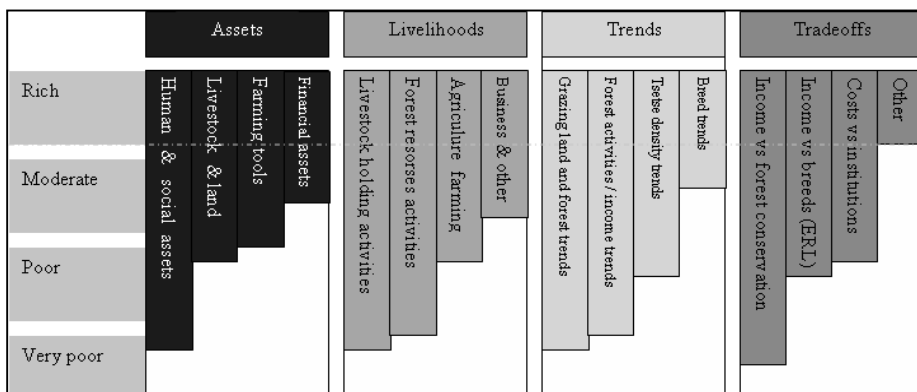


Figure 1: Conceptual framework: Trends and Tradeoffs analysis

The effectiveness of these interventions will depend on the impacts of the relative returns to forests and ERL compared to alternative investments.

Figure 1 presents a conceptual framework that depicts the above discussion, showing the links between assets, livelihoods and the likely trends and tradeoffs. The rest of this paper uses the results of the PRA in the Gambia to validate the model and its implications in terms of the opportunities and constraints that households face in terms of livelihood strategies and asset allocation and accumulation. It also begins to explore the size and determinants of the returns that households—disaggregated by poverty status—receive from investing their assets in alternative livelihood strategies and what these imply for possible intervention options. While the conceptual model in Figure 1 is generic, the rest of the paper will proceed to i) determine the different wealth categories and their proportions in each community; ii) determine the alternative livelihood strategies and their contribution to livelihoods; and iii) explore the trends in livestock production objectives and breed composition, grazing lands and forests.

Data collection: The PRA approach

A Participatory Rural Appraisal (PRA) survey was carried out during June 2009 in The Gambia. It covered the districts of Nianija, Nianina East and Kiang West. These sites were selected to reflect on different agro-ecological conditions; Kiang West is the Lower River Division, whereas Niamina East and Nianija are in the Central River Division. (See Table 1 for site characteristics). Three villages were selected in each site based on the number of households. The selection was stratified based on the number of households in the village – small, moderate, big. For each village in these sites, two days were spent to implement all PRA activities.

Participatory rural appraisal approach has been widely used in development studies and recognized as supporting a new development paradigm (Chambers, 1994). It is based on timely participatory feedback from the concerned local communities. It is argued that research that disregard farmers' views will risk non-adoption. Therefore, it is recommended that baseline studies include strong socio-economic focus to ensure the participation of farmers in the design of appropriate research/development interventions. This approach has, however, been facing empirical challenges, mainly related to data integration and aggregation and statistical analysis (Collinson, 2000).

To overcome the aggregation problem, this paper uses simple models of representative farm-households for each site. For policy analysis, it is recommended that these farm household systems be combined to provide assessment of aggregated impacts (Collinson, 2000). For statistical analysis, the nature of PRAs and their small sample size raise problems for conventional statistical analysis and interpretation. But a good deal of data “from analytical games” such as ranking, scoring and preferences are “amenable to analysis” based on Non Parametric Techniques (NPT) (Riley and Fielding, 2001; Abeyasekera, 2001). NPT include Kruskal Wallis Test, Friedman's test or the Kendall's association test, which we apply to questions such as (i) “do farmers perceive the contribution of different livestock/activities to have different impact?” or “How consistent farmers are in their evaluation?”

Livelihoods in ERL systems: Assets, activities and trends

The PRA has examined the livelihood systems in the three sites and focused on livestock and related resources. The PRA attempts to use a participatory wealth ranking to gain farmers' perception of wealth levels and criteria. The main assets and their levels were then identified and four wealth categories (Very poor VP, Poor P, Moderately Rich MR and Rich R) are recognized in local language³. We constructed representative farm-households for each site, which are presented in Table 1.

To illustrate these results, Figure (2) shows wealth ranking in Kang West. Physical capital assets (e.g. Livestock, land, and farm implements) and financial assets (e.g. off-farm income and household remittance) were the main assets perceived by these communities. This assumes also that natural assets (agro-ecological potential) and social and human assets are equally shared. But, based on PRA discussions⁴ a strong distinction between the poor and very poor reside in the human assets (e.g. the ability to work, children education).

³ *Miskeno* (very poor); *Fuwaro* (poor); *Temakundako* (moderately rich) and *Fankamaa* (rich).

⁴ In local language, *Miskeno* is the one who is unable to gain his basic needs (may afford one meal per day) and rely on community support. The very poor, does not have assets (or minimal assets) but has the ability to make his living (can afford two meals per day). The poor cannot meet the educational expenditure of their children, who always drop out at the lower, whereas the poor can educate children upto upper basic.

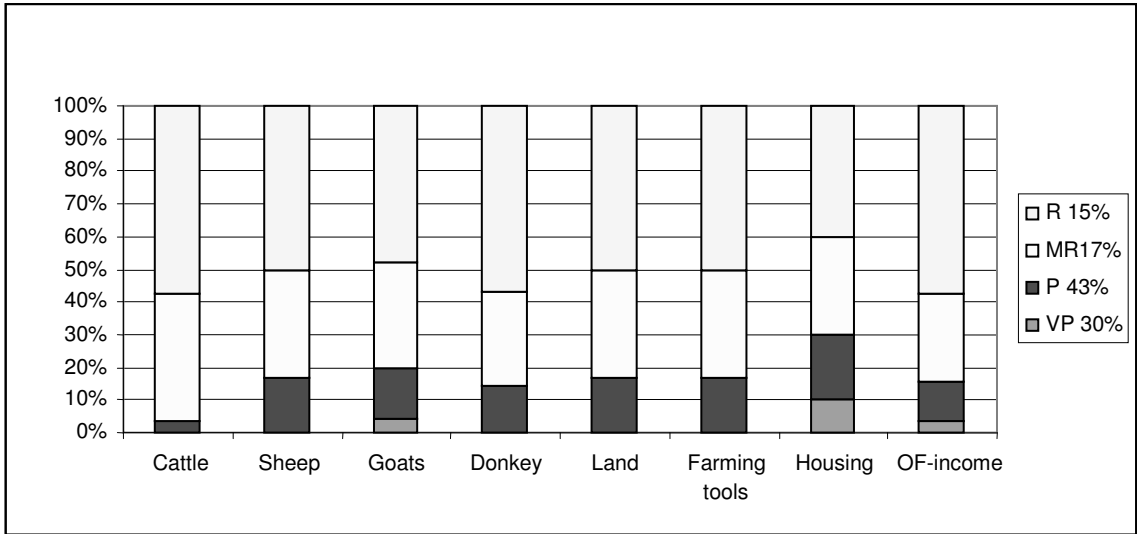


Figure 2: Wealth ranking in Kiang West site

Land access alone was not seen as sufficient for crop farming. A distinction between the poor and rich with regard to access to land is actually perceived from land productivity. For the poor and very poor categories, land access was restricted and/or constrained by limited farm implements for adequate use and productivity. Off-farm income comes from business and trading activities, saving (we assume more important to the rich) and household remittance (we assume very important to the poor given their limited sources).

The PRA attempts to capture livelihood strategies for all social categories combined (see Figure 2). It appears that the most important activities are crop farming, livestock holding and forest products harvesting (including cutting and collecting wood). These are well related to assets, i.e. land access and farm implements, livestock and labour (human assets). Moreover there is a trend that activities related to livestock and forests are becoming more important, but farming is said to become less important in Kiang west (2 villages) because of rural migration but also reduced prices for groundnut which is the main cash crops in the regions (Figure 2).

Table 1: Wealth ranking for representative farm-households in each site

	Very poor	Poor	Moderately rich	Rich
Kiang West	30%	43%	17%	10%
Livestock- Cattle	0	1-2	5-15	+15
Sheep	0	2-4	4-5	10-15
Goats	1	3-5	5-10	10-20
Donkeys	0	1-2	2-3	4-5
Horses	0	0	0	0
Land ¹	0	1	2	3
Farming implements ²	0	1	2	3
Housing ³	1	2	3	4
Income ⁴	1500	4000	10000	20000
Niamina East	12%	63%	17%	8%
Livestock- Cattle	0	1-2	10-20	+20
Sheep	0	1-5	10-15	15-30
Goats	1	2-5	5-10	15-30
Donkeys	0	1	2-3	+4
Horses	0	0	1-2	+4
Land ¹	1	2	3	4
Farming implements ²	0	1	2	3
Housing ³	1	2	3	4
Income ⁴	1000	5000	10000	25000
Nianiya	27%	53%	13%	7%
Livestock- Cattle	0	1-5	10-20	+50
Sheep	0	1-3	10-20	+30
Goats	1	2-31	5-10	+20
Donkeys	0	1	2	+3
Horses	0	0	1	+2
Land ¹	1	2	3	4
Farming implements ²	0	1	2	3
Housing ³	1	2	3	4
Income ⁴	500	2500	10000	20000

1. limited/restricted access and poorly used =1; adequate access but poorly used = 2
Adequate access and use = 3; Good access and high production = 4.
2. No implements = 0; limited (1 sine hoe and 1 seeders) = 1; adequate (2 sine hoes, 2 seeders and oxen) = 2; good implements (2-3 seeders, 2-3 sine hoes and pair of oxen).
3. Bad/inadequate (mud house, leaking-thatched roofs) =1; fair (mud house, corrugated roof) = 2; good (mud house plastered with cement) = 3; Very good (cement house) = 4.
4. Income is off-farm or remittance (financial assets)

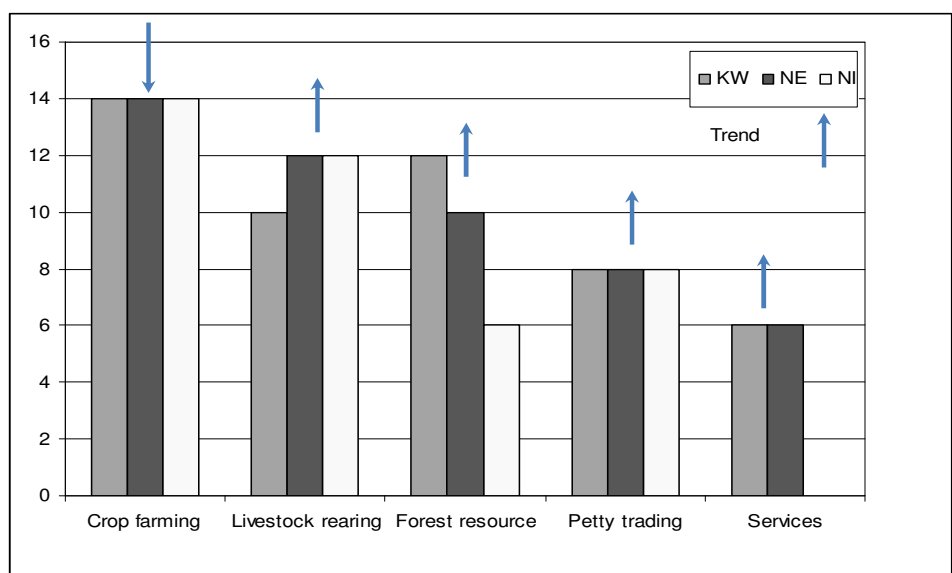


Figure 2: Livelihood strategies and trends in the three sites

The discussion above points to additional hypotheses regarding the link between assets, activities and wealth categories; which social category is more likely involved in which activity?). Households are likely to engage in different a ctivities based on their varying resources, according to our conceptual model.

Production of ERL: Role and production objectives

Using the PRA, we attempt to assess the contribution and the perceived value of endemic ruminants to farm and herding households. This section is devoted to livestock activities and focus on the role ERL play in the

livelihood of people, production objectives as perceived by farmers.

The objective of PRA was to capture the proportion of different breeds of cattle, sheep and goats currently used in the village areas. This was achieved by asking the PRA participants to divide 10 stones into groups representing each breed. In relation to the cattle population, Kiang West was reported to comprise close to 100% N'dama, whereas Niamina East and Nianija comprised 70-80% N'dama with the remainder N'dama by Zebu crosses or, less predominantly, pure-bred Zebu.

The pattern was similar for sheep, with close to 100% Djallonke indicated for Kiang West, and the other two sites comprising 70-80% Djallonke with the remainder Djallonke by Sahelian crosses or pure-bred Sahelian. For goats, the proportion of non-ERLs was reported to be lower than that for cattle and sheep. In Kiang West, close to 100% West African Dwarf (WAD) was reported, whereas for the other two sites about 90% were WAD, with the remainder Sahelian or Sahelian crosses.

Objectives for keeping livestock

It is worth to discuss the overall contribution of livestock to livelihoods to understand the objectives for keeping animals. The contribution to livelihoods was disaggregated into contribution to general livelihoods and contribution to cash income. This distinction will be more apparent when we discuss production objectives in the following section. Ranking was used to capture the relative contribution of the diverse species. Whereas Cattle was ranked first, i.e. is the most important to livelihoods in general, followed (in order of importance) by horses, donkeys⁵, goats and sheep (Figure 3), the contribution to cash income shows opposite direction; i.e. Goats and sheep are becoming more important, whereas Cattle, horses and Donkeys are less important.

The PRA assessed the objectives of the livestock keepers for rearing the different livestock species discussed above. This was performed by utilisation of a preference rating matrix, with species (as cows, bulls,

5 Horse and Donkey were ranked second and third because they are for many families the only means of transportation to to purchase or sell their products on local markets. Horses and Donkey are also preferred as draught power for field work because they work faster than cattle.

sheep, goats) as column headers, and production objectives (11 pre-set, as well as ‘other’) as row headings. Participants who had been divided into groups based on cattle herd size were asked to place between 0 to 10 stones in each cell, with the number of stones proportional to the importance of the production objective.

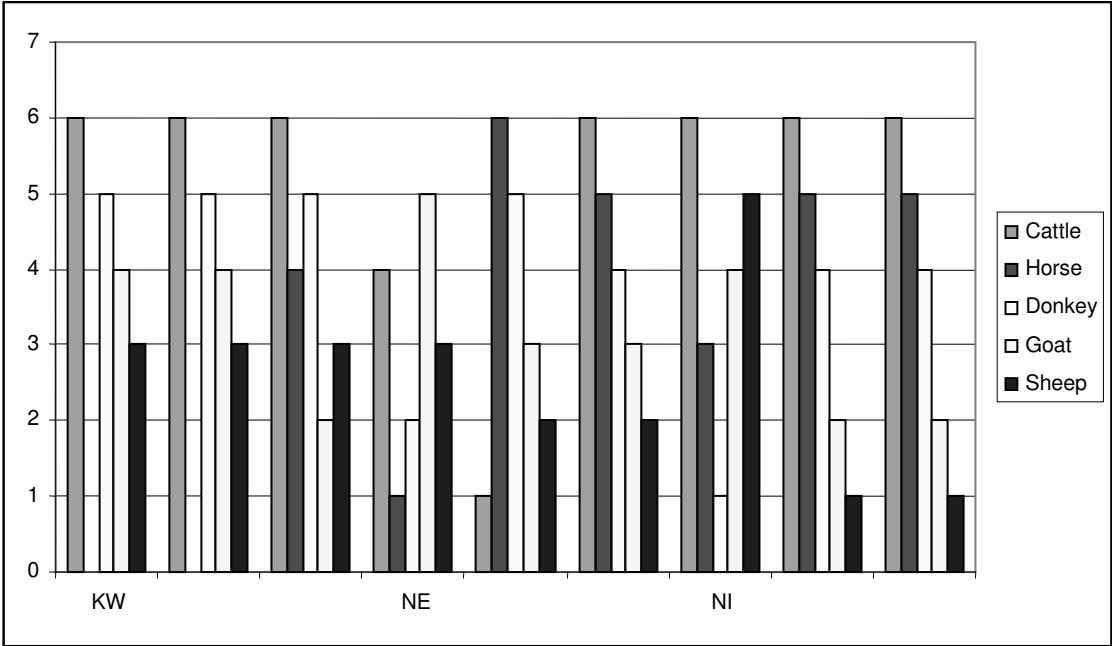


Figure 3: Ranking of the relative contribution to livelihoods of diverse species per site and village

Results are described here for livestock keepers owning less than 10 cattle, which represented the majority (70%) of the total PRA participants and also because this category was found in every village. For this level of cattle ownership, 9 groups (one per village) completed the matrix, with group sizes ranging from 8 to 25 persons. In total 136 persons were involved, about half (48%) of which were female. Realised herd/flock sizes per livestock keeper were 0-9 for cattle, 1-20 for sheep, and 1-14 for goats. 90 to 100% of animals owned by each group were pure-bred ERL.

Table 2 gives the mean and range of ratings for each production objective by species, over all PRAs. The most noticeable result is that the

objective of savings and insurance scored the highest for all species. For cattle the next highest scoring objectives were draught and manure, followed by (for cows) domestic milk consumption and milk sale, and then ceremonial/dowry. The other remaining traits (including income and domestic meat consumption) received relatively low ratings. For sheep and goats the next highest scoring objectives were income and ceremonial/dowry, followed by manure. Again ratings were relatively low for the other remaining traits (including domestic meat and milk consumption, and milk sale).

It is of interest to note that whilst cattle rated somewhat higher than the small ruminants for savings and insurance (with average ratings of 9.74 and 7.44, respectively) the opposite trend was observed for income where the small ruminants (averaging 6.98) were considerably more important than cattle (averaging 3.13). This indicates the tendency to sell sheep or goats, in preference to cattle, in times of cash needs.

Domestic milk consumption rated moderately high for cows (6.07) but low for the small ruminants (averaging 0.75), with the score given for goats was two times more than that for sheep (with implications for breeding programs for milk). In addition, only cows' milk was sold. Domestic meat consumption rated low for all species, but was highest for goats (2.73, though also with a high across village variation), followed by sheep (1.79) and then cattle (0.56).

The importance of cattle for draught (averaging 6.71), and all species for manure (6.67-4.49), is reflective of these livestock owners also engaging in cropping activities. The use of cows for draught was strongly location dependant, with the highest observed across village variation. The relatively low score of cattle for transport (average 2.45), is likely indicative of other species, such as donkeys or horses, fulfilling this role (Bennison et al., 1996).

All species were important for ceremonial / dowry purposes, with goats rating the highest (7.30), closely followed by sheep (6.68), and then cattle (averaging 5.00). One group indicated that goats were favoured for this purpose due to their high multiplication rate.

Hides / skin received ratings of zero in all villages bar one, indicating that this product is not commonly utilised. Ratings of zero were always

given for sale of breeding animals or their services, indicating that these small-holders do not supply this service.

Results of this study agreed well with a previous study of Bennison et al. (1996) on the production objectives of livestock keepers in the Gambia (with the only noteworthy discrepancy a higher average rating of cows for draught in this study compared to the previous). This indicates that the objectives for keeping cattle, sheep and goats have generally remained consistent over the last 10 or more years.

Table 2: Mean and range (in brackets) of ratings for each production objective by species*

Objective	Animal species					
	Cows	Bulls	Sheep	Goats		
Savings / insurance	9.61 (8-10)	9.86 (9-10)	7.75 (6-10)	7.79 (5-10)		
Manure	6.67 (4-10)	6.67 (4-10)	4.49 (2-8)	5.27 (0-9)		
Draught	6.28 (0-10)	7.13 (2-9)	0.00 (0-0)	0.00 (0-0)		
Domestic milk consumption	6.07 (2-9)	0.00 (0-0)	0.56 (0-5)	0.93 (0-5)		
Milk sale	5.86 (2-9)	0.00 (0-0)	0.00 (0-0)	0.00 (0-0)		
Ceremonial / dowry	5.34 (2-10)	4.77 (3-7)	6.68 (2-10)	7.30 (2-9)		
Income	2.93 (1-9)	3.33 (1-9)	6.30 (5-8)	7.65 (6-9)		
Transport	2.06 (0-8)	2.84 (0-8)	0.00 (0-0)	0.00 (0-0)		
Hides / skin	0.56 (0-4)	0.56 (0-4)	0.99 (0-9)	1.11 (0-10)		
Domestic meat consumption	0.56 (0-2)	0.56 (0-2)	1.79 (0-5)	2.73 (0-10)		
Sale of breeding animals* *	0.00 (0-0)	0.00 (0-0)	0.00 (0-0)	0.00 (0-0)		

* In the case where a group did not give any rating (over all objectives and species) as 10, all values were first adjusted by multiplication by (10/highest score). Ranges are rounded to the closer integer.

* *The full objective description was “sale of breeding animals or their services”

Habitat quality and Trends in ERL systems

As introduced in our conceptual framework, habitat degradation resulting from changes in the system (livelihood activities and interventions) may be considered⁶ as a major driver of crossbreeding in the study regions.

6 Results indicate (though not prove) that in two of our Gambia sites change in breeds is due to habitat degradation (less tsetse). We however acknowledge that

There is an extensive literature that shows the links between habitat-natural resources conservation and livelihoods (Cleaver and Schreiber, 1994; Gjertsen, 2005). Sustainable management of the endemic ruminant livestock breeds (Ndama cattle, Djallonke sheep and West African dwarf goat) in the study sites is inseparable from sustainable management of the natural resources (land, vegetation, water and forestry).

Current status of natural resources (Habitat)

Habitat quality is assessed based on three features: available land types, vegetation cover and the frequency of bush fires whether controlled or uncontrolled. Current status of different land types is assessed based on availability on the scale of 0 (Not available at all) to 5 (very abundant) (Figure 4). The value for each site is an average of the scores reported for three villages where the PRA was conducted in each site. Results show that cropland and rangeland are in much abundance in Kiang West compared to the other 2 sites (Niamina East and Nianija). Kiang-West also has a significant area of protected forest whereas there is none in Nianija. The degraded land reported in Nianija during the PRA is high compared to the other two sites. These results seem to portray two contrasting set of sites – one with abundant natural resources (Kiang West) and the other with depleting resources (Niamina East and Nianija).

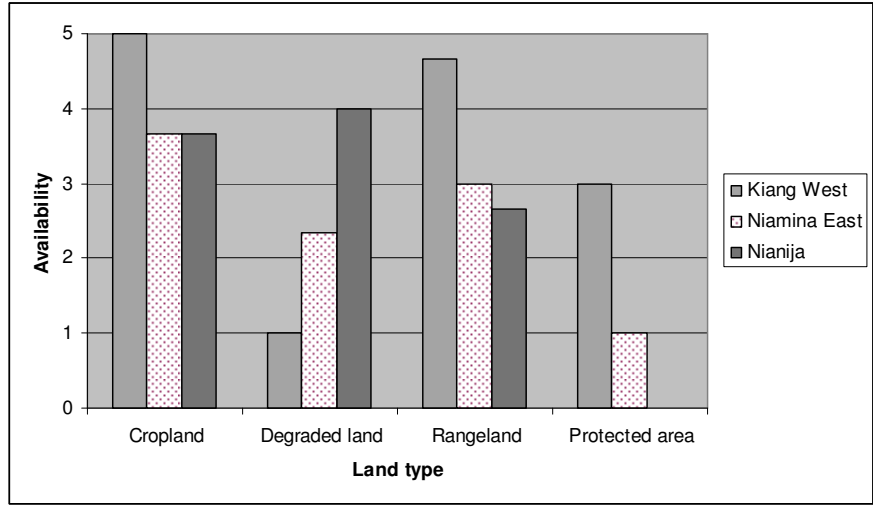


Figure 4: Current status of land types in the three sites

there may be other influencing factors not captured in our study.

Other aspects to capture the status of habitat are related to vegetation. Attributes of the vegetation in the three project sites was assessed based also on availability on a scale of 0 (None at all) to 5 (very high). The value for each site is an average of the scores reported for three villages in each site. The presence of invasive weeds was reported in all the study sites (Table 3) but seems more common in Niamina East and Nianija. The dominance of annual grasses in the vegetation of all the study sites is a possible explanation for the good pasture quality reported during the PRA. Given the availability of rangeland and protected forest, and relatively good quality vegetation in Kiang West, it is not surprising that the community reported an increase in population of Ndama breed in these villages. Uncontrolled bush fire is a major problem in all the project sites and this is a major threat to conservation of habitat for the endemic ruminant livestock. The controlled bush fire reportedly being practiced in the project sites at a small scale is inadequate to combat the menace of uncontrolled bush fire.

Trend and magnitude of changes in Habitat

The key question addressed here during the PRA was if there had been changes in availability of major natural resources in the project sites in the past 30 years and their trends and magnitudes. Trends and magnitude of changes in the past 30 years of natural resources in the three project sites in the Gambia on the scale of 0 (None at all) to 4 (widespread). The direction of the trend is indicated by a positive (negative) sign. The value for each site is an average of the scores reported for three villages where the PRA was conducted in each site. Negative value (sign) implies a decreasing trend while positive value implies an increasing trend (Table 4).

Table 3: Attributes of the vegetation in the three project sites in the Gambia

Attribute	Project site		
	<i>Kiang West</i>	<i>Niamina East</i>	<i>Nianija</i>
Annual / seasonal vegetation production	4.00	3.00	2.67
Quality / palatability	4.00	3.33	3.67
Presence of invasive weeds	2.00	3.00	3.33
Dominance of annual grasses	4.33	3.33	3.67

Dominance of perennial grasses	3.00	2.00	2.00
Dominance of annual legumes	3.33	0.33	0.67
Dominance of perennial legumes	4.33	0.00	1.00
Shrubs population	4.33	3.33	2.00
Incidence of controlled bush fire	2.67	1.67	2.33
Incidence of uncontrolled bush fire		4.33	3.67
			4.33

Results showed that the habitat (natural vegetation and agro-ecosystem in which these animals are found) for the endemic breeds has declined significantly in Niamina East and Nianija whereas it increased significantly in Kiang West (Table 4). The explanation given for the trend in Kiang West was significant emigration from the community to urban areas which led to abandonment of crop fields and conversion of cropland into grazing areas. This is supported by the declining trend of area of land cropped reported during the PRA workshop. The increasing trend in the habitat for endemic breeds might have encouraged increase in the population of Ndama breed being kept in the site (Kiang West).

In the case of Niamina East and Nianija, the reported decline in the habitat of endemic breeds could be attributed to expansion of crop field to grazing areas due to inadequate available land for cropping and significant decline in the population of tsetse fly in the sites. The population of Ndama cattle was reported to have declined significantly in Niamina East and this could be associated with decline in the habitat and consequently inadequate feed resources. Introduction of Zebu breeds (non-endemic breeds) and animal diseases were also reported as reasons for the decline of Ndama breed in the site. There was a general decline in population of tsetse fly in all the study sites as reported by the PRA workshop participants and this may create a favourable environment for introduction of non-trypanotolerant breeds as reported in Niamina East and Nianija.

Table 4: Trends and magnitude of changes in the past 30 years of natural resources

Natural Resource variable	Project site		
	<i>Kiang</i>	<i>Niamina</i>	<i>Nianija</i>
	<i>West</i>	<i>East</i>	
Land available for cropping	3.67	1.00	-0.67
Area of land cropped	-1.00	-1.00	2.00
Crop yield	-3.33	-4.00	-3.33
Grazing areas for livestock	3.67	-2.33	-2.33
Expansion of crop field to grazing areas	-3.00	0.67	3.67
Population of livestock in the territory	3.67	-1.67	0.67
Population of Ndama cattle	3.67	-3.67	0.67
Population of Djallonke sheep	-0.67	-1.67	-1.33
Population of West African dwarf goat	-0.67	-1.67	-1.33
Habitat of endemic breeds	3.67	-3.67	-3.33
Population of tsetse fly in the territory	-1.00	-3.33	-1.33
Clearing of forest for cropping	-4.00	-2.67	0.00
Harvest of forest products	1.67	0.00	2.67

Tradeoffs and Implications

In the sections above we attempted to lay down the main characteristics of livelihoods, livestock production and breed composition and prevailing/trends in natural habitat. Figure 5 shows asset ownership and access (given environmental and institutional constraints), and

households’ allocation of labour and land to different activities. In particular, there is risk that only households in possession of resources (the rich and moderate) are likely to take part of and benefit from any development intervention.

The observed trends suggest tradeoffs between livelihood decisions (such as crossbreeding, forest use and cash crop cultivation) and ecosystem preservation (endemic ruminant genetic resources and their habitat). The wealth ranking point to the social categories most affected or concerned by these changes. This is particularly important to draw related implications.

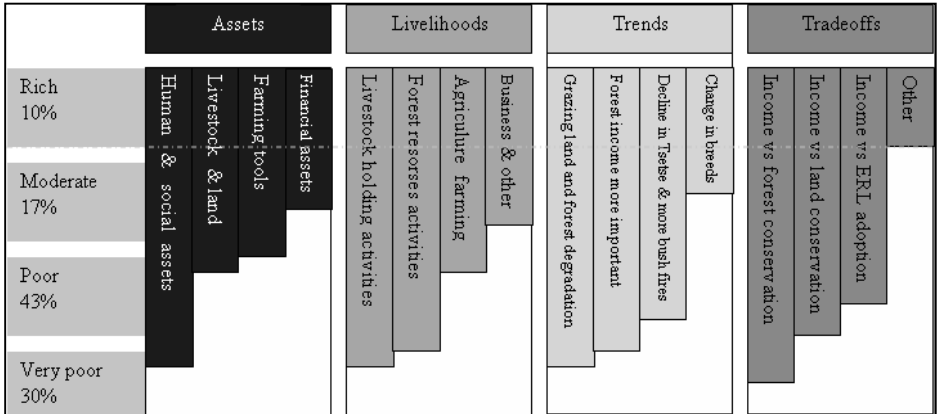


Figure 5: Wealth categories and corresponding assets and livelihood

Major trends and the implied tradeoffs could be summarized as below:

- In all sites, the poor and very poor categories amount to 70 to 80%. The major activities found in the sites and becoming even more important include crop farming, livestock holding, and forest products harvesting, including cutting and collecting wood. Production objectives for keeping cattle, sheep and goat have generally remained consistent over the last 10 or more years: Cattle are kept mainly for savings and insurance while small ruminants are the main source of cash income. The poor and very poor would rely more on livestock and forest resources given their asset base. Non sustainable forest activities would amplify degradation.

- Trends in habitat quality tend to drive changes in breed composition at the site levels but also changes (tradeoffs) in livelihoods (income generating activities). Habitat degradation as suggested by the results is related to an increase or decrease of particular animal breeds. The availability of rangeland and protected forest, and relatively good quality vegetation in Kiang West, was related to an increase in population of Ndama breed. On the other side, uncontrolled bush fire resulting from conflicts in interests between the livestock owners and crop producers is a major problem in all the sites and is a major threat to the endemic ruminant livestock. For instance, the population of Ndama cattle was reported to have declined significantly in Niamina East.
- There was a general decline in population of tsetse fly in all the study sites which may create a favourable environment for introduction of non-trypanotolerant breeds in Niamina East and Nianija; the introduction of Zebu breeds (non-endemic breeds) was reported as driving the decline of Ndama breed in these site. Fighting tsetse by the use of chemicals and bush fires imply not only breed changes by also more forest degradation.
- Another driver of change in natural resource use which invariably affects the habitat quality is the emigration from the project sites to urban areas. The immediate effect of this is in terms of labour availability for cropping activities which tend to contribute to decline in cropping area (case of Kiang West). The tradeoffs (rural/urban activities) may reduce pressure on forest resources but may imply other changes in livestock systems.
- Finally, livelihood options are largely defined by the assets base (resources), which are mostly common property in the Gambia. Community-based management of the natural resources is critical to sustainable endemic ruminant livestock, natural resources and livelihoods.

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**LAND USE AND ENVIRONMENT/
UTILISATION DES TERRES**

LIVESTOCK PRODUCTION SYSTEMS AND TSETSE IN EAST AFRICA/

UNE CARTE DES SYSTEMES D'ELEVAGE DANS LES ZONES D'AFRIQUE DE L'EST INFESTEES PAR LES GLOSSINES

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Résumé

Les trypanosomoses transmises par les glossines freinent encore l'utilisation du potentiel agro-écologique de vastes régions en l'Afrique de l'Est. Une carte des zones présentant un haut potentiel de croissance de l'élevage mais soumises à la maladie peut permettre de définir des zones prioritaires d'intervention. Une meilleure connaissance de la répartition géographique des différents systèmes d'élevage et de leurs déterminants environnementaux contribue à cet objectif. Dans le cadre d'une collaboration entre l'Autorité Intergouvernementale pour le Développement/Initiative pour les politiques de l'élevage (IGAD LPI) et le Programme de Lutte contre la Trypanosomose Africaine (PLTA), les

systèmes pastoraux, agro-pastoraux et mixtes ont été cartographiés, en utilisant des données issues d'études sur les moyens de subsistance. Les systèmes de productions ont été définis en fonction du rapport entre les revenus tirés de l'élevage et des cultures, et les résultats ont été mis en carte. Lorsque les données sur les moyens de subsistance n'étaient pas disponibles, des modèles statistiques basés sur des paramètres environnementaux ont permis d'extrapoler aux zones mal renseignées. Les systèmes ont aussi été décrits en termes de densité de population, de potentiel agricole et de couverture de terre, et ont été comparés à des cartes de systèmes d'élevages dressées antérieurement, révélant un niveau de correspondance élevé. Le produit de cette étude est une des composantes clés d'un outil de prise de décision pour la région de l'IGAD, qui vise à identifier des zones prioritaires d'intervention contre la trypanosomose en mettant en carte les bénéfices potentiels qui pourront être réalisés si la contrainte était éliminée.

Summary

Tsetse-transmitted trypanosomiasis still hinder achievement of full agro-ecological potential of vast areas of Eastern Africa. Mapping areas with potential for livestock sector growth, which are constrained by the presence of the disease, can point to priority areas for intervention. A better understanding of the geographical distribution of the different livestock production systems and of their environmental determinants can contribute towards this goal. In a collaborative effort between the Intergovernmental Authority on Development Livestock Policy Initiative (IGAD LPI) and the Programme against African Trypanosomiasis (PAAT), pastoral, agropastoral and mixed farming systems have been mapped for the IGAD region using information collected in the framework of livelihood analysis. Production systems were defined according to the ratio of livestock- to crop-derived income and were subsequently mapped for those areas where livelihood data were available. Gaps were filled using statistical modelling techniques based on environmental data, thus providing complete regional coverage. The resulting systems were described according to population densities, agricultural potential and land cover characteristics, and compared to earlier livestock production system maps. This comparison showed a high level of correspondence. The output of this study is a key component of a spatial decision-support system for the IGAD region, which identifies priority areas for trypanosomiasis interventions by

mapping the potential economic benefits that would result from trypanosomiasis removal.

Introduction

That African Animal Trypanosomiasis (AAT) imposes significant constraints on Africa's rural economies is widely recognised and documented (e.g. Swallow, 2000; Shaw, 2004). The African Union declaration at Lomé in 2001 commits Africa's leaders to measures to deal with tsetse and trypanosomiasis. However, a wide range of livestock production systems exist within the estimated 8.7 million square kilometres infested by tsetse, which themselves reflect the variability of human and natural resources as well as the nature and severity of the tsetse-trypanosomiasis problem. This diversity has profound implications for the feasibility and cost of dealing with the disease in livestock as well as for the potential benefits from its removal. Thus, understanding the nature of the livestock production systems affected is an important prerequisite for policy-makers.

A recent study linked quantitative economic variables to a Geographic Information Systems (GIS) to derive a map of the economic benefits that would result from the removal of trypanosomiasis from West Africa (Shaw *et al.*, 2006). This approach is now being applied to East Africa (Djibouti, Eritrea, Ethiopia, Kenya, Somalia, Sudan and Uganda) through a collaborative effort by the Intergovernmental Authority on Development - Livestock Policy Initiative (IGAD LPI) and the Programme against African Trypanosomiasis (PAAT).

The methodology developed by Shaw *et al.* (2006) used breed production systems to stratify the analysis into groups where the response to trypanosomiasis removal can be expected to be similar. This is not feasible in East Africa, and it was therefore necessary to develop a map of livestock-oriented production systems.

This study proposes mapping livestock production systems using data collected for livelihood analysis, most notably in the framework of the Household Economy Approach (HEA) (Seaman *et al.*, 2000; FEG Consulting and Save the Children, 2008). This was developed to account for the diversity of coping strategies utilized in developing countries, especially by the rural poor and uses information on sources of food and cash income as a key component of baseline assessments. Income data are linked to well-defined, mappable areas.

The map of livestock production systems based on livelihood analysis was used to show how tsetse hinder the achievement of the full agro-

ecological potential in East Africa. Production systems were matched with tsetse distributions, cattle density and agro-ecological potential, to illustrate how tsetse presence constrains stocking rates and livestock-derived income.

Materials and methods

A map of livestock production systems in East Africa based on livelihood analysis

A 'livelihood' can be characterized as the sum of activities and resources through which households fulfil their needs. Various conceptual frameworks for livelihood analysis have been developed, including the sustainable livelihood framework (Scoones, 1998; Carney, 2003) and the HEA (Seaman *et al.*, 2000). The HEA, which has been extensively used in East Africa, was developed in the early 1990s by Save the Children-United Kingdom (SC-UK) with the initial goal of improving the ability to predict short-term changes in access to food.

Two central components of livelihood analysis are 'livelihood zones' and 'baseline assessments'. Livelihood zones are areas within which people share broadly the same livelihood - including farming practices, consumption patterns, expenditure, trade and exchange. Livelihood zones are depicted in maps that are increasingly available in GIS format. Data on wealth breakdown and livelihood strategies within livelihood zones constitute the baseline assessment from which valuable information relating to production systems can be derived. In particular, the contribution of crops and livestock to the food basket and to cash income is clearly identified and can be used to rank or classify livelihood zones by their degree of reliance on livestock and livestock products.

Livelihood studies conducted for all or parts of the IGAD member states provided the input to this study (FEWS-NET, 2004; FEWS-NET, 2006-2007; FEWS-NET, 2006a; FEWS-NET, 2006b; FSAU and SC-UK, 2000-2001-2002; KFSM, 2006; NFIS, 2006; SC-UK and DPPA, 2001-2002-2004-2005; SSCCSE and SC-UK, 2005; UDSS *et al.*, 2005, see also the section 'Abbreviations').

In the present analysis, the dominant livestock production system was defined within each livelihood zone according to the relative dependence of households on livestock. If *L* and *C* are defined as the total household

income derived from livestock and crops respectively, and ‘total income’ is the sum of the value of the marketed and subsistence production (see Otte and Chilonda, 2002), then the ratio L/C can be used to define three systems as follows:

- pastoral systems: where $L/C \geq 4$;
- agro-pastoral systems: where $1 < L/C < 4$; and
- mixed farming systems: where $L/C \leq 1$.

L/C can be estimated for all households, or groups thereof, including those for which waged labour, remittances or commerce represent an important component of income. An ‘urban and other areas’ category was also introduced for households where crop and livestock agriculture contributes less than 10 percent of the total income, e.g. in urban areas, or those where tourism, trade or fishing predominate. A fifth class, designated ‘protected areas’ accounts for national parks and other reserves.

Quantitative information on the livestock- and crop-derived income was not available in some livelihood studies (most notably in Uganda and Eritrea). In these areas, production systems were assigned according to the livelihood profiles and the descriptions of production systems therein. These normally include explicit qualitative reference to the dominance of either pastoral, agro-pastoral or mixed farming systems. Livelihood information in these areas was complemented by expert opinion (see ‘Acknowledgments’).

The combined use of quantitative and qualitative information enabled a map of livestock production systems to be derived for all areas of East Africa where livelihood analysis has been conducted.

Modelling the distribution of livestock production systems

Livelihood data are not yet available for large areas of Ethiopia and Sudan. One way to fill the gaps in the observed distribution of the livelihood-based livestock production systems, and thus provide complete regional coverage, is to use stochastic spatial modelling techniques based on logistic regression. These techniques have been widely used to model a range of agro-ecological and epidemiological parameters that are likely to be related to climate, topography, and other environmental variables (Wint *et al.*, 2002; Gilbert *et al.*, 2005). For this

study, they were used to model the presence or absence of each of the three livestock production systems.

Logistic regression models were generated using data extracted for each of a regular grid of sample points spaced approximately 15 km apart. For each point, the presence or absence of a livestock production system was extracted, together with values for a wide range of potential predictor variables.

Separate models were constructed for each of the three defined livestock production systems. The regression relationships were then applied back to the selected predictor datasets to provide 1 km resolution images giving the predicted probability of each of the three livestock production systems occurring. Each pixel was then assigned the system with the highest predicted probability.

Agro-ecological potential, cattle stocking rates and tsetse

The map of livestock production systems based on livelihood analysis identifies areas where income derived from livestock exceeds that derived from crops (i.e. pastoral and agro-pastoral areas). Given that tsetse poses a major constraint to livestock rearing, it seems useful to investigate the relationships among tsetse presence, agro-ecological potential and cattle stocking rates, in the context of these production systems.

The length of growing period (LGP) is the number of days when both water availability and prevailing temperatures permit crop growth (Fischer *et al.*, 2002) and provides an environmental characterization that can be used as a surrogate for agro-ecological potential. The LGP map was derived from the WorldCLIM dataset (Hijmans *et al.*, 2005), by Thornton *et al.* (2006).

Cattle stocking rates (densities) were taken from a series of 1 km resolution digital maps for the IGAD region, created within the framework of the IGAD LPI using the methods described in Wint and Robinson (2007), and based on the most recent national statistics available for the period 2000-2005.

A map of the predicted presence of tsetse was taken from the PAAT Information System (Wint and Rogers, 2000). Predicted areas of suitability for each tsetse group (*fusca*, *palpalis* and *morsitans*) were

converted into masks of presence, defined as 50% probability of presence, and then combined to derive a single map of predicted absence/presence of tsetse.

Results

A map of livestock production systems in East Africa based on livelihood analysis

Figure 1 shows the distribution of livestock production systems in East Africa as derived from livelihood data (Figure 1a) and from subsequent statistical modelling (Figure 1b).

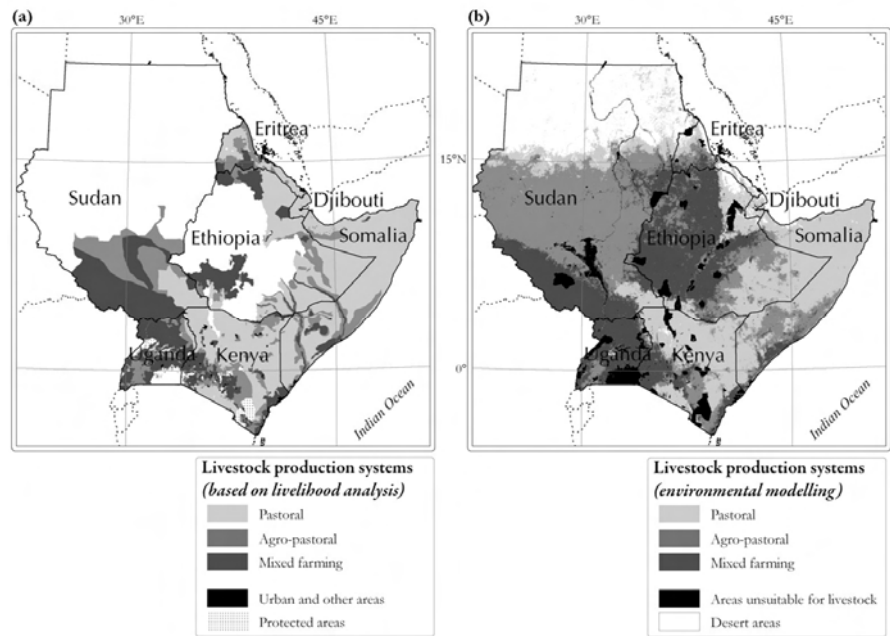


Figure 1: Livestock production systems in the IGAD region

The systems are defined as a function of the ratio L/C , where L is the total household income derived from livestock and C is the total household income derived from crops. $L/C \geq 4$: pastoral; $1 < L/C < 4$: agro-pastoral; $L/C \leq 1$: mixed farming. L/C is calculated or estimated for each livelihood zone, and the resulting systems are shown in Figure 1a (data are unavailable for areas in Sudan and

Ethiopia). Figure 1b shows the modelled distribution of the systems derived from logistic regression of those shown in Figure 1a against a wide range of environmental datasets. Areas unsuitable for livestock - including high elevation areas, urban centres, water bodies, forests and protected areas - have been masked out (black). A second mask (white) depicts desert areas where environmental conditions are not suitable for rearing ruminant livestock.

These results demonstrate the strong link between environmental factors (used as predictors in the modelling) and the geographic distribution of livestock production systems. The statistical accuracy of the models (Table 1) provides good grounds for using them to fill the gaps in the livelihood-based map of livestock production systems shown in Figure 1a.

Table 1: Accuracy metrics for the models of livestock production systems (confusion matrixes)

Value	Pastoral			Agro-pastoral			Mixed farming		
	0	1	% correct	0	1	% correct	0	1	% correct
0	5,543	751	88.1	1,861	523	78.1	2,697	369	88.0
1	391	5,199	93.0	446	1,887	80.9	349	2,601	88.2
All	90.4			79.5			88.1		

Agro-ecological potential, cattle stocking rates and tsetse

The relationships among agro-ecological potential (LGP), cattle stocking rates (densities) and tsetse distribution was investigated using standard GIS functions. Within the areas in East Africa that are covered by livelihood studies (Figure 1a), LGP and cattle density values and the presence or absence of tsetse were extracted for a regular sampling grid (sampling points spaced approximately 10 km apart). The average cattle density was estimated for each LGP value. The results are plotted in Figure 2, where the hollow diamonds (and the related dashed trendline) represent areas free of tsetse and black diamonds (and related solid trendline) represent tsetse-infested areas.

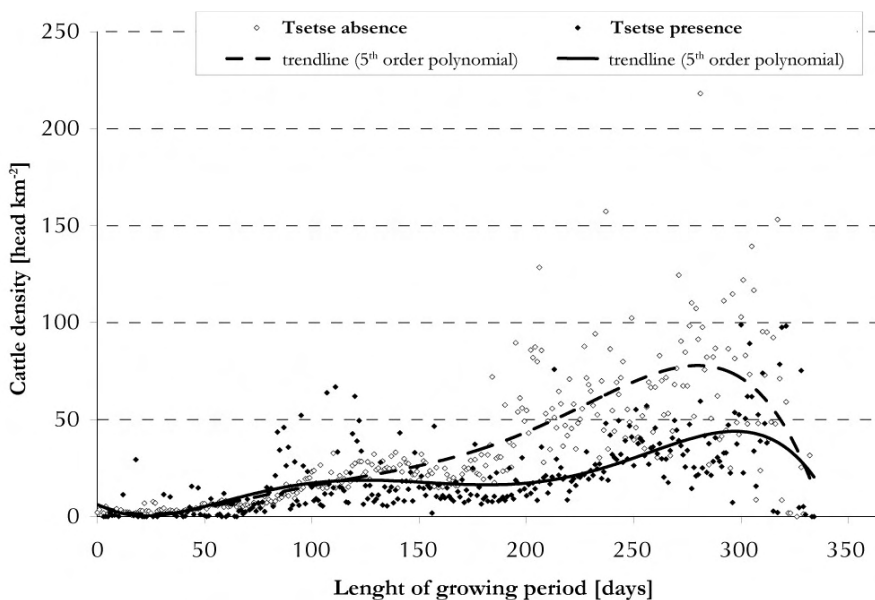


Figure 2: Cattle densities and length of growing period (study area: zones of East Africa covered by livelihood analysis, as shown in Figure 1a). Hollow diamonds represent tsetse-free areas, and black diamonds represent tsetse-infested areas

Differences in stocking rates between tsetse-infested and tsetse-free areas start to become apparent for LGP values higher than 120 days, and increase as LGP increases up to an LGP of about 280 days. For a large range of LGP values (between approximately 170 and 280 days), cattle densities in tsetse free areas are more than double the densities in tsetse-infested area. As LGP values increase beyond about 280 days, stocking rates start declining – probably because very high LGP values are associated with tropical rain forest ecosystems. Consequently the differences in stocking rates between tsetse-free and tsetse-infested areas become progressively marginal.

The absence of any apparent impact of tsetse on cattle densities for LGP values lower than 120 could be interpreted in different ways. However, the fact that tsetse are almost completely absent at these LGP values, as shown in Figure 3, provides perhaps the most obvious explanation.

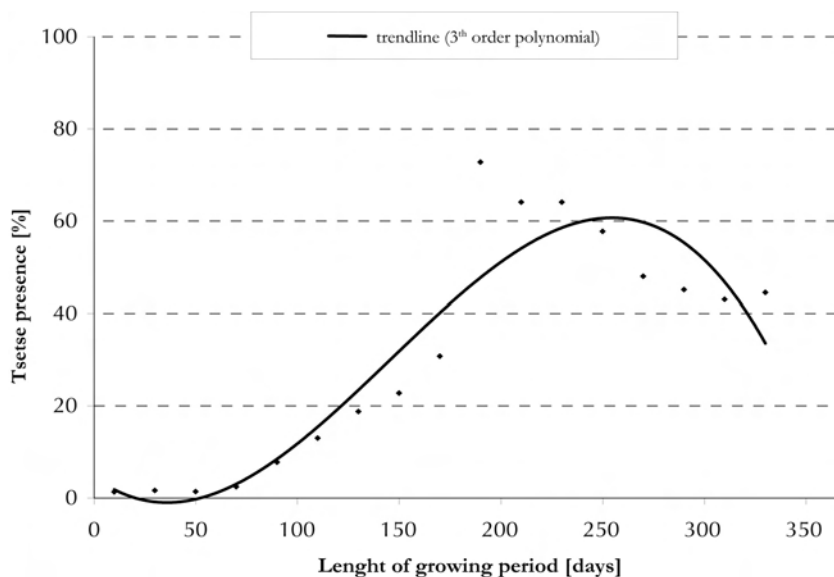


Figure 3: Tsetse-presence and length of growing period. LGP and tsetse absence/presence values were extracted pixel by pixel; they were subsequently ranked by LGP and binned by using 18 equally spaced LGP classes before calculating the proportion of tsetse presence in each bin.

Figure 3 also shows that in East Africa, the probability of presence of the tsetse fly increases with LGP to approximately 200 days and then falls. This pattern can largely be explained by the highlands of Ethiopia and Kenya where, at high LGP values, cooler temperatures associated with higher altitudes limit distribution of tsetse.

Figure 2 shows how higher stocking rates are associated with the absence of tsetse within a wide range of LGP values. The map of livestock production systems presented in Figure 1a allows an analysis of the presence of tsetse in relation to the distribution of livestock production systems, and thus the dependence of livelihoods on livestock.

To this end, values of LGP and production systems were sampled both for tsetse-infested and tsetse-free areas. Values were subsequently ranked by LGP, and binned using 18 equally spaced LGP classes before calculating the proportion of each production system in each bin (Cecchi *et al.*, in press). Figure 4 summarizes the results by showing the percentage of livestock-dominated systems as a function of LGP. Livestock-dominated systems are those where $L > C$, and thus include the categories of pastoral and agro-pastoral systems. The pattern for

mixed-farming systems is not shown as, for each LGP value, it is simply the inverse of the line for livestock-dominated systems.

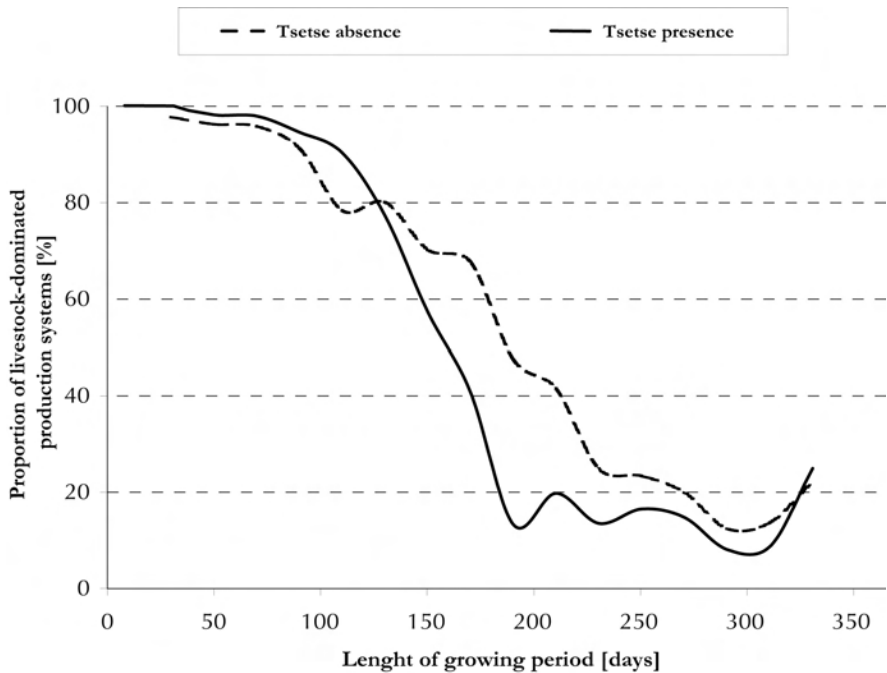


Figure 4: Proportion of livestock-dominated production systems and length of growing period (LGP). Livestock-dominated production systems are those where income derived from livestock exceeds that derived from crops. They correspond to pastoral and agro-pastoral systems as depicted in Figure 1a. The solid line represent tsetse-infested areas and the dashed line the tsetse-free areas.

Figure 4 shows how the relative dependence of rural households on livestock and crops is correlated with agro-ecology: areas with low LGP values are dominated by pastoral and agro-pastoral systems, whose frequency decreases at growing LGP values, where cropping becomes progressively more feasible. Figure 4 also shows that the drop in the proportion of livestock-dominated systems is sharper in tsetse infested areas. At LGP values of 180 days, for example, the percentage of livestock-dominated systems in tsetse-infested areas is lower than 30, whereas it is close to 60 in tsetse-free areas. A higher proportion of livestock-dominated systems, which is a measure of higher income

derived from livestock, thus appears to be correlated with tsetse-free areas.

Conclusions

The study presented here demonstrates that livelihood maps can help improve our knowledge of the geographic distribution of livestock production systems. Data generated through livelihood analysis were used to incorporate a socio-economic dimension into the mapping of livestock production systems in East Africa. Incorporating socio-economic and livestock related information into the systems' definition may further contribute to the usefulness of agricultural systems maps, linking them more closely to aspects of the rural livelihoods than has hitherto been feasible.

The combined analysis of production systems, agro-ecology, tsetse distribution and livestock data would suggest that AAT hinders the achievement of the full agricultural potential in vast areas of East Africa. Tsetse presence is shown to be correlated with lower cattle stocking rates, altered patterns of production systems and lower income derived from livestock. Despite these clear correlations, this type of analysis can not prove causality. Indeed, it is likely that in tsetse infested areas other factors contribute to limiting cattle rearing, tick-borne diseases arguably among the most important.

The results of this analysis will feed into a broader study concerned with mapping the potential benefits of dealing with tsetse in East Africa. It is also believed that the regional map of livestock production systems presented here could benefit policy development and technical decision-making beyond the field of tsetse and trypanosomiasis control.

Abbreviations

Abbreviations used in the text, tables or references: Department for International Development (DFID), Disaster Prevention and Preparedness Agency Ethiopia (DPPA), Early Warning Unit/Ministry of Agriculture, Animal Industries and Fisheries of Uganda (EWU-MAAIF), European Commission (EC), European Commission's Humanitarian aid department (ECHO), Food Economy Group (FEG), Famine Early Warning System Network (FEWS-NET), Food and Agriculture Organization of the United Nations (FAO), Food Security Analysis Unit-Somalia (FSAU), Geographic information systems (GIS), Household

Economy Approach (HEA), Intergovernmental Authority on Development (IGAD), International Fund for Agricultural Development (IFAD), International Livestock Research Institute (ILRI), Italian Cooperation (IC), Kenya Food Security Meeting (KFSM), length of growing period (LGP), Livestock Policy Initiative (LPI), National Food Information System of Eritrea (NFIS), Programme against African Trypanosomiasis (PAAT), Save the Children-United Kingdom (SC-UK), Southern Sudan Centre for Census, Statistics and Evaluation (SSCCSE), Swedish International Development Cooperation Agency (SIDA), total household income deriving from crops (C), total household income deriving from livestock (L), Ugandan Department of Disaster Management (UDDM), United Nations Office for the Coordination of Humanitarian Affairs (UNOCHA), United States Agency for International Development (USAID), World Food Programme (WFP).

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URBANISATION ET DISPARITION DES GLOSSINES A BRAZZAVILLE, CONGO/

URBANIZATION AND THE DISAPPEARANCE OF TSETSE IN BRAZZAVILLE, CONGO

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Summary

As early as 1906, Brazzaville had been the gathering and treatment place of all Human African Trypanosomiasis (HAT) cases detected in French Congo. This may have led to the establishment of the disease in the locality, mainly due to the presence of tsetse (*Glossina* spp). In the 1970s, several breeding sites were found in the suburbs and in the city centre. As a result of vector control activities and urbanization, tsetse has since disappeared for the most part, except in the zoo which became reinfested between 1977 and 1980. However, in 1987, an entomological survey confirmed that all *Glossina* had once again totally disappeared from the zoo. This area is the only urban environment with living conditions that are conducive to the survival of tsetse flies, and some imported HAT cases have from time to time been diagnosed in Brazzaville. In order to ascertain that there is no case of local transmission, we conducted an entomological survey in the zoo: twenty pyramidal traps, placed over four days and checked twice a day did not

catch any tsetse. This finding shows that the city-center of Brazzaville has been free of tsetse since the vector control campaign of 1985 - 1987. This is as a result of the strong urbanization that took place here: from 1985 to 2002, the population has multiplied by 8 and urbanized areas extending by 6.7 times. This urbanization has prevented the reinfestation of the zoo by tsetse flies present a few kilometers away, on the island of Mbamou. Urbanization contributes effectively to the disappearance of *Glossina* and consequently, to the elimination of HAT.

Résumé

Dès 1906, Brazzaville a été le lieu de rassemblement et de traitement de tous les malades de Trypanosomiase Humaine Africaine (THA) dépistés au Congo français. Ceci a pu favoriser l'implantation de la maladie dans la localité, du fait notamment des glossines présentes dans de nombreux gîtes. Dans les années soixante-dix, plusieurs gîtes étaient encore dénombrés en périphérie et au centre-ville. Grâce aux activités de lutte antivectorielle et à l'urbanisation, ces gîtes ont disparu pour la plupart, à l'exception de celui du parc zoologique qui a été réinfesté entre 1977 et 1980, mais en 1987, une enquête entomologique faisait à nouveau état de la disparition totale des glossines du zoo. Cette zone est le seul milieu urbain qui offre des conditions de vies favorables à la survie des glossines et des cas de THA importés sont quelquefois diagnostiqués à Brazzaville. Afin de nous assurer de la non-existence d'une transmission locale, nous avons conduit une enquête entomologique dans le zoo, vingt pièges pyramidaux, posés pendant quatre jours et contrôlés deux fois par jour n'ont capturé aucune glossine. Ce résultat montre que le centre-ville de Brazzaville est exempt de tsé-tsé depuis la campagne de lutte antivectorielle menée entre 1985 et 1987. Ceci est le résultat de la forte urbanisation opérée en ce lieu, de 1985 à 2002, la population a été multipliée par 8 et les espaces urbanisés par 6,7. Cette urbanisation a empêché la réinfestation du zoo par les glossines présentes à quelques kilomètres dans l'île Mbamou. L'urbanisation contribue efficacement à la disparition des glossines et conséquemment, à l'élimination de la THA.

Introduction

Brazzaville est la capitale de la République du Congo. Cette ville a été pendant longtemps considérée comme un foyer urbain de

Trypanosomiase Humaine Africaine (THA), du fait des cas de THA qui y étaient diagnostiqués et de la présence de nombreux gîtes à tsé-tsé.

Les gîtes à glossines de Brazzaville ont été signalés pour la première fois au début du vingtième siècle (Martin *et al.* 1909). C'est une ville située à la limite sud de la poche de distribution de la sous-espèce *Glossina fuscipes quanzensis* (Pires 1948). Ces gîtes étaient localisés particulièrement dans les ravins de la Glacière, du Tchad, de la Mission ; le long des rivières M'Foa, Tsiéma, Makélékélé, M'Filou, Djoué, le ruisseau du jardin d'Essai et dans le parc zoologique. Des mises au point périodiques sur l'évolution de ces gîtes ont été faites successivement jusqu'en 1986 (Martin et Ringenbach, 1910 ; Maillot, 1950, 1952 et 1955 ; Maillot et Ceccaldi, 1956 ; Taufflieb, 1965 ; Frézil *et al.* 1972 ; Gouteux *et al.* 1986).

La présence des glossines et la quasi-permanence d'un réservoir humain de parasites, entretenue par la forte main d'œuvre en provenance des zones hyperendémiques de l'arrière-pays y ont favorisé l'installation d'un cycle de transmission. En 1971, 15 cas de THA ont été diagnostiqués en plein cœur de la cité (Anonyme, 1975). Des activités de lutte antivectorielle, le développement urbain et l'extension des quartiers ont conduit à la diminution de la densité des glossines et à la disparition de certains gîtes (Frézil *et al.* 1972). De 1980 à 1985, le parc zoologique était considéré comme le seul gîte intra-urbain productif de glossines. A cette même période, les gîtes temporaires (aux densités trop faibles pour permettre une croissance autonome des populations) étaient la concession de l'ORSTOM, le club hippique et le jardin d'Essai (faculté des sciences) (Gouteux *et al.* 1986). Les autres gîtes actifs étant localisés à la périphérie, notamment dans les élevages porcins et sur le Djoué.

En 1989, des cas passifs de THA ont été dépistés à Brazzaville, sans preuve de contamination locale (Jannin *et al.* 1990). Toutefois, il a été admis qu'une souche de *T. b. gambiense* à faible virulence circulait au cœur de la ville. Certains auteurs ont évoqué l'existence de deux cycles de transmission, un cycle prédominant d'homme à homme à faible degré de virulence et un cycle mineur impliquant un réservoir animal (Noireau *et al.* 1989). Des malades de THA continuent d'être passivement dépistés à Brazzaville (Rapport d'activités du PNLTHA, 2007 ; non publié). Ce sont des patients qui ont résidé dans des foyers connus de THA dans un passé plus ou moins proche du moment de leur diagnostic.

Y a-t-il encore possibilité de l'existence d'une chaîne de transmission urbaine de la THA à Brazzaville, En 1986, il avait été supposé que les glossines qui réinfestaient le parc zoologique provenaient du Djoué ou de

l'île M'bamou situé en amont de la ville, dans le Stanley Pool (Gouteux *et al.* 1986).

Quelle est la situation entomologique de ce parc vingt années après la dernière campagne de lutte antiglossinienne,

Les réponses à ces questions constituent l'objet de ce travail.

Méthodologie

Zone d'étude

Créé le 26 juin 1952, le parc zoologique de Brazzaville a été l'un des plus grands parcs zoologiques d'Afrique. Il est situé dans la réserve forestière de la Patte d'Oie, en plein cœur de la ville de Brazzaville et est entouré de grandes voies de circulation urbaine (fig.1). A l'origine, ce parc servait de centre de transit de nombreuses espèces animales sauvages de l'Afrique Equatoriale Française, destinées aux ménageries et jardins zoologiques de la métropole. Il présente encore les reliques des derniers lambeaux de forêt naturelle, et est cerné d'arbres exotiques. En son sein, on retrouve une zone active dite de récréation et une zone d'exhibition des animaux.

La zone d'exhibition des animaux compte quatorze enclos à ciel ouvert destinés à héberger des antilopes et autres ruminants, 3 volières pour oiseaux, 1 vivarium pour serpents, 2 bassins pour les crocodiles varans et les tortues, 2 cages pour carnivores, 6 singeries et une nursery (pour les gorilles). Avant les troubles socio-politiques qu'a connus le Congo en 1997, ce parc comptait 42 animaux dont 12 mammifères, 21 oiseaux et 9 reptiles. Aujourd'hui, seuls quelques singes, tortues et serpents y sont présents.

Piégeage

Seize pièges pyramidaux (Gouteux et Lancien, 1986) munis de boîtes de capture ont été utilisés. Ces pièges ont été posés aux abords des allées, à la lisière de la forêt et autour des cages à animaux. Les alentours de ces pièges ont été bien nettoyés afin de permettre une visibilité raisonnable pour les glossines. Ils étaient visités deux fois par jour, à 10 heures et à seize heures et ont été laissés en place pendant quatre jours.

Analyse probabiliste de capture

La probabilité de capturer une glossine a été calculée telle que décrite par Barclay & Hargrove (2005),

$$p = \exp (-S\sigma\lambda)$$

avec, λ , la densité de mouches dans la zone considérée = N/A

N , la population minimale que l'on souhaite détecter

A , la surface étudiée

σ , l'efficacité du système de piégeage

T , le temps de pose des pièges

S , le nombre de pièges posés

En cas de non capture des glossines, le piégeage est considéré suffisant si $p < 0,05$, insuffisant si $0,05 < p < 0,1$ et très insuffisant si $p > 0,1$.

Résultats

Aucune glossine n'a été capturée. La probabilité de capturer une glossine dans cette aire est de 0,002.

Discussion

Le piégeage conduit lors cette étude a été suffisant au regard de la valeur de la probabilité obtenue (Barclay & Hargrove, 2005). A cet effet, nous pouvons considérer le parc zoologique de Brazzaville est exempt de tsé-tsé. Les pièges n'ont pas été posés à l'intérieur des vestiges de forêt mais il nous semble évident que les pièges posés à la lisière auraient capturé des glossines à la recherche de la nourriture ; les vertébrés présents dans ce zoo étant essentiellement ceux apprivoisés dans le parc à animaux. Il nous a été signalé que seuls des serpents et des rats pourraient être retrouvés dans la relique forestière.

Plusieurs raisons pourraient expliquer la disparition des glossines dans ce milieu autrefois infestés de glossines, des travaux d'essai de lutte antivectorielle conduits au début des années 1980 (Dagnogo et Gouteux, 1985 ; Gouteux *et al.* 1986), la disparition des animaux du parc consécutive aux troubles socio-politiques et donc l'absence de nourriture pour les glossines, et les difficultés de réinvasion du fait de la forte urbanisation autour du zoo.

Il est en effet surprenant de constater que le parc zoologique n'a pas été réinfesté comme ce fut le cas après les mesures de lutte effectuées dans les années 1970 (Frézil *et al.* 1972 ; Gouteux *et al.* 1986) et ceci, malgré

la présence incessante des glossines dans l'île M'bamou. En 1992, il a été observé un remplacement progressif de la sous-espèce *G. fuscipes quanzensis* par *G. palpalis palpalis* à la périphérie de la ville de Brazzaville, du côté du Djoué (Gouteux, 1992). Ces espèces ont toutes les deux des grandes facilités de colonisation et d'adaptation. Au moins l'une d'entre elles auraient pu recoloniser le zoo, vu que les conditions écologiques à l'intérieur du zoo sont restées inchangées.

Il est connu que la croissance démographique pourrait contribuer à la disparition des glossines sans aucune activité de lutte antiglossinienne (Jordan, 1986). Sachant que la densité des populations humaines est corrélée à la surface des terres débarrassées de végétation qui offre des conditions de vies favorables aux glossines (McDermott *et al.* 2001), nous pouvons confirmer l'observation des auteurs qui avaient signalé une mise en place naturelle et progressive d'une lutte antiglossinienne dans la ville de Brazzaville, consécutive au lotissement et à l'implantation des jardins maraîchers le long des rivières et dans les ravins (Gouteux *et al.* 1986).

La ville de Brazzaville a connu une forte croissance démographique depuis le début des années 1980. De 1985 à 2002, le nombre d'habitants a été multiplié par 8 et les surfaces urbanisées par 6,7 (Ziavoula, 2005). Cette forte urbanisation a eu pour conséquence l'extension de la ville au-delà de ses frontières naturelles matérialisées par la rivière « Tsiémé » au nord et « N'Filou » à l'ouest. L'extension de la ville aurait rendu impossible toute tentative de réinvasion du zoo par les glossines en provenance du Djoué ou de l'île M'Bamou.

L'impact de la pression démographique sur la distribution des glossines a également été observé en Côte d'Ivoire (Fournet *et al.* 1999) et en République Démocratique du Congo (Mansinsa *et al.* 2009) où les glossines ont disparu des gîtes de Buma, Ndjili et Kimbanseke de la ville province de Kinshasa, sans aucune activité de lutte antivectorielle.

L'absence des glossines du parc zoologique de Brazzaville ne signifie pas que les habitants de cette ville sont à l'abri de toute transmission de la THA. Une transmission péri-urbaine pourrait exister si les glossines continuent d'être présentes à la périphérie de la ville. Dans la ville province de Kinshasa, malgré la disparition de certains gîtes à glossines, il a été observé une transmission péri-urbaine de la THA avec 66,7% de repas de tsé-tsé pris sur les hommes (Simo *et al.* 2006).

Conclusion

Le développement urbain de la ville de Brazzaville a contribué à la disparition des glossines du seul gîte intra-urbain encore présent dans les années 1980.

Aucune étude n'ayant signalé la disparition des glossines en périphérie, il serait important que des études complémentaires soient réalisées dans tous les gîtes à glossines potentiels situés à la périphérie de la ville afin d'avoir une idée précise sur la présence des glossines et sur la situation de la THA épidémiologique dans la ville.



Figure 1: Le parc zoologique dans la ville de Brazzaville. www.quid.fr

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MAPPING THE BENEFITS: EXTENDING THE CONCEPT OF MONETARY MAPS OF TRYPANOSOMIASIS LOSSES FROM WESTERN TO EASTERN AFRICA/

LA CARTOGRAPHIE DES BENEFICES: ETENDRE LE CONCEPT DE LA CARTOGRAPHIE MONETAIRE DES PERTES DUES AUX TRYPANOSOMOSSES DE L'AFRIQUE DE L'OUEST A L'AFRIQUE DE L'EST

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Summary

In 2005 the results of an innovative approach to identifying key areas where interventions to deal with the problem of tsetse and trypanosomiasis in livestock would be particularly cost-effective, were presented at the ISCTRC. A series of maps was created, culminating in a monetary map. This approach has been extended to the tsetse-infested parts of the Intergovernmental Authority on Development (IGAD) region in Ethiopia, Kenya, Somalia, Sudan and Uganda through collaborative work by the Programme against African Trypanosomiasis (PAAT) and the IGAD Livestock Policy Initiative (LPI). The same methodology was used as before: firstly defining and mapping livestock production systems, then assessing maximum stocking rate and mapping the expansion of cattle populations in the absence and presence of trypanosomiasis and, finally, attaching to these monetary values obtained from bio-economic cattle herd models. A new livelihoods-based map of pastoral, agro-pastoral and mixed farming systems was further subdivided, using cattle population data, expert opinion, livelihood surveys and documented information to define three different levels of draught power use and areas where more than 20% of cattle were grade animals used for dairy production. It is in these systems where livestock and agricultural production are closely linked, using high value animals, at high cattle population densities, often on the fringes of the tsetse distribution that the greatest potential benefits from dealing with the trypanosomiasis in livestock are to be realised.

Résumé

En 2005, les résultats d'une approche innovatrice pour identifier les zones clés où la lutte contre les glossines et les trypanosomoses animales seraient particulièrement rentables, ont été présentés à l'ISCTRC. Une série de cartes a été créée, culminant en une carte monétaire. Cette approche a été étendue aux zones infestées de glossines dans la région de l'Autorité Intergouvernementale pour le Développement (IGAD) en Ethiopie, au Kenya, en Ouganda, au Soudan et en Somalie, dans un travail collaboratif entre le Programme de Lutte contre la Trypanosomose Africaine (PLTA) et le l'Initiative pour des Politiques d'Elevage (LPI) de l'IGAD. La méthodologie utilisé a été la même, d'abord définir et mettre en carte les systèmes de production, ensuite évaluer la capacité de charge des zones et créer une carte de la diffusion

spatiale des populations bovines en présence et en l'absence de la trypanosomiase et finalement attribuer des valeurs monétaires obtenues de modèles bioéconomiques des populations bovines. Une nouvelle carte des systèmes d'élevage pastoraux, agropastoraux et mixtes, basée sur des données sur les moyens de subsistance, a été subdivisée en utilisant des données sur les populations bovines, opinions d'experts et enquêtes sur les moyens de subsistance, pour refléter trois niveaux d'utilisation de la traction animale et les zones où plus de 20% des bovins étaient des croisements exotiques utilisés pour la production laitière. Ceci a démontré que c'est dans ces systèmes où les productions agricoles et animales sont étroitement liées, souvent aux limites des distributions des glossines, que la lutte contre les trypanosomoses animales peut réaliser les bénéfices les plus élevés.

Introduction

For many years, livestock development issues in general, and those relating to animal health and Tsetse and Trypanosomiasis (T&T) in particular, faced declining interest on the part of donors and governments. The declaration of Africa's leaders at Lomé in 2000, the formation of the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), and WHO's campaign to deal with a serious resurgence of Human African Trypanosomiasis (HAT), have done much to bring these issues back into public prominence and to attract funding and manpower.

Clear goals and priorities are vital in order to tackle these diseases effectively on a national, and especially a continental level. These inevitably reflect the perceived severity of the problem in the affected human and livestock populations. In the field of livestock disease, whilst tsetse reinvasions and local reports identified disease hotspots, and knowledge of the disease and its vector showed where intervention would be technically feasible, it has nevertheless been more difficult to highlight areas where the problem was most economically significant.

In 2003 work started on a project to add monetary values to the growing range of mapped variables (cattle population density, probability of tsetse presence) which already supported prioritisation in the field of T&T control in the sub-humid zone of West Africa (Gilbert *et al.* 2001, Pender *et al.*, 2001, Wint *et al.*, 2002). The preliminary results were reported at the 2003 ISCTRC meeting, and the final study was presented at the 2005

ISCTRC (Shaw *et al.*, 2007 and published as a PAAT paper, Shaw *et al.*, 2006). With the establishment of the IGAD Livestock Policy Initiative (LPI), the need for similar information to assist decision-making in the field of T&T as well as livestock policy in general was identified. Within the IGAD region, the work covers the tsetse-infested parts of Ethiopia, Kenya, Somalia, Sudan and Uganda and is supported by collaboration between FAO's IGAD LPI and PAAT.

Materials and Methods

Tsetse infested areas and cattle population densities

As for West Africa, the starting points were the tsetse distributions and the current cattle population densities. The IGAD LPI data archive⁷ was used to provide the underlying geographic data used in the analysis.

The kilometre resolution tsetse distributions were modelled using remotely sensed variables as predictors (Hay and Lennon, 1999; Scharleman *et al.*, 2008; Wint and Rogers, 2000 Wint *et al.*, 2003). There are five tsetse species in the area, *Glossina pallidipes*, *G. fuscipes*, *G. morsitans*, *G. swinnertonii*, and *G. austenii*. Figure 1 shows all the areas within the study region which are infested by one or more of these species. It thus defines the areas within which livestock populations exist that could benefit the removal of animal trypanosomiasis. This map shows the fragmented nature of the vector distribution in the study area, with some more isolated populations in Ethiopia, contrasting with the south and west of Sudan, Kenya and Uganda, where the tsetse populations are on the fringes of solidly infested areas in the Democratic Republic of Congo and Tanzania.

The cattle populations are illustrated in Figure 2 (from FAO, 2004), also modelled at 1km resolution using environmental, climatic and demographic variables as predictors, as updated in 2007 using the methods set out in FAO's Gridded Livestock of the World, (FAO 2007). In the present study, as in the previous one carried out for West Africa (Shaw *et al.*, 2006), the focus was on cattle populations, as the vast majority of livestock losses due to trypanosomiasis occur in this species, and it thus acts as a proxy for livestock losses in general. A notable feature of cattle populations in the study area are the zones of extremely

⁷ <http://ergodd.zoo.ox.ac.uk/igadweb>

high stocking rates, especially in the Ethiopian and Kenyan highlands and on the shores of Lake Victoria.

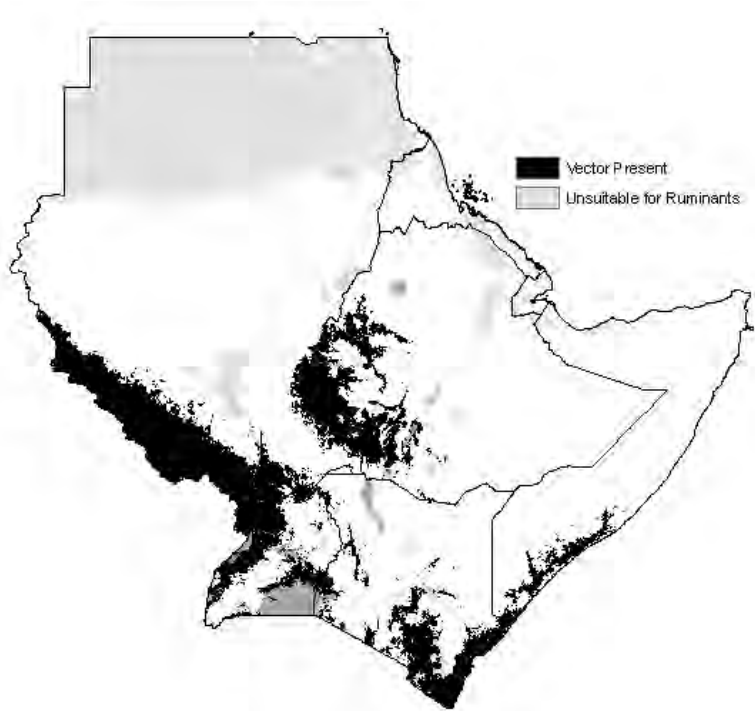


Figure 1: Tsetse distribution in the study area: all species combined

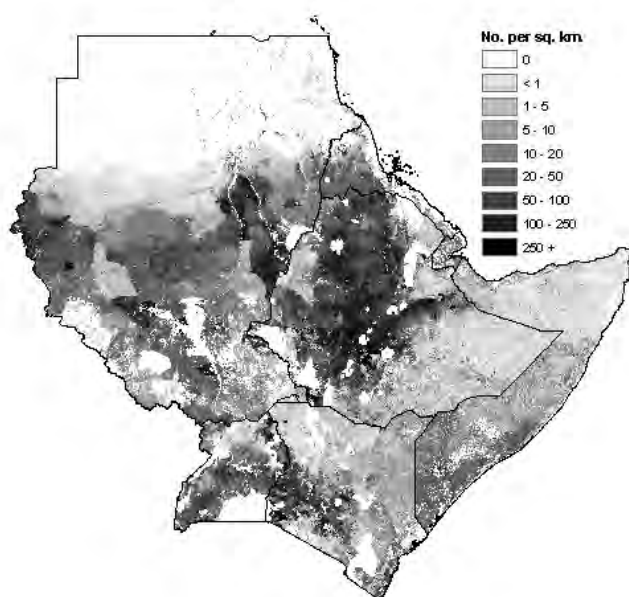


Figure 2: Current cattle population densities
Source: Adapted from FAO, 2004 and FAO, 2007.

Mapping livestock production systems

The livestock production systems in the study area are especially diverse, reflecting the range of ecosystems and human populations. Defining and mapping the cattle production systems for the purposes of the economic analysis was undertaken as a two-stage process. Firstly, the systems were divided into three broad categories: pastoral, agro-pastoral and mixed, characterised according to the ratio of livestock income (L) to crop income (C), where for pastoral $L/C \geq 4$, agro-pastoral $1 < L/C < 4$ and mixed $L/C \leq 1$. Information from a series of livelihood analyses was used to map these three broad categories (Cecchi *et al.*, in press; and Cecchi *et al.*, 2009 in this volume). This initial classification was further modified incorporating production characteristics which strongly influence each system's economic performance, particularly relating to the high value animals whose productivity is strongly affected by the presence of trypanosomiasis: work oxen and high yielding dairy cattle.

Quantitative data on the presence of work oxen was only recorded within Ethiopia. For the other countries, information was obtained from national

livestock experts and various published and grey literature sources citing herd compositions. The resulting distribution is shown in Figure 3. Three levels were defined for the analysis: with fewer than 10% of cattle being used as work oxen (low), between 10% and 20% (medium) and over 20% (high). The medium and high oxen use systems fell mainly within the mixed farming zone, but some, notably in central Kenya and parts of the Ethiopian highlands, were within the agro-pastoral areas.

For smallholder dairying, based on crossbred stock, only rural dairy production was considered, thus excluding specialised intensive production units associated with peri-urban, urban or irrigated areas. Qualitative evidence for substantial rural dairy production was restricted to Kenya and Uganda, and specific enumeration of dairy cattle was limited to Kenya. Numbers were also available, however, for exotic cattle from the Uganda livestock census, and these were accordingly taken as a proxy for dairy, based on the assumption that the use of exotic livestock for beef production is very limited in the rural parts of Uganda. These assumptions are corroborated by the evidence from local sources, showing high concentrations of dairying in the districts surrounding Entebbe and Kampala and in the south-western highland areas. Figure 4 shows the proportion of dairy/exotic cattle in the two countries. For the economic analysis, areas with over 20% dairying were selected, as with the higher use of work oxen, most of these fell within the mixed farming zone, but a proportion were within the agro-pastoral areas, notably in central Kenya.

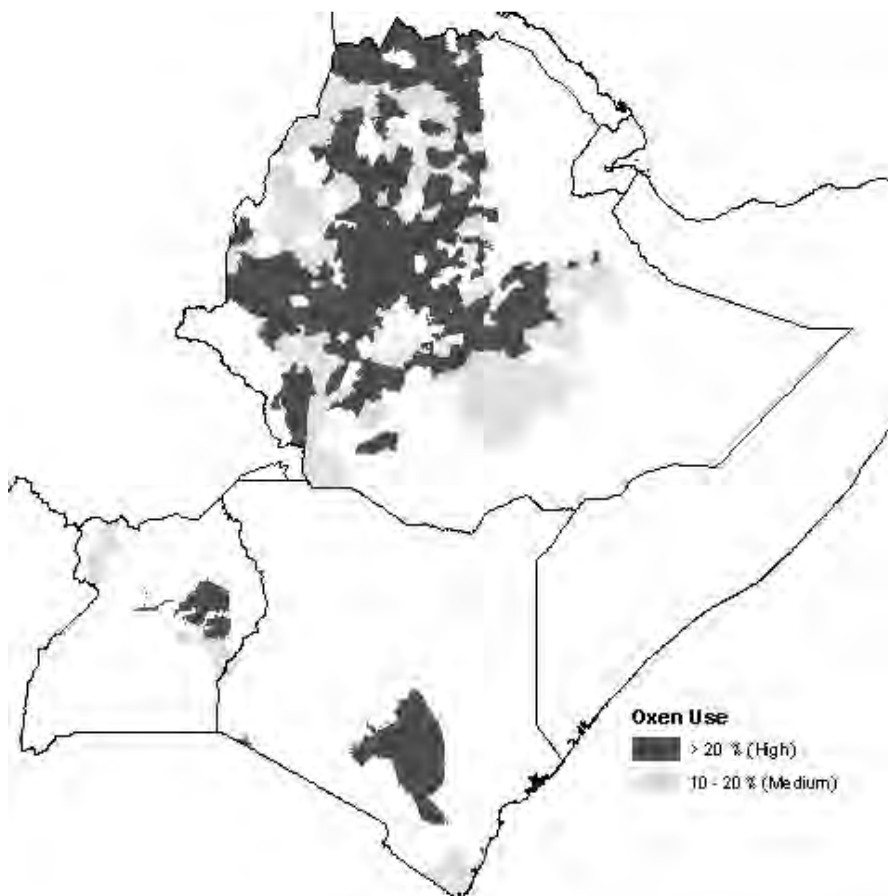


Figure 3: The use of work oxen

Based on these distinctions, the herd modelling also proceeded in two stages. Initially five basic bio-economic herd models were constructed. Pastoral and agropastoral systems were modelled separately. Because of the importance of work oxen in the economics of T&T, special attention was paid to this. From published and grey literature sources, it was evident that not only were work oxen used differently in the Ethiopian region – mainly for ploughing, with most farmers reliant on them for this and donkeys being used for other tasks, especially transport of crops and other materials during the rest of the year (e.g Rutebuka, 2006) but also the impact of trypanosomiasis was particularly severe in T&T-affected areas (e.g Jemal Ahmedin and Hugh Jones, 1995). Thus, within the mixed farming system, two basic models for zebu herds were

constructed, one for the Ethiopian region and one for the other areas. Lastly, smallholder dairy production is based on stocks which have some exotic blood, usually called 'grade cattle' enabling far higher milk yields to be obtained. Although, geographically, these animals co-exist with the other agro-pastoral or mixed farming cattle, they are reared separately and are a separate population, based on a different breed. Thus the fifth basic model was of 100% smallholder dairying. The main assumptions and key results for these five models are given in Table 1. The proportion of grade dairy cattle and work oxen has a big effect on the value of output and the economics of disease control. Accordingly twelve 'combination' livestock production systems were modelled:

One pastoral, four agropastoral with 3 levels (low, medium and high) of work oxen use and one with a high proportion (30%) of grade dairy animals

four general mixed farming with 3 levels (low, medium and high) of work oxen use and one with a high proportion (30%) of grade dairy animals

three mixed farming for the Ethiopian region, where there were no large populations of grade smallholder dairy cattle and so only the 3 levels (low, medium and high) of work oxen use were modelled.

Figure 5 shows the distribution of these systems. For visual clarity, the mixed farming (general and Ethiopian region) systems are merged, so that nine categories are shown. However, the block of mixed farming centred on the Ethiopian highlands stands out clearly as a separate mass.

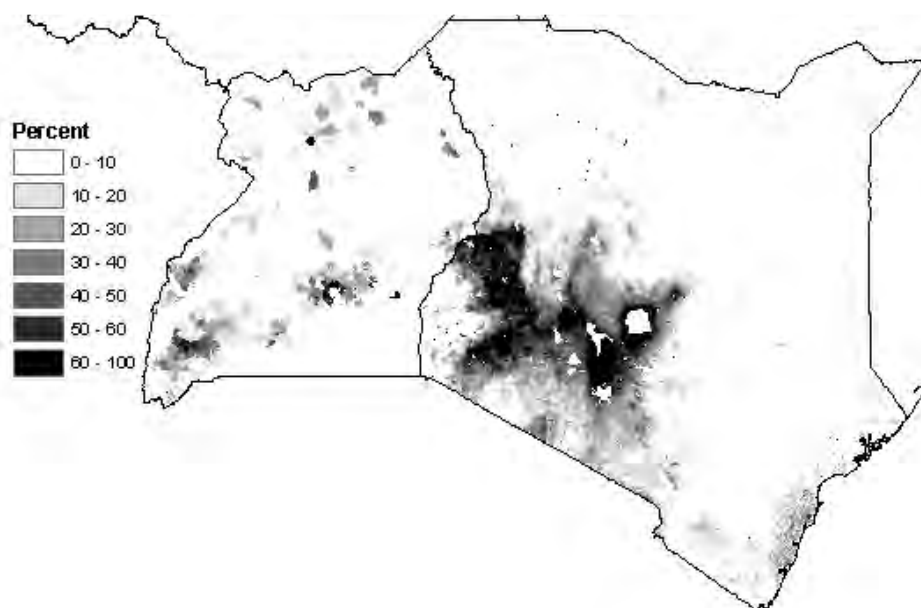


Figure 4: Dairy and exotic cattle in Kenya and Uganda

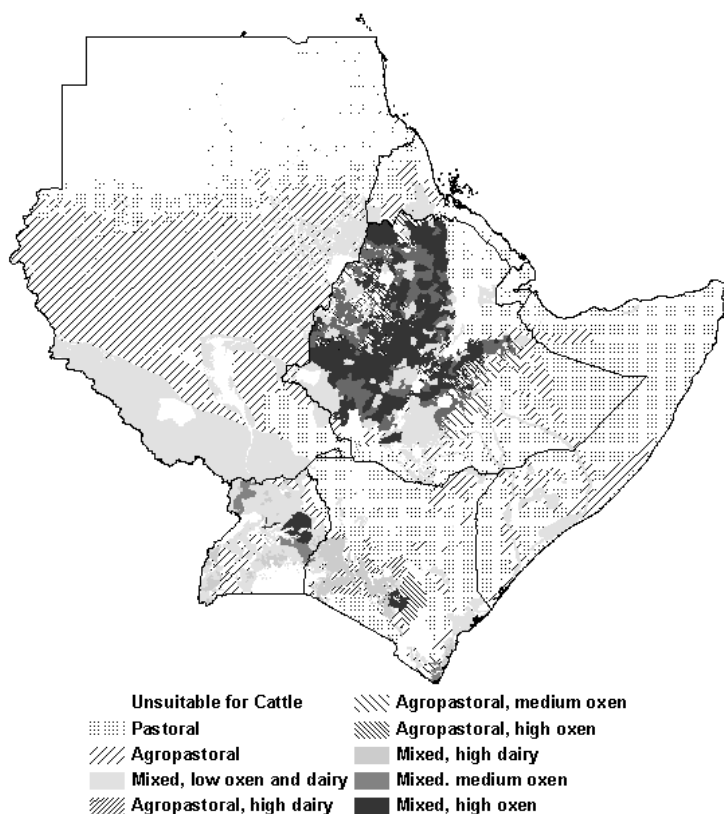


Figure 5: Production systems for which bio-economic herd models were constructed

Herd models

The economic calculations are based on a series of deterministic herd models combining information about the key production parameters in the absence and presence of trypanosomiasis (milk yields, traction performance, fertility, mortality and offtake rates) with relevant prices. The methodology follows that for the West African study (Shaw *et al.*, 2006) and models were constructed for each of the five basic production systems (pastoral, agropastoral, mixed farming general, mixed farming Ethiopian region and smallholder dairying) and then adjusted to reflect varying proportions of dairy cattle and work oxen so as to model all twelve combination systems. A wide range of published and unpublished studies were consulted. Earlier studies on the impact of trypanosomiasis

are summarised in Swallow (2000) and Shaw (2004), and summaries of livestock production parameters can be found in Otte and Chilonda (2002) and Peeler and Omere (1997). In addition to these, in order to characterise representative tsetse-infested production situations, a number field level studies in each country were consulted. These included Jemal Ahmedin and Hugh-Jones 1995; Morton, 2001 and Rutebuka, 2006 (Ethiopia), Gitonga, 2000 and Roderick *et al.*, 1998 (Kenya); Musa *et al.*, 2006 (Sudan); Hanks and Hogg, 1992 (Somalia) and Fyfe *et al.*, 2003, Laker, 2002 and Occaido *et al.*, 2005 (Uganda).

Table 1 shows some of the key assumptions and results from the basic herd models. The proportions of the herd consisting of oxen were determined according to typical herd compositions within the tsetse-infested areas of each production system. Within the bands of low, medium and high oxen use, these were highest in the Ethiopian region (Table 1). One of the 'indirect' effects of removing trypanosomiasis would be an increase in the use of draught power, this was modelled based on 10% to 20% increases at different levels of pre-existing oxen use. Other key assumptions were that removing trypanosomiasis would increase milk yields by 7.5%, and calving rates by 4 or 5%, but reduce draught oxen mortality by 20%. Table 1 also shows the modelled impact of the removal of trypanosomiasis on growth rates and herd mortality, giving results in line with similar studies (see Shaw, 2004). Overall, annual herd mortality in the tsetse infested areas of the region is high, reflecting the presence of tick-borne diseases and high stocking rates as well as of trypanosomiasis.

Table 1: Basic cattle production systems: key herd model parameters

Parameter	Pastoral	Agro-pastoral	Mixed farming General	Mixed farming Ethiopian Region	Small-holder Dairy
Work oxen as % of herd*					
With trypanosomiasis (L, M, H)	4.0	6.0, 13.0, 20.0	4.0, 13.0, 22.5	5.0, 15.0, 25.0	0
Without trypanosomiasis	5.0	7.5, 15.0, 22.5	5.0, 15.0, 25.0	6.3, 17.3, 27.5	0
Days worked by work oxen					
With trypanosomiasis	80	100	130	80	-
Without trypanosomiasis	88	108	139	86	-
Annual % herd mortality					
With trypanosomiasis	10.1	9.0	9.2	11.4	14.0
Without trypanosomiasis	8.9	7.8	7.9	9.6	12.0
Annual % population growth rate					
With trypanosomiasis	1.4	1.3	0.9	-0.6	0.5
Without trypanosomiasis	3.4	3.3	3.0	2.3	3.0

Note: * L,M,H refer to low, medium and high work oxen models respectively. Figures show the assumptions made for all three situations. For all other variables the low oxen model parameters are given, this being the basic model as well as the most widely distributed system.

Table 2: Baseline results by breed/production system: gain per head of cattle present at the end of 20 years without trypanosomiasis (US \$)

Livestock production system	No exports situation US \$	With exports situation: core areas US \$	With exports situation: export areas US \$
Pastoral	62.8	69.8	51.3
Agro-pastoral			
Low oxen, low dairy	81.8	95.6	58.6
Medium oxen, low dairy	97.7	109.8	77.9
High oxen, low dairy	118.5	128.8	101.8
Low oxen, high dairy	142.1	164.4	102.3
Mixed farming - General			
Low oxen, low dairy	89.7	106.0	58.5
Medium oxen, low dairy	122.4	136.8	95.9
High oxen, low dairy	152.4	165.5	128.1
Low oxen, high dairy	147.6	171.7	102.2
Mixed farming – Ethiopian region			
Low oxen, low dairy	102.3	119.5	61.3
Medium oxen, low dairy	135.4	154.6	87.9
High oxen, low dairy	161.1	177.2	125.7

Source: Model projections.

Note: Benefits are discounted to their present value at an annual rate of 10%

The models projected the cattle populations and calculated the income derived from them over a period of 20 years. They then modelled the situation both with and without the presence of trypanosomiasis in the ‘core population’ area, where cattle populations are currently located, and in the ‘export’ areas into which cattle populations are likely to expand over the period analysed. For the purposes of the study, each herd model had two main outputs: an estimate of cattle population growth and an estimate of income. Income from cattle was calculated as the value of meat, milk, animal traction and herd growth less basic production costs. By comparing income in the absence and presence of trypanosomiasis, the potential benefits of T&T interventions could be estimated for the

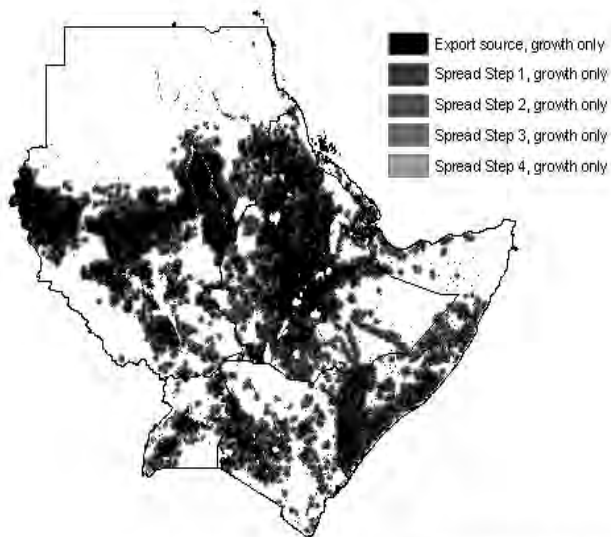
different cattle breed/production systems over the 20-year period. These were then discounted to their present value and converted to a single dollar amount, expressed as benefits per head of cattle present at the end of the time period and split between those generated by cattle remaining in the core area and those expanding into export areas. Table 2 gives these results for each of the twelve systems. The baseline gain per head of the herd (situation without exports) ranges from US\$ 63 to US\$ 161, increasing in line with the proportion of work oxen or dairy animals in the herd. Within the mixed farming system, these are highest in Ethiopia, because within each oxen use category the proportion of work oxen is higher than in the other livestock production systems and because of the severe impact of trypanosomiasis on production parameters.

Mapping the spread of cattle populations

The final part of the study mapped likely future cattle population distributions. Firstly, the potential cattle carrying capacity of the rangeland was estimated and mapped, using carrying capacity estimates derived from rainfall and knowledge about the quality of the rangeland and its carrying capacity (Jahnke, 1982; Shaw *et al.*, 2005, Wint *et al.*, 2003) and then modelled to take into account current stocking levels. Then, by applying the estimates of the cattle population growth rates provided by the herd models to maps the current distribution of cattle, it was possible to map the estimated distribution of livestock in 20 years' time. This future population was compared to the land's estimated carrying capacity to identify those areas where cattle numbers exceeded resources available to sustain them. For these situations, an innovative step-wise spatial expansion model, based in part on the method outlined by Gilbert (2003) was applied to show how 'excess' cattle populations might spread into nearby areas where grazing was available. In each case, spread was prevented into areas defined as unsuitable for livestock by FAO (Wint *et al.*, 2003) or already overstocked, and was scaled according to accessibility to markets (Pozzi *et al.*, 2008) so that spread was least into areas less likely to support livestock trading. The resulting potential stepwise spread of the cattle population in four time-steps was mapped in Figure 6a, for the situation as it would evolve over 20 years with trypanosomiasis and in Figure 6b, showing the further expansion due to the incremental growth in cattle populations if trypanosomiasis were removed. This spatial expansion model enabled us to quantify the potential benefits of the removal of trypanosomiasis from areas into which new cattle populations would migrate. The need to estimate the

benefits from this type of expansion of livestock production has been a major unresolved issue in analysing the T&T problem and, with the extremely high cattle populations found in some parts of East Africa, this has been shown to be very significant, notably more so than was the case for West Africa.

A) Exports over 20 years due to growth of the herd with trypanosomiasis present



B) Exports over 20 years due to incremental growth of the herd with trypanosomiasis removed

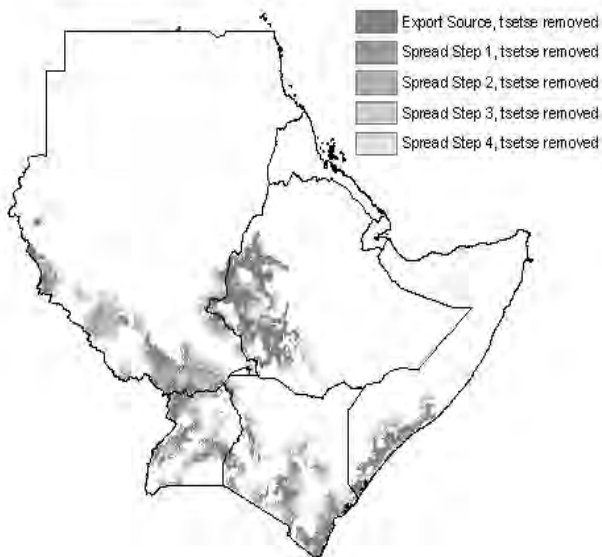


Figure 6: Areas identified for sequential spread of cattle over 20 years - a) for the situation in the presence of trypanosomiasis and - b) for that were trypanosomiasis removed

Results and discussion

The work culminated in a set of three maps showing the total benefits achievable over twenty years (Figure 7). Figure 7a shows the total benefits. These are the sum of the benefits realised by cattle that are exported (Figure 7b) and the benefits from cattle that remain in their core areas (Figure 7c). The range is extremely wide, from as little as \$10 to over \$15,000 per square kilometre. The greatest benefits are shown to accrue along the borders of the tsetse belts in south west Ethiopia, parts of western, central and coastal Kenya, and south western and central Uganda, *i.e.* close to established cattle rearing regions. Comparatively little benefit is estimated for much of the Sudanese tsetse belt, with the notable exceptions of those areas bordering northwest Uganda, and parts of western Ethiopia. This is at least partly because the calculated spread of animal into the central western Sudanese tsetse belt is somewhat limited because the neighbouring areas are relatively lightly stocked, and even with the substantial growth rates envisaged, do not increase greatly in absolute terms over the twenty year period incorporated by the herd models. This is not to say that the potential benefits of tsetse removal are much lower in these areas, but merely suggests that the final benefits will take substantially longer than 20 years to reach comparable totals per square kilometre.

Because dollar benefits are calculated for each pixel the methodology also allows a total calculated benefit for any defined areas. Whilst dangerous for small areas, in that the results of any modelling process for a small number of pixels is likely to be representative rather than precise, the summed results for each country should be more reliable. As with all such modelling and mapping exercises, care must be taken not to interpret the figures as absolute values providing exact answers, but should rather be taken as indicative as no error estimates are available. The results are summarised in Table 3. The total benefit to the area amounts to nearly 2.5 billion dollars – an average of approximately \$3,300 per kilometre. The greatest benefits accrue to Ethiopia, because of the very high livestock densities the country supports and its high use of animal traction, but even the lowest national benefits shown for Sudan amount to a substantial sum – at over \$500m or about \$1,500 per square kilometre.

Whilst restricted to a smaller area, the overall benefit levels turn out to be very similar to those obtained in the earlier study for West Africa (Shaw *et al.*, 2006). Because of the low inflation rate in meat and milk prices in

Africa, changes in the value of the dollar and a tendency for prices in eastern Africa to be lower than in western Africa when converted to US dollars, the prices of the main livestock outputs and inputs are virtually identical to those used in the West African study. This means that the results and the maps are comparable and can be laid side by side. In each case they highlight priority areas for intervention where high losses are currently being sustained and provide guidance on what level of expenditure are appropriate in each area over a particular time period.

Having now demonstrated that combining tsetse distributions, cattle populations and cattle population growth spread together with economic parameters, is viable in two very different parts of tsetse-infested Africa, there is a strong argument for extending this approach to cover southern and central Africa, so as to provide a complete set of comparable maps to help underpin continental targeting of resources in this area.

Table 3:Total Benefits over 20 years of removal of trypanosomiasis per country

Country	Area of tsetse fly belt (sq. km.)	Total benefit (US \$ million)	Mean benefit (US \$ per sq. km.)
Ethiopia	156 793	833.7	5 317.3
Kenya	128 905	589.9	4 576.1
Somalia	37 733	157.8	4 181.0
Sudan	310 084	485.1	1 564.4
Uganda	103 051	390.1	3 785.9
Total	736 566	2 456.6	3 335.2

Note: Benefits are discounted to their present value at an annual rate of 10%

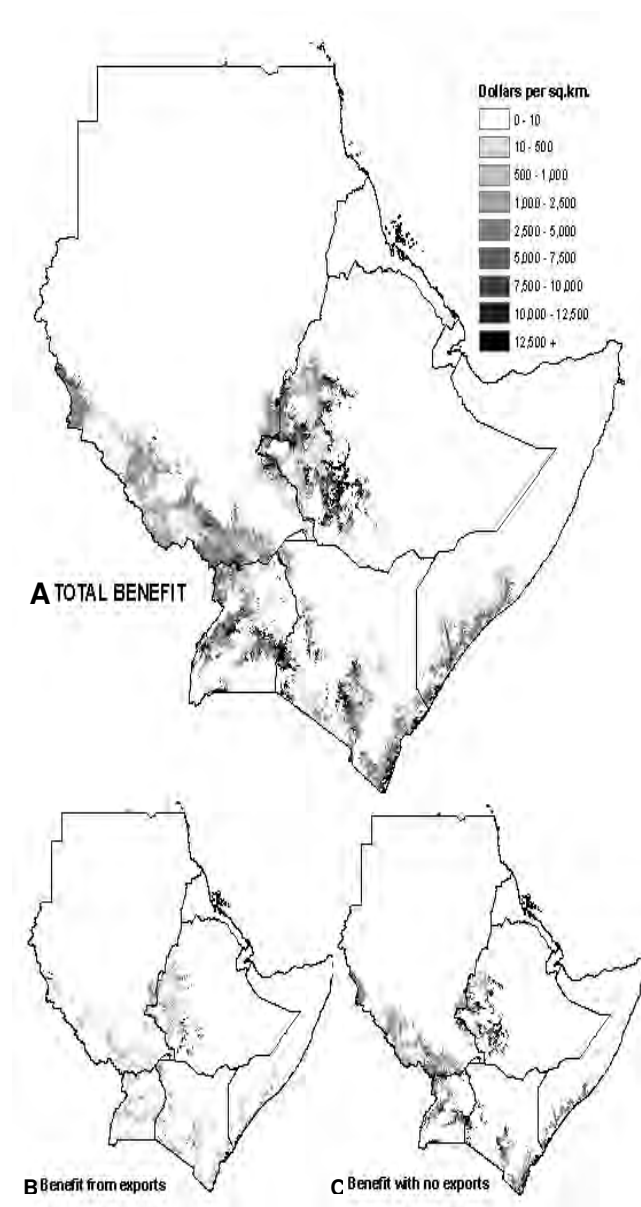


Figure 7: Total benefits after 20 years from the removal of trypanosomiasis

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**FOCAL POINT DEVELOPMENT APPROACH (FPDA): A
CONCEPT FOR COMMUNITY PARTICIPATION IN
TSETSE CONTROL PRACTICED BY COASTAL
COMMUNITIES IN KENYA/**

**APPROCHE DE DEVELOPPEMENT A TRAVERS LES
POINTS FOCAUX (FPDA), UN CONCEPT POUR LA
PARTICIPATION COMMUNAUTAIRE A LA LUTTE
CONTRE LES GLOSSINES PRATIQUE PAR LES
COMMUNAUTES COTIERES AU KENYA**

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Résumé

Le contrôle des mouches tsé-tsé à un niveau qui conduirait à un élevage profitable requiert une contribution significative du Gouvernement et un financement substantiel des bailleurs. La viabilité des initiatives entreprises à cet effet n'est pas assurée vu que la plupart de celles-ci s'effondrent quelques mois après l'achèvement du projet convoité ou le retrait du financement public. L'approche du développement par les points focaux est un concept de participation communautaire qui vise à maintenir la vision de la vie en communauté tout en donnant au paysan individuel la capacité d'identifier et de quantifier les avantages qu'il peut tirer lui-même de l'éradication de la mouche tsé-tsé. Les résultats des données collectées au bout de cinq ans depuis son démarrage et son adoption par les agriculteurs des districts de Kwale et de Makueni au Kenya montrent que la viabilité et la continuité du concept FPDA est tributaire de divers facteurs, parmi lesquels la politique gouvernementale, la vision de la communauté et les avantages acquis au niveau individuel par l'agriculteur. Aujourd'hui, ces communautés parviennent à élever des bovins laitiers et à reconstituer leurs stocks autochtones.

Summary

The control of tsetse flies to a level that would result in profitable livestock farming requires enormous Government inputs and donor funding. The sustainability of the same is not assured with most of such undertakings collapsing a few months after the coveted project completion or withdrawal of government funding. Focal Point Development Approach (FPDA) is a community participation concept that seeks to keep alive the communal vision while individual farmer is able to identify and quantify benefits gained by him from tsetse suppression. Results from data collected after five years since its inception and adoption by farmers in Kwale and Makueni Districts of Kenya show that the sustainability and continuity of the FPDA concept is dependent on various factors, among them government policy, vision of the community and benefits gained at individual level by the farmer. Today the said communities have managed to rear dairy cattle and restock their indigenous herds.

Introduction

Focal Point Development Approach is an innovative model initially designed to mobilize community to control tsetse flies in organized groups. It is an integrated approach which places emphasis on participatory and sustainable approaches in addressing tsetse and trypanosomiasis menace. The model identifies 10 critical steps that are crucial in impacting knowledge, eliciting interest and commitment by community in order to undertake tsetse control activities in sustainable way. It is therefore a qualitative process that helps farmers to conceptualise on communal and individual gains that would accrue on successful communal control activities and relate the same to improved livelihood. It recognizes the importance of group cohesiveness in meeting the objective and it therefore identifies generative themes (Paul F., 2000) that keep the group dynamism alive thus creating willingness to act to change the situation for the better and the ability to ensuring sustainability of those efforts (Flo F. *et al* 1999; Angela S. *et al* 2003; IFAD 2008). In a communal setup, comprehensive approaches are required in addressing the problems of distressed neighbourhoods because such complex, interrelated problems are better addressed in tandem than individually (Judy A. 1995). Hence the importance of developing FPDA model to solve some of community problems.

Objective of the study

The main objective of the study was to develop and evaluate a community based approach to tsetse control. The specific objectives were: (i) to assess psycho-social factors at household level that affect livestock rearing and production (ii) evaluate the level of adoption of the control technology and policy (iii) quantify the changes in cattle population and structure that has occurred over the study period.

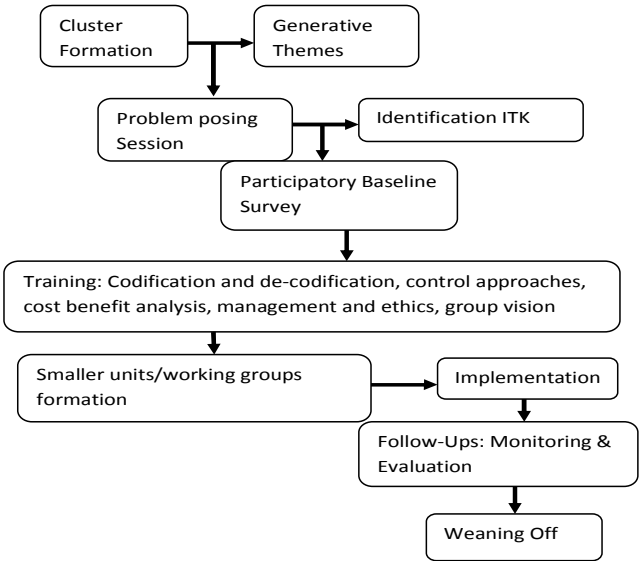
Research methods and methodology

This study was carried out in Kwale District, which lies in the Southern Coast of Kenya. Kwale District has an area of 8,257 km² with arable land comprising 7,313 km². It is divided into four major topographical zones ranging from 0 m to 842m above sea level (a.s.l.). The Coastal Plain Zone lies below 30m contour with a coral reef of 300m to 1000m from the shoreline whereas the Foot Plateau Zone rises abruptly and lies at 60m to 135m above sea level. The area borders Shimba Hills National Game Park from which are found several of the wildlife that acts as hosts to tsetse flies which infest the area, and as a source of bloodmeal. The land is generally flat with undulating hills and seasonal rivers. The vegetation consists of the relic tropical forest. The main source of income to the community is livestock keeping and crop farming. The tsetse species infesting the area are *Glossina pallidipes*, *G. brevipalpis* and *G.austeni* (Owaga *et al.*, 1995; Moloo *et al.*, 1980, Kamau *et al.*, 2008).

The research on Focal Point Development Approach (FPDA) as a community concept for tsetse control in Kwale District took 5 years from year 2003 to 2009. Five cluster groups were formed; Mrima, Mazumalume, Mafisini, Makobe and Mivumoni cluster each comprising of 50 to 70 household units. The clusters were thereafter subdivided into smaller working units or villages. The members of clusters were then taken through the critical steps of FPDA; problem identification, identification of generative themes, identification of indigenous knowledge on tsetse control (ITK), participatory baseline survey on the tsetse flies and psycho-social aspects of the community, training and empowerment, implementation of action plans, follow-ups and weaning off. The steps involving cluster formation to participatory baseline survey took three months while implementation and follow-ups was a continuous process for a period of not less than four years. Training was undertaken at two levels; staff training and community levels. These

were scheduled once in a year throughout the life of the study before its weaning-off. Community members used either spraying of livestock at a communal crush pen or used communal cattle dip in the village for to control tsetse flies using recommended synthetic pyrethroid. A cost benefit analysis was done by farmers to enable them decide on the right acaricides to use. At the end of the study period, data was collected from farmers using questionnaires on the benefits and changes realized within their homesteads following control of tsetse through initiatives of FPDA.

Fig 1: Critical steps involved in FPDA



Results

The study was intended to assess changes within the community and households in Kwale District who were participants in research on Focal Point Development Approach for tsetse control. The respondents were interviewed using a questionnaire and the data analysed using SPSS software with results presented in descriptive form.

A total of 70 respondents were interviewed; 14 drawn from each cluster at random. The respondents were all adults who were involved in implementation of the project to its weaning – off phase.

Psyco-social factors affecting livestock rearing within the coastal community

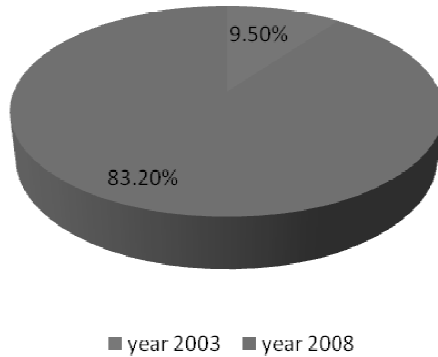
he age and gender factor were found to be significant in adoption of livestock and tsetse control technology within the coastal community. Of the total household respondents, 57.4% had the man as in charge of livestock records such as milk, birth, drugs and treatments. The households where the wife played an active role were 26.5%. The youth showed least interest in livestock related projects, with young men role standing at 4.4% on record keeping and nil for girls.

The formal education was also important in uptake of the technology. Those who had at least a formal education and felt that it was important in uptake and implementation of FPDA were 75.3% in total. Of this, 63.2% had primary education and 22.1 % had attained secondary school level of education.

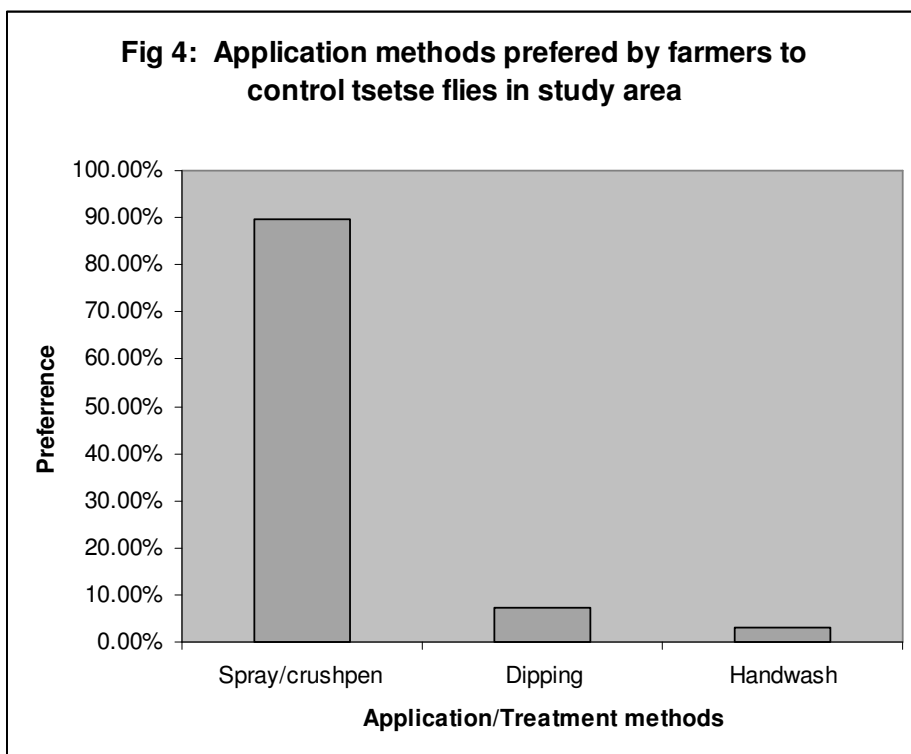
Impact of cattle cleansing policy on success of the tsetse control and sustainability

All households interviewed indicated that they used recommended pesticides by Government for control of the tsetse and ticks. Those who were using synthetic pyrethroids in year 2008 on their animals were 82.3% compared to 9.5% before the start of the study in 2003. The remaining 17.7% were either using organophosphates or amitraz on their livestock. However of those using synthetic pyrethroids alpha-cypermethrin, cypermethrin, deltamethrin, 60.3% preferred alpha-cypermethrin (Dominex^R) due to cost benefit with 10.3% using whatever other synthetic pyrethroid was available in the market.

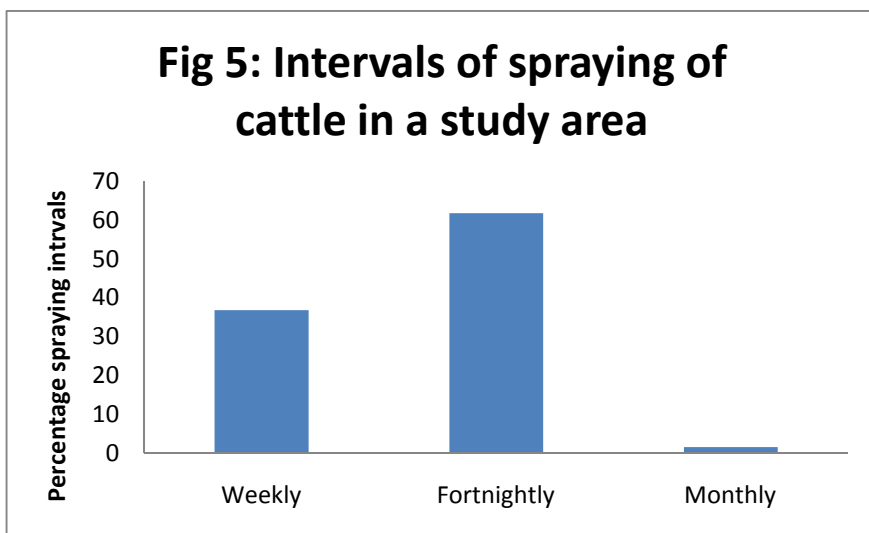
Fig 2: households using synthetic pyrethroids before and after the research



The results also showed that apart from cattle dipping and use of traps, other methods of controlling tsetse flies by community gained currency with community following the training on their use during the study period. Whereas prior to the inception of the study, the only practiced method for controlling tsetse in the study area was trapping, 96.8% had adopted and changed into using livestock as mobile targets by the end of the study. The crushpens were the most preferred technology of tsetse control with its adoption and usage standing at 89.8% compared to cattle dips whose usage stood at 7.4%.



The results also indicated that of those farmers using crushpen, the spraying intervals of cattle in order to control tsetse flies differed with 61.5% of respondents spraying their cattle on fortnightly basis, 36.6% weekly and 1.5% on monthly basis respectively.



Changes in cattle population and structure following implementation of FPDA in the study area

Before the inception of the study in year 2003, only 8.4% of farmers had dairy cows aged below 5 years, 4.4% between 5-10years. Those who had dairy cows aged above 10 years formed only a 1.5%.

On the other hand, farmers keeping dairy cows below 5 years in year 2008 were 20%. Of this the households with calves aged two years and below formed 16.2% showing that most of the calves were born or bought by farmers two years after the inception of the study. Another 12.3% had dairy cows that were aged between 5-10years. Only 2.9% had dairy cows aged above 10 years.

Fig 6: Farmers keeping dairy cows

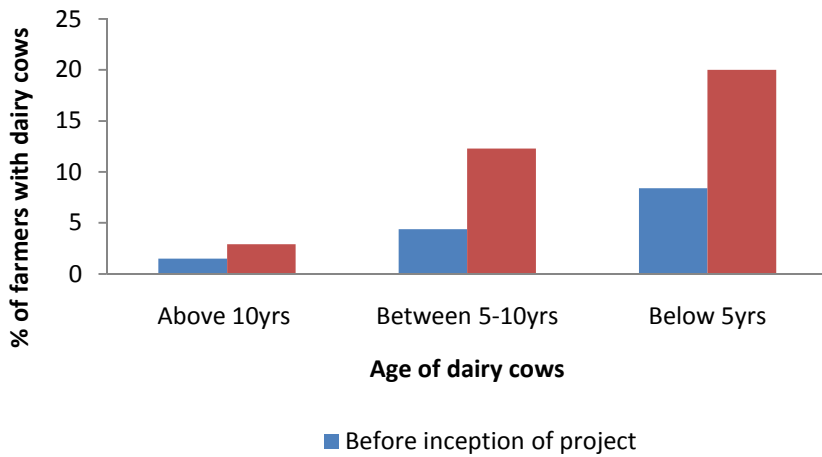
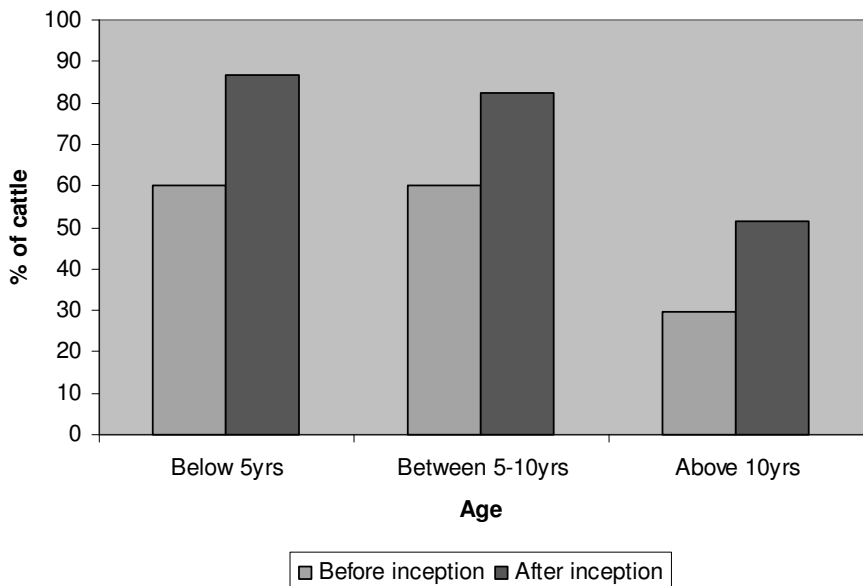


Fig 7: Population of indigenous cattle before and after inception of the project in the study area



The population of the indigenous cattle also increased during the life of the study. Those below 5 years increased by 26.5% while those aged between 5-10 years increased by 22.1%. Similarly the population of indigenous cattle aged above 10 years increased by 22.1% within a 5 year period.

Community perception on changes to their life in the course of the study

The community were positive that the project had changed their lives. Of the total respondents, 91.4% were in agreement that their livelihood had changed significantly for the better than it was before the inception of the project in terms of making decisions, savings and venturing into livestock rearing as a gainful economic activity.

Discussion

The ownership and responsibility over cattle in a household is important and need to be emphasized when implementing a community livestock project in the study area. Since the wife has been shown to be involved in making decisions on cattle to some extent, it is then essential to involve both in trainings on tsetse flies control and use of resources. Formal education was shown to play a pivotal role in adoption of a tsetse flies control technology within a community. The level of education was seen also as pivotal in training of the farmers on FPDA approach and influencing of community to contribute resources towards its tsetse control.

The preference to use livestock as mobile target instead of traps by community was more so due to the fact that they could graze them in the forests and that it did not require much attention unlike traps which would be emptied after every 24 hours. Farmers could easily identify with crushpens since they had experience of controlling ticks using different acaricides within their homesteads.

In Kenya, the structural adjustment programme of the in mid 1990s affected the diseases control programme. The cattle cleansing activities against ticks by use of cattle dips were placed under community. The government was then left with supervisory and technical advisory role after liberalisation policies on livestock sector (Baltenweck, *et al.*, 2000).

This move has continued to affect delivery of livestock services with many of cattle dips failing to operate and having collapsed leading to loss of livestock to tickborne and other vector borne diseases.

Increase in cattle population could be attributed to low mortality rates, reduced calving intervals, new calving and purchase of new animals into the study area. This would only be possible with an effective control measure for tsetse flies. Under FPDA concept, members of the community were able to gain confidence in their efforts to control tsetse flies and this had an effect of farmers restocking their livestock in the knowledge that they will not succumb to trypanosomiasis disease. The increase in cattle population below 5 years could be attributed to the low trypanosomiasis and tsetse challenge following adoption of FPDA concept in the five clusters by farmers. The high rate of uptake and adoption of crushpens and use of synthetic pyrethroids showed that with appropriate training, the community members would adhere to the guidelines set out in cattle cleansing policy.

Studies conducted by Okoth, (Okoth *et al*, 1991; Okoth, 1999a, 1999b) have indicated that some successes on community participation in tsetse control in Uganda when farmers are able to identify with a specific motivation. In his study, Okoth (Okoth *et al*, 1991; Okoth, 1999a, 1999b) was able to identify concerns of own health and likelihood of infection with sleeping sickness as the main motivation. In this study, it also shows that individual benefits are the overriding motivation factors in adopting use of crushpens as a technology for controlling tsetse flies in Kwale District. The findings of this study further shows that involvement of community and extension staff in the initial design of FPDA is important for it addresses jointly hindrances to control programmes. This is supported by the study conducted by Sindao (2008) which found that lack of involvement and participation by community in formulating the control strategies leads to their abandonment and collapse.

Prolonged and organized regular dipping or spraying of livestock with pyrethroid was cited by Hangrove (2000) as a factor that is thought to influence the success of control of tsetse flies. The FPDA is therefore able to organize the community living in an area and undertake a sustained campaign against flies for a longer period.

Recommendation

It is conclusive that community participation must address and outline to members on gains and benefits that are bound to be realized by individual members once they join their efforts together. Shared village and group vision should form basis of articulating the envisaged benefits to the group collectively and to households individually.

Training at different modules level forms the most important component of the FPDA in empowering the farmers. These should be encouraged and planned with emphasis placed on the generative theme in order to sustain attention of the learners who are adults and farmers. This study therefore conclusively shows that a well designed community approach and process of impacting knowledge and skills would bear fruits after one to two years into life of a FPDA project.

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ANNEXES

ANNEXE I: COMMUNIQUE

30TH ISCTRC MEETING KAMPALA, 21-25 SEPTEMBER 2009

The 30th ISCTRC Meeting was held at the Speke Commonwealth Resort in Kampala, Uganda between the 21st and 25th of September 2009. This meeting coincided with the 60th anniversary of the ISCTRC and the theme for the meeting was “Towards consolidating strategies to manage trypanosomiasis in Africa”.

The meeting was officially opened by the Rt Hon. First Deputy Prime Minister and Minister for East African Federation Hon. Enya Kategaya. The occasion was also graced by Hon. Hope Mwesigye Minister for Agriculture, Animal Industry and Fisheries. In a keynote address the Deputy Prime Minister, highlighted the impact of tsetse and trypanosomiasis on rural development and human welfare on the African continent. He urged scientists to focus on research areas that would contribute to the effective management of tsetse and trypanosomiasis.

Presentations focused on the following thematic areas: The Pan African Tsetse and Trypanosomiasis Campaign, Human African Trypanosomiasis, Animal African Trypanosomiasis, Glossina Biology and Control, Socio-Economics, Environment and Land Use. Position papers were delivered under each of the thematic areas.

During this meeting ninety seven (97) scientific presentations were made. Seventy one (71) papers were presented orally while twenty six (26) were presented as posters. Twenty (20) African countries presented their reports on progress of tsetse and trypanosomiasis activities since the last meeting held in Luanda Angola in the year 2007.

The meeting recognized the progress made in the implementation of the PATTEC initiative. However, the meeting observed that there were great variations in tsetse ecology requiring a careful selection of intervention areas, methods and tools for tsetse control/eradication. This calls for an integrated approach that is environmentally sensitive and which brings together all the relevant stakeholders. Developmental initiatives should be considered as an integral part of the planning process for tsetse and trypanosomiasis eradication projects. The meeting observed an increased participation of countries in the presentation of country reports. In

particular the meeting commended those countries that were presenting for the first time.

The meeting appreciated the efforts made by International Organizations to ensure that disease endemic countries have access to the right tools for tsetse and trypanosomiasis interventions. These tools include improved diagnostics/treatment, data collection, and analysis, reporting and sharing of information.

It was recognized that the training and mentoring of young scientists was crucial in addressing the growing manpower demands of tsetse and trypanosomiasis research/control. In this regard the meeting appreciated the efforts of organisations that have supported capacity building and appealed to others to contribute.

The meeting observed with great concern the widespread occurrence of counterfeit drugs and/or drug misuse and the resultant phenomenon of treatment failure. The general consensus was that private sector should work more closely with the relevant government arms to address the crisis in the shortest possible time.

The meeting appreciated the efforts made by scientists to generate new information on tsetse genetics which would be very useful in the planning of control/eradication programmes

The mandate and functions of the ISCTRC were discussed and the meeting passed a resolution which called for the strengthening of the council to enable it responds to emerging challenges.

The meeting made a number of recommendations that require implementation. Deserving scientists were awarded certificates and gold medals in recognition to their contributions to the research and control of tsetse and trypanosomiasis. Awards were also given to the first five best posters as a means of encouraging the production of high quality and educative posters in future meetings.

Participants expressed their gratitude to the Government and people of Uganda for accepting to host the meeting and for the warm hospitality that they received.

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