Identification of *Trypanosoma brucei rhodesiense* in wild Tsetse flies in Nkhotakota Wild Life Reserve by PCR

32\textsuperscript{nd} International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), September 8-12\textsuperscript{th}, 2013, Khartoum, Sudan.

JE Chisi
College of Medicine, University of Malawi
Introduction

Human Africa Trypanosomiasis

Nagana in Animals

Transmitted by tsetse flies (*Glossina* species)

From South of Sahara to Zimbabwe

Two major forms:

- *T. brucei rhodesiense*  
  (acute form: E.& Southern Africa)

- *T. brucei gambiense*  
  (chronic form: C. & Western Africa)
Areas Of HAT

HAT in Malawi has been reported in 3 districts

Rumphi/Mzimba – Vwaza Game Reserve

Nkhotakota – Nkhotakota Game Reserve

Kasungu – National Parks

Other District sporadic
Tsetse Areas

G. *morsitans* & G. *pallidipes* found in Vwaza, Mwabvi Game Reserves & Matandwe Forest Reserves

G. *m. morsitans* found in Kasungu, Nkhotakota, Dedza-Salima, Tuma Forest, Mangochi Forest, Liwonde & Lengwe

G. *pallidipes* found in Majete Game Reserve, Lengwe National Park

G. *brevipalpis* found in Elephant Marsh
INFECTION RATES
T.b=86/307 (28.0%)
T.s=78/307 (25.4%)
T.s.t=56/307 (18.2%)
T.g=45/307 (14.7%)
T.v=36/307 (11.7%)
T.c.s=17/307 (5.5%)
T.c.k=13/307 (4.2%)
T.c.f.=9/307 (2.9%)

T. brucei = ~480; T. c. f. = ~714;
T. c. k. = ~620; T. simiae = ~400;
T. s. tsavo = ~370; T. godfref = ~280;
T. vivax = ~250; T. c. savanna = ~69;
Rationale for Tsetse studies

There have been published reports of tourists getting infected with HAT after being bitten by tsetse flies while having animal safari in our Game Reserves. (Darby et al., 2008).

British Soldiers in Kasungu National Park have been infected (Croft et al., 2006., 2007.), which has negative implications on tourism.


Detection of trypanosomes in the vector indicates the presence of potentially infective human trypanosomes as well as Nagana in the surrounding areas.
Methodology – Two types of traps were used: Bicon and Epison.
Methodology-2: Microscopy-Samples of infected mid-guts
Methodology-3 Polymerase Chain Reaction Methods

**PCR (polymerase Chain Reaction)** - One set of primers is used

*T. brucei PCR* *(Moser et al., 1989)* which is specific for *T. brucei* species.

**ITS PCR** *(Njiru et al., 2004)* which identifies all species of trypanosome.
## Tsetse fly infections in the Midgut in Nkhotakota Game Reserve

<table>
<thead>
<tr>
<th>Country/Study sites</th>
<th>Total Catches</th>
<th>Total Dissected</th>
<th>Tsetse Species</th>
<th>Infected Organ Midgut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nkhotakota</td>
<td>257</td>
<td>242</td>
<td>G.morstans and G.pallidipes</td>
<td>206</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Locality/Sites</th>
<th>Tsetse Species</th>
<th>Condition</th>
<th>M</th>
<th>F</th>
<th>Total</th>
<th>Infection rates</th>
<th>Midgut pools</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malawi</td>
<td>Infected</td>
<td></td>
<td>90</td>
<td>11</td>
<td>206</td>
<td>85.1%</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>Non Infected</td>
<td></td>
<td>19</td>
<td>17</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dead</td>
<td></td>
<td>7</td>
<td>5</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Teneral</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>11</td>
<td>14</td>
<td>257</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Nkhotakota Game Reserve

**TBR PCR**

- 116/206 (56.3%) came out positive with TBR PCR.
- This represents a high potential infection risk to humans.
ITS PCR

- 206 samples
- 109/206 positive
- Of positives 75/109 T. brucei
- 68% of infection rates T. brucei
SRA Gene

- 75 samples analysed for T. brucei SRA gene
- 4/75 come out positive
- 5.3% infection rate of T. brucei rhodesiense
SUMMARY

Nkhotakota Game Reserve harbours a very effective transmitter for trypanosomes, the G. morsitans

Unexpected results of very high infection rates of trypanosomes in tsetse flies demonstrated by both microscopy as well as molecularly (similar trend has been established in Kasungu & Liwonde game reserves: Infection rate 45%)

There are mixed infections of tryps in some tsetse flies

J.E. Chisi et al 2013
Implications of the Results

- Tsetse flies in Malawi have the highest infection rates of *T. brucei* (spps).

- Microscopy & PCR have detected several species of Trypanosomes in Malawi.

- Malawian Disease is sub-acute (most parasites hide in lymph nodes & Bone Marrow) (Songa et al., 1991; Moore et al., 2002).

- Need to sample blood from both animals and human to establish whether the risk translates into infections in the human beings and in cattle, pigs, goats and wildlife in and around the reserves.

- Intensify tsetse trapping to reduce the number of flies in the park and blood meal analysis for the source of infection.
Thank you for your attention and Keep Smiling

Questions – Are Welcome
Acknowledgements

- Ministry of Health in Malawi
- Ministry of Tourism, Parks & Wildlife
- Ministry of Agriculture
- Makerere University for Sub-Award
- College of Medicine, University of Malawi