

Seasonal Variation and the Effect of Land use patterns and on the epidemiology of Trypanosomiasis in a previously tsetse free area - the Jos Plateau, Nigeria

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Introduction

Animal African trypanosomiasis (AAT), also called Nagana is a parasitic disease of great economic importance. 50 - 70 million cattle are at risk and 3 million die every year. It restricts livestock production over 10 million km and is responsible for economic losses of up to \$4.75 billion a year (1). Infection with these trypanosomes causes anorexia, anaemia, diarrhoea, staring coat, excessive lachrymation, emaciation, weakness and eventually death (2). The Jos Plateau in North Central Nigeria covers an area of 8000km² and an average altitude of 1280m. It was previously free of tsetse flies and trypanosomiasis. This lack of disease and abundant pasture attracted large number of cattle keeping pastoralists to settle in the area which now holds over a million cattle and plays an important role in the national/regional cattle industry. However, over the past twenty years tsetse flies have reinvaded the Jos plateau (3) and trypanosomiasis is now a significant problem. Little is known about the distribution and overall prevalence of the disease across the Jos plateau which is essential if successful interventions to reduce its impact are to be put in place. A longitudinal cluster survey was carried out in 2008 to determine the prevalence of trypanosomiasis in cattle using a suite of state of the art molecular tools. The Results on prevalence and how land use patterns and seasonal variations affect the epidemiology of trypanosomiasis in this area are presented here.

Materials and Methods

Sampling frame: The prevalence of trypanosomiasis in cattle on the Jos plateau was estimated using a longitudinal, stratified two stage cluster survey designed to give 95% confidence. The study area was stratified by river basin, with villages as primary clusters and a fixed number of cattle to be sampled in each village. Study sites were selected by drawing a grid over the area and selecting one village per cell. 80 cows were randomly selected for sampling from individual herds within selected villages during the dry season, early wet season and late wet season to enable assessment of seasonal variations. Blood was collected from each by venipuncture and applied directly onto Whatman FTA cards. Cards were air dried at room temperature for at least one hour and then stored in sealed envelopes with desiccant. The sex, age, breed and condition score of each animal was also recorded.

Extraction of DNA: Five 3mm discs were cut out of each sample using a Harris Micropunch. The discs were given two fifteen minute washes in 1ml of Whatman FTA purification reagent to remove haemoglobin which inhibits the PCR reaction. Used reagent was discarded after each

wash. They were then given two fifteen minute washes in TE buffer (10mM Tris, 0.1 mM EDTA, pH 8.0) to remove the FTA purification reagent and the used buffer discarded after each wash. Discs were dried for 30 minutes in an oven at 37°C. 100µl of 5% chelex suspension was added to dry discs and incubated at 90°C for 30 minutes to elute the DNA off the FTA discs. Eluted DNA solution was used for the PCR reactions as it was found to be more sensitive than using the dried FTA disc as recommended by the manufacturers (4, 5)

T. brucei Primer sequences (6)

TBR 1: 5' - CGA ATG AAT AAA CAA TGC GCA GT - 3'

TBR 2: 5' - AGA ACC ATT TAT TAG CTT TGT TGC - 3'

PCR amplifications were carried out in a 30µl reaction mixture containing 2.5µl Super Taq 10 x buffer, 0.5µl TBR1 (100pmoles/µl), 0.5µl TBR2 (100pmoles/µl), 0.2 µl 100mM dNTP, 1µl BIOTAQ Red DNA polymerase (1U/µl), 14.55µl Distilled water, 0.75µl MgCl and 5µl **sample** DNA. Thermal cycling was carried out in a Dyad Peltier thermal cycler as follows: Step 1: 94C for 3 minutes; Step 2: 94C for 1 minute; Step 3: 55C for 1 minute; Step 4: 72C for 30 secs; Step 5: Repeat steps 2 - 4 x 34 times; Step 6: 72C for 5 minutes.

T. congolense savannah Primer sequences (7)

TCS 1: 5' - CGAGAACGGGCACTTTGCGA - 3'

TCS 2: 5' - G GACAAACAAATCCCGCAC - 3'

PCR amplifications were carried out in a 30 µl reaction mixture containing: 2.5µl Super Taq 10 x buffer, 1.5µl TCS1 (100pmoles/µl), 1.5µl TCS2 (100pmoles/µl), 0.2 µl dNTP (100mM), 1µl BIOTAQ Red DNA polymerase 1U/µl, 12.55µl Distilled water, 0.75µl MgCl, 0.75µl Sample DNA. Thermal cycling was carried out in a Dyad Peltier thermal cycler using as follows: Step 1: 94C for 3 minutes, Step 2: 94C for 1 minute, Step 3: 55C for 2 minute, Step 4: 72C for 2 secs, Step 5: Repeat steps 2 - 4 x 29 times, Step 6: 72C for 5 minutes.

T. vivax Primer sequences (8)

ILO1264:5'-CAGCTCGCCGAAGGCCACTTGGCTGGG-3'

ILO1265:5'-TCGCTACCACAGTCGCAATCGCAATCGTCGTCTGAAGG-3'

PCR amplifications were carried out in a 30µl reaction mixture containing: 2.5µl Super Taq 10 x buffer, 1.5µl TCS1 (100pmoles/µl), 1.5µl TCS2 (100pmoles/µl), 0.2 µl 100mM dNTP, 1µl BIOTAQ Red DNA polymerase (1U/µl),

12.55µl Distilled water, 0.75µl MgCl, 5µl sample DNA. Thermal cycling was carried out in a Dyad Peltier thermal cycler as follows: Step 1: 94C for 3 minutes, Step 2: 94C for 1 minute, Step 3: 55C for 2 minute, Step 4: 72C for 2 secs, Step 5: Repeat steps 2 – 4 x 35 times, Step 6: 72C for 5 minutes.

Gel Electrophoresis: 1.5g of agarose was added to 150ml of 1 x Tris Borate EDTA (TBE) buffer (36mM Tris, 30mM NaH₂PO₄, 1mM EDTA, pH 8.0) stained with Ethidium bromide (0.5µg/ml). This was poured into a 30cm x 20cm mould. 15µl of the PCR product from the second round was run on this 1.5% Agarose gel alongside a 100bp graduation marker at 100V for 1hour on a Bio Rad Power Pac 300 machine. The gel was examined on a Bio Rad Gel Doc 2000 using Bio Rad Quantity One software.

Results and Discussion

Prevalence: Trypanosome infections were detected at 46.4% prevalence representing 3314 infected animals out of 7148 sampled. Of these 3314: 59% were *T. congolense*, 56% were *T. vivax* and 3% *T. brucei brucei* and 21% were mixed infections.

Table 1 Seasonal Prevalence of Trypanosomiasis

	Dry Season	Early Wet Season	Late Wet Season	Overall
<i>T. b. brucei</i>	3.0%	5.1%	1.4%	3.3%
<i>T. congolense</i>	30.8%	24.8%	26.0%	27.2%
<i>T. vivax</i>	23.0%	18.8%	29.9%	26.1%
All species	44.1%	46.7%	47.0%	46.4%

With village level data, 3 different patterns of seasonal variation were observed.

Group 1: In these villages, the prevalence of trypanosomiasis is lowest in the dry season and increases significantly in the wet season (dry season vs. early wet season $p = 0.008025$; dry season vs. late wet season $p = 0.003547$). This is the expected seasonal variation of tsetse transmitted trypanosomiasis: tsetse populations are low and of limited distribution in the dry season and increase and become more widely distributed as the wet season progresses (9)

Group 2: In these villages, the prevalence of trypanosomiasis remains at a constant level all year round. There with no significant difference in seasonal preference. This disease pattern points to a persistent population of tsetse flies which transmits trypanosomiasis all year round and is not affected by seasonal variations in the environment.

Group 3: In these villages, the prevalence of trypanosomiasis is highest in the dry season, decreases as the wet season begins and increases again towards the end of the wet season (dry season vs. early wet season $p = 1.1 \times 10^{-6}$; dry season vs. late wet season $p = 0.032598$; early wet season vs. late wet season $p = 0.003986$). This is contrary to the expected pattern of seasonal variation. There are three likely drivers for this reversed epidemiological picture: The dry season lasts for ~ 7months. It is a time of intense stress

for cattle on the Jos Plateau as there is very little food and water available. Cattle must walk several miles a day in search of adequate grazing and malnutrition is a grave concern; the tsetse flies found on the Jos Plateau are the riverine species *Glossina tachinoides* and *G. palpalis* (10) Only a few streams and rivers persist through out the rainy season and both cattle and the small residual population of tsetse flies are concentrated at these points, providing a very high level of host-vector contact; recent agricultural expansion by farming communities has prompted reclamation of pasture lands that pastoralists previously used. Introduction of irrigated farming also means that several ponds and rivers are surrounded by vegetable plots, restricting the access of cattle to drinking water. This exacerbates the effects of the harsh dry season and in many villages, has forced pastoralists to migrate to areas with lower land pressure and more abundant resources to avoid conflict and livestock mortality. This combination of natural resource conflict, stress, malnutrition, poor condition and high tsetse challenge has succeeded in ensuring very high transmission from a small population of vectors, and high prevalence of trypanosomiasis in the cattle population.

Table 2 Seasonal Prevalence of Trypanosomiasis in three epidemiological groups.

Group	Average Prevalence		
	Dry Season	Early Wet Season	Late Wet Season
Group 1	15.8%	62.25%	26.5%
Group 2	42.75%	43.4%	40.6%
Group 3	68.8%	17.4%	48.1%

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