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Distribution of trypanosomes within tsetse in northern Nigeria

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Background

Tsetse-transmitted trypanosomes are the etiological agents of African trypanosomiasis. This disease, a major contributor of poverty in Africa has been estimated to cause financial losses worth billions of dollars in potential agricultural production with *T. congolense*, *T. vivax*, *T. simiae*, *T. evansi* and *T. brucei brucei* being the major causative parasites. In Nigeria, several studies on the prevalence and distribution of trypanosomes in tsetse and mammalian hosts have shown varying infection rates of *T. vivax, T. congolense, T. brucei*, *T. simiae* and *T. suis* [1,2,3,4,5]. These authors have used conventional parasitological tools in the diagnosis of trypanosomes. However, this method that employs microscopy has been greeted with some shortcomings and therefore not completely reliable. For instance, microscopic investigation cannot be specific for genetically different but morphologically similar trypanosomes including its inability to detect immature or mixed infections. Another limitation can be seen in cases of very low infections where parasites may be very difficult to detect.

In order to overcome these challenges, specific and sensitive molecular tools have to be applied. ITS1-PCR is now widely used for trypanosomes diagnosis [6,7,8]. The application of these universal primers has been seen to considerably reduce the time and costs used in trypanosome diagnosis compared to species-specific PCR diagnosis. However, the use of any of these PCR tools is still perceived to be relatively expensive and therefore scarcely considered in disease diagnosis in Nigeria.

Presently, there is no epidemiological data showing the distribution of trypanosome parasites in Nigeria (NITR-conference communication, 2013). In order to attain elimination through an effective control programme, accurate information regarding the prevalence and distribution of *Trypanosoma* species in tsetse and mammalian hosts is urgently needed. In an attempt to partly achieve this, we screened tsetse flies collected in endemic areas of Niger and Bauchi (Yankari) States using PCR diagnostic tools. Information arising from this investigation will provide evidence in reassessing the disease risks in Nigeria.

Results and Discussion

We screened 488 tsetse flies of three species for the presence of trypanosomes using ITS1 primers. All samples that were PCR positive for *T. congolense* were then screened to determine which clade of this species they belonged to. The overall prevalence data for the three species and Savannah clade shows prevalence levels of *T. brucei, T. congolense* and *T. congolense* Savannah in the range 2.5–14 %. In addition, one case of *T. godfreyi* was detected in *G. tachinoides*, one case of *T. simiae* detected in *G. morsitans* and five cases of *T. congolense* Forest were detected—four in *G. tachinoides* and one in *G. morsitans.* This low infection rate of *T. godfreyi* and *T. simiae* in tsetse could be an indication of low transmission rate of these parasites in Yankari, a game reserve which is home to some wildlife animals [9].

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To assess transmission potential to a little extent, DNA preparations from abdomen (ABD) and head + proboscis (H+P) samples were screened from the majority of the flies. For *T. congolense,* there were very few flies that were positive in both locations and most infections were found only in the abdomen samples (χ^2=8.47, d.f.=1, p>0.01). We would offer two potential explanations for this result. Firstly, these data may simply reflect the known biology of the *T. congolense* life cycle [10,11]; the positive samples in the abdomens reflect immature infections and only a subset of these would expect to develop into mature infections in the mouthparts. Also, some flies would expect to be too young when trapped for mature infection to have developed. Second, many abdomen samples will have contained blood which is an inhibitor of PCR. In contrast, *T. vivax* was detected only in the H+P samples (χ^2=20.05, d.f.=1, p>0.001), reflecting that these parasites develop only in the mouthparts [12]. The data for this parasite however give useful guidance that only active infections have been detected; any *T. vivax* parasites ingested into the midgut in the act of tsetse feeding will have been killed and the PCR procedure employed has not detected any signal from the degraded remains of the DNA from the dead parasites.

The differences in prevalence between male and female flies of each of the three species of tsetse are shown in Table 1 for all trypanosome infections, *T. congolense*, *T. congolense* Savannah and *T. vivax*. The general trend is that infection prevalences were higher in female than in male flies, except for *T. congolense* and *T. congolense* Savannah in *G. palpalis*. To investigate the differences in prevalence between the sexes and host species more rigorously we undertook a forward step-wise regression analysis, the outputs of which are summarized in Table 2. As these results show, the higher prevalence of infection in female flies compared with male is almost entirely caused by the differences in prevalence in *T. vivax* infections (Table 1). The differences for *T. congolense* show the same trend but are not significant. In contrast, the data for species differences show that it is the lower prevalence of *T. congolense* in *G. palpalis* compared with the other two species (Table 1) that is statistically significant (Table 2). This finding needs to be treated with considerable caution however, as there is the confounding variable that the *G. palpalis* samples are from Niger whereas *G. tachinoides* and *G. morsitans* are both from Bauchi (Yankari) i.e. the real effect may be a difference in site rather than species but we do not have multiple species from both sites that would allow us to analyse this potential difference.

Table 1. Comparison of % prevalences of trypanosomes in male and female flies.

Species	sex	n	All trypanosomes (%)	*T. congolense*	*T. congolense* Savannah	*T. vivax*
G. *palpalis*	Male	98	6.1	3.1	3.1	3.1
	Female	100	8.0	2.0	2.0	6.0
G. *tachinoides*	Male	40	2.5	2.5	2.5	0
	Female	161	14.3	9.9	6.2	5.6
G. *morsitans*	Male	17	11.8	5.9	0	0
	Female	72	13.9	8.3	8.3	5.6

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Table 2. Logistic regression analysis of the effects of sex and tsetse species on infection with trypanosomes. Significant results are shown in bold.

Output variable	Sex		Species	
	t-values	p	t-values	p
All trypanosomes	**1.97**	**0.05**	1.43	0.16
T. *congolense*			**2.40**	**0.02**
T. *congolense* Savannah			**2.02**	**0.04**
T. *vivax*	**1.98**	**0.05**		

Conclusion

The screening of tsetse reveal that there is active transmission of trypanosomes in these areas in which *T. vivax* and *T. congolense* Savanna are likely to be the main parasites responsible for African Animal Trypanosomiasis (AAT) in livestock. We advocate that screening for trypanosomes should be extended to livestock in these endemic regions and beyond using molecular tools. This undoubtedly will provide better and more reliable information on the distribution of *Trypanosoma* species in Nigeria with the view of effectively adopting the most appropriate control measures in achieving disease elimination.

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