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## Presence of Trypanosome Species and Determination of Anaemia in Trade Cattle at Sokoto Abattoir, Nigeria

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### ABSTRACT

A survey for the prevalence of trypanosomes species and the anemic status of trade cattle presented for slaughtering at Sokoto main abattoir, Northwest Nigeria was conducted between January and June, 2008. Blood samples were collected at ante-mortem via jugular vein and examined by Standard Trypanosome Detection Methods (STDM). Anaemic status was determined by Packed Cell Volume (PCV) and FAMACHA® Anaemic Guide technique while Polymerase Chain Reaction (PCR) technique was used to detect the presence of *Trypanosoma brucei* group. Out of 500 samples analyzed by STDM, 9 (1.8%) were positive out of which 6 (66.7%) had *Trypanosoma vivax*. The PCR technique detected 22 (4.4%) positive cases of *Trypanosoma brucei* group while 45 (9.0%) cattle were anaemic using the PCV and FAMACHA® techniques, respectively and White Fulani breed had the highest infection rate with 5 (55.6%) cases. These findings are significant as the study area has earlier been declared as tsetse free zone, transhumant activities as practice largely by the cattle herders may be responsible for these detections. Presence of other mechanical vectors may lead to rapid spread of the infection which may have adverse effects on productivity of the animals with resultant economic losses. The detection of the *T. brucei* group in the examined trade cattle may also portend danger to public health as some ruminants and pigs have been incriminated as reservoir hosts of the Human African Trypanosomosis (HAT) agents in some parts of Africa. Statewide surveillance is therefore, needed to establish the true prevalence of the infection in all domesticated animals in the study area.

**Key words:** Bovine, trypanosome species, Famacha® Anaemia Guide, packed cell volume, polymerase chain reaction

### INTRODUCTION

African trypanosomosis is a debilitating disease of man and domestic animals. It is caused by haemoflagellate of the genus: trypanosome, family: trypanosomatidae transmitted by tsetse flies (*Glossina spo*) (WHO, 1998) and its characterized by parasitaemia, fever, anemia, loss of condition reduced productivity and frequently high mortality which among the other factors limit the pace of rural development in tropical Africa (Abenga *et al.*, 2002; Swallow, 2000; Fajinmi *et al.*, 2007). Trypanosomosis complex is described by the World Health Organization (WHO) as serious diseases

lacking effective control measure and all mammalian species are susceptible to the infection (Cattand *et al.*, 2005). The lack of effective control measures may be attributed to the emergence of conventional trypanocidal drug resistant strains which may be overcome by the use of some medicinal plants and natural products (Bala *et al.*, 2010; Faremi and Ekanem, 2011).

In Africa countries where trypanosomosis occurs, it is one of the major public health problems. The epidemiological trend indicates that it is wide spread in 36 sub-saharan countries for Human African Trypanosomosis (HAT) and 37 countries for Animal African trypanosomosis (AAT) covering over nine million square kilometers between Latitude 14°N and 29°S, approximately one third of Africa's total land area (NITR Annual Report, 1989; Swallow, 2000; Steverding, 2008). About 60 million people and 50 million cattle are risk of being infected with up to three million livestock death annually. Less than 15% of cases in human are diagnosed and treated (Kristjanson *et al.*, 1999; Kamuanga, 2003; Cecchi and Mattoli, 2009). The control of the disease has further been complicated by evidence of potential wild and domesticated animal hosts (Abenga and Lawal, 2005; Chreihen and Smoak, 2005). 21.3% of 402 small ruminants and pigs examined in Kenya were positive for trypanosomes of which 20 (5.0%) were positive for *T.b. rhodisiense* through the identification of Serum Resistance Associated (SRA) gene by PCR technique (Ngayo *et al.*, 2005).

The epidemic solely affects individual, families/communities and disrupts their economy posing a serious obstacle to agriculture and livestock development. Generally human population such as farmers, shepherds, herdsmen, hunters, tourists, merchants, researchers are at risk of exposure to trypanosomosis (PHAC, 2001; WHO, 1998). Human infection is caused by *Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodensiense* resulting in sleeping sickness (Picozzi *et al.*, 2002; 2005). Sleeping sickness is hallmarked by sleep and wakefulness disturbances with the sleep pattern fragmented (Lundkvist *et al.*, 2004). *T. vivax* and *T. congolense* on the other hand affect the animals predominantly. The risk of transmission is primarily linked to the intensity of the encounters between vectors and host (Putt *et al.*, 1988; Michel *et al.*, 2002). Apart from tsetse flies which are major transmitter of the African animal trypanosomosis, other mechanical fly vectors have also been incriminated. *Trypanosoma evansi* in camels however are known to be transmitted mainly by haematophagus flies such as *Tabanus*, *Lyperosae* and *Haematobia* species (Kamal, 2008; Derakhshanfar *et al.*, 2010).

Animal trypanosomosis has been ravaging several parts of the agro-ecological zones of Nigeria and the distribution follows the pattern of tsetse fly spread covering about 80% of the land mass between latitude 4°N and 13°N. Jos, Mambilla and Obudu highlands which had been hitherto declared as tsetse and trypanosomosis free zones are now infested (Onyiah *et al.*, 1983; Onyiah, 1997; Dede *et al.*, 2005; Ahmed *et al.*, 2005). A recent report from a North central state of Nigeria revealed an overall trypanosome infection rate of 1.5% among sheep and goat over three seasonal variations (Samdi *et al.*, 2010).

In Sokoto State, the status of animal trypanosomosis is not well documented as the state had been unofficially declared a tsetse free zone. Transhumant activities intra and inter-state and across the international borders abound and may encourage introduction of the infection to the hitherto declared free zone. There is thus the need to establish and update knowledge on the existence of the infection in the study area in order to prepare effectively for control and eradication.

## **MATERIALS AND METHODS**

**Study area:** The study was conducted between January and June 2008 at the Sokoto main abattoir. The nearby feeder cattle market is a converging area for animals from the twenty three

local government areas of the state, neighbouring states and some other West African countries. Sokoto is within Sudan Savannah zone, lies between latitude 10° and 14° North and longitude 3° and 9° East with mean annual rainfall of 1022 mm, mean relative humidity of 50% and daily temperature range of 27 and 48°C. Majority of the rural dwellers are predominantly subsistent crop farmers and herdsmen who migrate with their animals during hot dry season towards the middle belt areas of the country in search of greener pasture. There are sedentary animal population within major towns that are kept semi-intensively and allowed to scavenge for food and water or herded together to a common grazing and watering sources on daily basis. Sokoto state has a land area of 102,500 sq km<sup>-1</sup> (39,5655 sq miles), human population of 4,392,400 and an estimated population of 1,772,830 cattle (RIM Report, 1992).

**Sample size:** The abattoir was visited daily for seven days to estimate slaughter capacity per day. The obtained figures were pooled and averaged to obtain the sample size frame. Animals to be sampled were selected by systematic sampling technique whereby every nth unit in the population is sampled (Putt *et al.*, 1988). The sampling interval (K) was determined by the use of formula  $K = N/n$  (where N= total number of animals available and n= the number rpequired in the sample). A random number (r) falling between one and K is then chosen by the use of card shuffling physical randomization technique (Cameron, 1999; FAO Animal Health Manual, 1999).

**Clinical examination:** The selected animals were examined at ante-mortem for classical clinical manifestations of trypanosomiasis signs such as fever, emaciation, bi-ocular discharge, enlargement of superficial lymph nodes and palour of mucous membrane. Eye colour chart (FAMACHA Anaemia Guide) was also used to detect change in colour of eye mucous membrane to determine the anaemic status (Grace *et al.*, 2007).

**Sample collection:** Five milliliters of blood was collected from the jugular vein of each sampled cattle into ethylene tetra-acetic (EDTA) bottles, kept in cooler box and transported immediately to the Veterinary Public Health laboratory, Usman Danfodio University, Sokoto for processing. Likewise, a drop of whole blood from each sampled animal was placed on DNA binding matrix (Whatman® FTA classic cards); air dried, placed in container with hydroscopic dessicant and then stored at room temperature before processing (Picozzi *et al.*, 2002, 2005).

**Parasitological analysis:** The obtained blood samples were analysed using the following Standard Trypanosome Detection Methods (STDM): Wet blood film, thick blood film, thin blood films, microhaematocrit centrifugation technique and animal inoculation method (WOAH, 2004). Motility in wet film and morphological appearance in Giemsa stained films were used to identify the trypanosome species. Organism with a free flagellum, very well developed undulating membrane and a small subterminal kinetoplast was classified as *T. brucei* organism with a medium sized marginal kinetoplast but without a free flagellum and inconspicuous undulating membrane was identified as *T. vivax* while organism with no free flagellum, inconspicuous undulating membrane and marginal and sub-terminal kinetoplast was identified as *T. congolense* (Stephen, 1986; Onyiah, 1997; Abenga *et al.*, 2002).

Packed Cell Volume (PCV) was also determined for each of the blood samples examined as haematological index for anaemic conditions.

Table 1: Reaction mixture used for PCR amplification

Reaction mixtures	Conc. ( $\mu\text{L}$ )
Super taq 10 x buffer	2.5
TBR1 (100 pmoles $\mu\text{L}^{-1}$ )	1.00
TBR 2 (100 pmoles $\mu\text{L}^{-1}$ )	1.00
100 mM dNTP 0.2 $\mu\text{L}$	
BIOTAQ red DNA polymearse 1U	1.00
Distilled water	14.55
MgCl	0.75
Sample DNA	5.00

**PCR analysis:** DNA was extracted from Whatman<sup>®</sup> FTA cards that were spotted at the Sokoto abattoir as described by Picozzi *et al.* (2002, 2005). The samples were tested specifically against *T.brucei* using TBR-PCR due to its public health significance. The TBR PCR uses the kinetoplast minicircle DNA of *Trypanosoma brucei* as a target and gives rise to a 177 bp PCR product. It can be used for the detection of *Trypanosoma brucei* infection in human, animals as well as tsetse flies (Katakura *et al.*, 1997) and it is approximately equivalent to one parasite. The following oligonucleotide primers sequences were used in the PCR analysis:

TBR1, 5' CGA ATG AAT AAA CAA TGC GCA CT 3'

TBR 2, 5' AGA ACC ATT TAT TAG CTT TGT TGC 3' (Artama *et al.*, 1992).

**Amplification of DNA:** PCR amplification was carried out in a 25  $\mu\text{L}$  reaction mixture as shown in Table 1.

**Thermal cycling:** Thermal cycling was carried out in a Dyad Peltier Thermal Cycler using the following conditions.

- 94°C for 3 min
- 94°C for 1 min
- 55°C for 1 min
- 72°C for 30 sec
- Repeat steps 2-4, 35 times
- 72°C for 5 min

The PCR amplifications were conducted in 25  $\mu\text{L}$  reaction mixtures containing as final concentrations: 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200  $\mu\text{M}$  each of the four deoxynucleoside triphosphates (dNTPs) and 1 unit to Taq DNA polymerase (REDTaq SuperPak DNA Polymerase, Sigma). These concentrations were the same for both primer sets. 0.4  $\mu\text{M}$  (TBR1/2). Two milliliter of DNA eluate extracted from a disk (2 mm) from the sample prepared on FTA<sup>®</sup> cards were added as template DNA to the final PCR reaction. A DNA-positive control, as well as a negative control, was used every time a set field samples was tested. Amplifications were carried out using a Peltier thermal Cycler (DNA engine DYAD). For TBR1/2, cycles consisted of an initial denaturing step at 94°C for 45 sec, an annealing step at 60°C for 1 min and an extension step at 72°C incubation for 5 min.

**Gel electrophoresis:** 1.5 g of agarose was added to 150 mL of 1x tris Borate EDTA (TBE) buffer (36 Tris, 30 mM NaH<sub>2</sub>PO<sub>4</sub>, 1 mM EDTA, pH 8.0) stained with Ethidium bromide (0.5 µg mL<sup>-1</sup>). This was poured into a 30 x 20 cm mould. 15 µL of the PCR product from the second round was run on this 1.5% Agarose gel alongside a 100 bp graduation marker at 100 V for 1 h 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.

The total 25 µL of amplification mixture was loaded onto a 1.5% agarose gel and electrophoresis was processed for 40 min at 100 V. The gel was stained with Ethidium bromide and the samples were examined under UV light.

**Data analysis:** Spearman correlation coefficient (SAS, 1999 version) was used to examine the relationship between the eye scores, PCV and the trypanosomes. Arithmetic means and standard errors were also used to determine the relationship between trypanosome infected animals and their PCV.

## RESULTS

Out of the total 500 cattle examined 199 (39.8%) were males with 5 (2.5%) positive cases and 301 (60.2%) females with 4 (1.3%) cases. The overall prevalence rate obtained was 9 (1.8%) out of which 6 (1.2%) were *T. vivax*, 2 (0.4%) *T. brucei* and 1 (0.2%) *T. congolense*. The haematocrit centrifugation technique detected more trypanosome species than others in the parasitological analysis (Table 2). The White fulani breed harboured most of the trypanosomes above other breeds encountered in the course of this study (Table 3).

The FAMACHA eye score chart rated as ordinal (ranking) scale (Thrusfield, 1997) indicating scores 1, 2 and 3 as non anaemic while 4 and 5 scores are anaemic. The obtained values for non-anaemic through FAMACHA corresponds to non-anaemic through the PCV values obtained and vice-versa for the anaemic animals (Table 4).

The haematological parameters obtained are as shown in Table 5 and there are significant variations in the normal values and the obtained values for infected and uninfected cattle.

Two of the rats that were inoculated with infected blood died four days later and the histopathology of the kidney revealed fatty degeneration, coagulative necrosis and liquefactive necrosis. Other haemoparasites encountered through parasitological analysis include *Babesia bovis*,

Table 2: Prevalence of bovine Trypanosomes species in slaughtered cattle (500) at Sokoto abattoir using STDM

Method	No. positive for trypanosomes
Wet blood	5(0.1)
Thin smear	3(0.6)
Thick smear	3(0.6)
Microhaematocrit	9(1.8)

Note: In parentheses are the percentages

Table 3: Breed distribution of Trypanosomes species in examined slaughtered cattle at Sokoto abattoir using STDM

Breed	No. of animals sampled	No. of animals + ve	<i>T. vivax</i>	<i>T. congolense</i>	<i>T. brucei</i>
White Fulani	120	5 (4.2)	3 (2.5)	1 (0.8)	1 (0.2)
Red Bororo	140	3 (2.1)	2 (1.4)	0 (0.0)	1 (0.7)
Sokoto Gudali	140	1 (0.7)	1 (0.7)	0 (0.0)	0 (0.0)
Ketekou	100	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	500	9 (1.8)	6(1.2)	1 (0.2)	2 (0.4)

Note: In parentheses are the percentages

Table 4: Presence of trypanosomes in relation to FAMACHA scores and PCV values in the examined slaughtered cattle at Sokoto abattoir

Famacha Score	PCV % Score Grade	No. of cattle	Mean±SE	No. infected with trypanosome (n=9)
1	> 28	352	32.90±0.9	1
2	23-27	103	29.4±0.9	2
3	18-22	40	21.4±0.3	2
4	13-17	5	15.4±0.3	4
5	> 12	0	0.0±0.0	0
Total		500		9

Table 5: Average haematological values of trypanosome infected and uninfected slaughtered cattle at Sokoto

Parameters	Infected cattle	Uninfected cattle	Normal values
Hb (gm %)	8.20±0.20	10.40**±0.00	11.6±10.05
PCV	38.50±0.50	44.50*±0.50	44.50±0.37
TEC x 10 <sup>6</sup> /μL	5.65±0.15	7.70**±0.10	8.14±0.05
TLCx 10 <sup>6</sup> /cumm	13.25±0.25	11.10**±0.10	11.30±0.03
DLC			
Neutrophils (%)	45.50±0.50	26.50±0.50	26.12±0.85
Lymphocytes (%)	40.00±0.00	62.00*±1.00	62.00±0.85
Monocytes (%)	6.50±1.5	6.00±0.00	5.98±0.48
Eosinophils (%)	8.00±2.00	5.50±0.50	5.40±0.65
Basophils (%)	0.00±0.00	0.00±0.00	0.50±0.29

\*p<0.05, \*\*p<0.01, PCV = packed cell volume, TEC= Total erythrocytic count, TLC= Total Leucocyte count, DLC= Differential leucocyte count, Values are presented as Mean±SE

*Babesia gibsoni*, *Babesia bigemina*, *Babesia divergens*, *Anaplasma bovis*, *Anaplasma centralis*, *Anaplasma maginale* and *Microfilaria* species.

Out of the 500 blood samples examined for the presence of *T. brucei* group using the polymerase chain reaction technique, 22 (4.4%) were positive. The two positive samples from STDM were also positive at this level.

## DISCUSSION

The study area is a converging place for trade animals coming from different local government areas of the State, neighbouring States and across the international borders of Republic of Niger, Republic of Benin, Republic of Chad and occasional from the Northern parts of other West African States. Also the animal rearing activities in the study area is basically on extensive method where there is little restriction to the movement of animals. All these make it difficult to ascertain the origin of the animals presented at the abattoir for slaughter, thus inability to trace back the infection (Thrusfield, 1997).

The Standard Trypanosome Detection Method (STDM) is a combination of different techniques used to avoid misdiagnosis of the infection. The microhaematocrit centrifugation technique detected most of the infection than others and this is in agreement with Omotainse *et al.* (2004) and Kalejaiye *et al.* (1995, 2004) that obtained a higher infection rates of between 8.5 and 15% in some ruminants by the use of buffy coat technique in some parts of Nigeria. Buffy coat and haematocrit centrifugation are still regarded as the best parasitological techniques for quick detection of the parasite as they concentrate the trypanosomes allowing for quick and clear visualization (Chappuis *et al.*, 2005).

The obtained prevalence rate of 1.8% through the STDM is significant as the study area has been unofficially declared a tsetse and trypanosomosis free zone. This declaration might have been borne out of the fact that the study area is in the extreme northwest of the country and always experience high environmental temperature and low humidity all year round which is unfavourable for the development of tsetse flies (the major vector of the infection) (Ahmed *et al.*, 2005). It has been found out that the *Morsitans* and *Palpalis* groups are most prevalent in the Savannah zones (covering mainly the northern parts of Nigeria) and are found along the drainage lines and riverine vegetation (Bourn *et al.*, 2001). The study area is drain mainly by River Sokoto and River Rima and there is the tendency of tsetse flies that incure into this microclimate to survive and become focal source of infection. Biting flies such as *Tabanids* and *Stomoxys* have been incriminated also as mechanical vectors (Ahmed *et al.*, 2005) and environmental conditions of the study area favour their development.

Most of the cattle reared within the study area are kept semi-intensive and extensively and there is seasonal migration of the Fulani herdsmen who are in possession of a large number of cattle herds during dry season towards the middle belt in search of greener pasture and water. The middle belt region is known to be endemic for trypanosomosis as there is abundance of tsetse flies. The exposed cattle at the beginning of rainy season in the northwest returned possibly with the infection and this can be spread by mechanical vectors on their arrival. Transhumant activities of the herdsmen encourages the long range flying tsetse to follow the animals along the cattle route thus spreading the infection among the sedentary herds closer to routes (Onyiah, 1997). Gibson and Brady (2008) reported that wild male and female tsetse are known to have the ability to travel at ground speed of 5-5-6.5 ms<sup>-1</sup> and at angular velocity of 350-400° sec The white Fulani breed haboured most of the parasites in the examined cattle, this breed is the most predominant breed in the study area (RIM report, 1992) and are known to be highly susceptible to trypanosomosis (Putt *et al.*, 1988).

*Trypanosoma vivax* was found to occur mostly in this study and they are known to be dominant species in ruminants in most parts of northern Nigeria (Omotainse *et al.*, 2004). This parasite is haematinin which are found mainly in the plasma. Its infestation leads to rapid destruction of erythrocytes which leads to anaemia and rapid weight loss thus reduction in market value of the animals (Losos and Ikede, 1972). In Nigeria an estimated 837.2 million Naira annual losses from cattle has been attributed to trypanosomosis (NITR Annual Report, 1996). Death of two mice at the fourth day of inoculation with infected blood is suggestive of the presence of *T. brucei* group. The vascular tissue destruction at histopathology also lends credence to their presence. *T. brucei* groups are usually found in extravascular and intravascular areas leading to destruction of the invaded tissues resulting in abortion, perforation of intestine, haemorrhagic anaemia, lower milk production and central nervous system involvement (Anosa *et al.*, 1992).

The overall average mean value of haemoglobin, PCV, TEC and TLC for uninfected cattle was higher than the infected ones. This may be due to destruction of red blood cells by the hematinin trypanosomes in infected ones (Anosa, 1988) and may also be attributed to the inhibition of erythrocyte formation in the bone marrow or their lysis by endotoxin liberated by trypanosomes. High environmental temperature as obtainable in the study area may equally altered the biochemical and haematological values alongside the presence of the parasites (Azab and Abdel-Maksoud, 1999; Anosa, 1988). The total WBC counts in all the infected animals were higher than the uninfected ones, this is as a result of the response of the affected animals to the infection (Anosa, 1988). There were no appreciable changes in monocyte and basophil counts between the



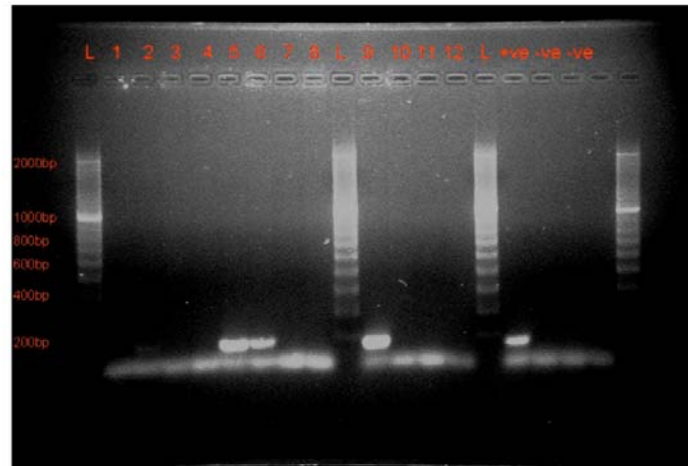


Fig. 1: PCR-TBR gel images of some positive samples from cattle slaughtered at Sokoto abattoir (Note: Lanes 5,6,9 show clear bands at 177 bp as in the positive control)

two groups and this is in agreement with Rajkhowa *et al.* (2003) who found out that during naturally occurring trypanosomosis the values of monocytes and basophils are only slightly affected.

Anaemia is a major hallmark for trypanosome infections and the use of FAMACHA eye chart in the study compliment what was obtained through the PCV values (Grace *et al.*, 2007). The high percentage of animals recorded with the anaemic condition may be as a result of extraneous factors such as the state of nutrition of the host, the presence of other inter-current infections of infestations and stresses caused by work, thirst, parturition, lactation and trekking (Bourne *et al.*, 2001) all of which are obtainable in the study animals and the study area. Other haemoparasites encountered include *Babesia bovis*, *Babesia gibsoni*, *Babesia bigemina*, *Babesia divergine*, *Anaplasma bovis*, *Anaplasma cetralis*, *Anaplasma maginale* and *Microfilaria* species. These blood parasites may occur concurrently with trypanosomes species in animals contributing to anemic condition and some other indistinguishable clinical and pathological signs (Hursey, 2001).

Out of 500 bovine blood samples examined for the presence of *T. brucei* groups using polymerase chain reaction (TBR-PCR) technique 22 (4.4%) were positive (Fig. 1). The obtained value is higher than the STD values in multifold and this is in agreement with Clausen *et al.* (1998) and Picozzi *et al.* (2002). Though a study in some two eastern states of Nigeria used ELISA technique to detect 3.03% infection rate due to *T. brucei* group (Ezeani *et al.*, 2008) the PCR technique is known to combine the advantages of a greater sensitivity than the parasitological or immunological techniques with the added advantage of differentiating trypanosomes which have a similar morphology but very different economic impacts (Duvallet *et al.*, 1999; Picozzi *et al.*, 2002; Thumbi *et al.*, 2008). The *T. brucei* groups have severe medical and economic impact on human and animals in most parts of sub-saharan Africa. The *T. b gambiense* causes a chronic, slow wasting disease that can eventually lead to death in West and Central Africa while *T. b rhodesiense* is acute and rapidly fatal, found mostly in East Africa. *T. b brucei* is found throughout the sub-Saharan

Africa and have profound effect on animal population but its not infective to man due to its rapid lyses by human serum (Krafsur, 2009).

The use of PCR technique in this study has revealed that there are far more trypanosomes infected animals circulating in the study area than previously thought of, thus the need for a comprehensive epidemiological survey to establish the true prevalence of the infection in order to prepare effectively for its control.

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