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Full Length Research Article

INVESTIGATION OF LIVESTOCK FOR PRESENCE OF *TRYPANOSOMA BRUCEI GAMBIENSE* IN TAFA LOCAL GOVERNMENT AREA OF NIGER STATE, NIGERIA

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ABSTRACT
The study investigated the presence of *Trypanosoma brucei gambiense* in livestock to ascertain their reservoir role and also screened for other pathogenic trypanosomes of animals in Tafa Local Government Area of Niger state, Nigeria. A total of 460 livestock comprising (cattle, sheep, goats, and dogs) selected at random were bled, examined using the buffy coat and Giemsa stained thin film and packed cell volume estimated. Questionnaire was filled for each animal on demography, awareness and management practices. An overall prevalence of 2.17% with *Trypanosoma brucei, T. congolense, T. vivax* and a mixed infection of *T. brucei* and *T. congolense* observed microscopically awaiting characterization. Interviews revealed high awareness (82.8%) of tsetse and trypanosomiasis described as bush disease and abortion in four cows. The PCV values were within the normal range, however, a significant decrease ($P<0.05$) was observed in sheep aged 7months to 4years in two communities. Therefore, the study indicated the presence of *T. brucei* and other trypanosomes suggesting that animal trypanosomiasis is still a problem to animal health and wellbeing in the study area. The study recommends effective integrated chemotherapy and vector control including livestock rearing under intensive management system to boost livestock production and productivity.

Keywords: African trypanosomiasis, bush disease, livestock, reservoir host

INTRODUCTION
African trypanosomiases are complex debilitating diseases of man and animals caused by various trypanosome species of the genus *Trypanosoma* and transmitted by arthropod vectors in remote agrarian areas of sub-Sahara Africa. Human African trypanosomiasis (HAT) or sleeping sickness transmitted by tsetse flies is caused by *Trypanosoma brucei gambiense* in West and Central Africa and *T. brucei rhodesiense* in East and Southern Africa. In Latin America, *Trypanosoma cruzi* transmitted by an insect triatomine bug causes Chagas disease or American trypanosomiasis. Animal trypanosomiasis known as Nagana/samore is caused by different species of trypanosomes in domestic animals namely *T. congolense*, *T. vivax* and *T. b. brucei*- a species morphologically indistinguishable from the two human African infective trypanosomes. Others are *T. simiae* and *T. suis* in pigs. In recent times Saror *et al.* (2017) reported *T. evansi* in pigs in Benue state. Animal trypanosomiasis ravages the agricultural sector and leads to annual agricultural production loss put at US$ 4.0 billion (FAO, 2019). The trypanosomiases generally are potentially life threatening with grave socio-economic impacts if not promptly diagnosed and treated.

Unlike animal trypanosomiasis, HAT is typically restricted to historic foci and had caused serious epidemics in Africa including Nigeria in the last century leaving massive trails of death. However, control efforts through large scale vector control and mass treatment by mobile teams successfully brought the disease close to elimination by the mid-1960s (Lester, 1945; Duggan, 1962; NITR Annual Report, 1989, WHO, 1998). Later in the 1980s, HAT re-emerged in parts of Central and West Africa in epidemic proportions occasioned by war (population displacement into bushes), existence of animal reservoir and dismantling of control programmes. For Nigeria, outbreaks of the disease occurred in the Abraka foci, Delta state which led to deaths, though many patients were successfully treated at Eku Baptist hospital with drug from the WHO (Edeghere *et al*., 1989, Elhassan *et al*., 1993). Again concerted efforts of national governments and the international community in the past decades helped reverse the upward trend of new cases (WHO, 2012). In 2002, Enwezor and Ukah reported a case of advanced HAT in a child domiciled in Abraka who was referred to NITR; confirmatory test done and hospital care and treatment provided at Barau Dikko but later died. Later from the same foci and environs were the reports by Osue *et al*., 2008; Franco *et al*., 2014 and Luintiel *et al* 2017. In Benue state, surveillance studies conducted indicated zero prevalence for HAT (Enwezor *et al*., 2010).

The global successes in control efforts had led to drastic decline in number of new cases reported to the World Health Organization (WHO) and have raised high expectations of HAT elimination. In 2017, only 1,445 new cases as compared with

2,184 in 2016; 9,875 in 2009 and for the first time in 50 years, between 50,000 and 500,000 deaths recorded (WHO, 2018, and 2019). Consequently, the WHO targets the year 2030 for achieving zero cases for *T. b. gambiense* and 100% elimination of transmission.

Prominent in the framework for the elimination goal is clarifying the extent of the threat posed by cryptic infections /animal reservoirs in *gambiense* HAT epidemiology (Franco *et al*., 2018) as it had been proven that *T. b. gambiense* animal reservoir was a major factor in the persistence of the disease in endemic areas (Abenga and Lawal, 2005; Majekodunmi *et al*., 2013; Simwango *et al*., 2017). Their identification will aid development of effective integrated approach for control and management of the disease and its targeted elimination in endemic countries including Nigeria. The study was aimed at screening livestock for possible presence of *T. b. gambiense* within Tafa Local Government Area (LGA) of Niger State, Nigeria.

MATERIALS AND METHODS

Ethical Permit

Ethical permit was sought and approval given for conduct of the study by Tafa Local Government, Niger state. The Institute field officer in the area mobilized and sensitized the relevant Tafa LGA personnel, village leaders, the ardos/sarkin Fulani and the pastoralists with regard to mission of the study. This greatly facilitated understanding and cooperation received.

Study areas

Azhi Bichi (AZ), Dogon Kurmi (DK), Garam (G), Ija Gwari (IG) and Sabon Wuse (SW) communities in Tafa LGA of Niger State, Nigeria. Niger state was an old HAT endemic focus. At present the Institute tsetse outstation is located within the state, precisely in Suleja. Tafa LGA shares border with Kaduna state and the Federal Capital Territory Abuja. The LGA lies between latitude 9°15'N and longitude 7°15'E. It is characterized by woodland and grass land with marked wet and dry seasons. The inhabitants of the study area engage majorly in farming, hunting, fishing and livestock rearing. Only a few are civil servants.

Sample size determination

The following parameters were used to obtain the sample size at 95% confidence level, 5% desired level of precision and a prevalence rate of 16.1% (Odeniran and Ademola, 2018) applying the formula by Thrust Field (2005); $N=Z^2P(1-P)/d^2$ and arrived at 207.56 for N. This becomes the minimum sample size (208) for collection.

Sampling frame

During the field study, many of the herders had moved out with their animals to other locations and therefore households met on ground were enlisted with their consent and animals sampled. Within the herd, the head was drawn at random without replacement. Also, animals reported sick by their owners and apparently healthy animals were sampled. Furthermore, a questionnaire was administered to obtain information on demographic data of each animal concerning age, breed, physical condition, management practices and sex of each animal. The level of awareness of trypanosomiasis by respondents and other diseases of medical importance were also ascertained. Overall, 460 animals (177 cattle, 209 sheep, 61 goats, 12 local dogs and 1 monkey) were sampled and their blood specimens collected. Prohibition of pig rearing in the study areas attributed to crops destruction resulted in their exclusion in the sampling frame. It is pertinent to mention that at Azhi Bichi, the pastoralist/ herdsmen had left for grazing by the time the team arrived and therefore samples were taken from two animal farms and those residing within the community.

Collection of blood samples

About 3 ml blood was collected from each animal (cattle, sheep and goats) through the jugular vein using 10ml syringe and 21G needle. 2.5 ml was discharged into labelled blood sample bottles containing Lithium Heparin as anticoagulant. The dogs restrained by their owners and mouth guard to avoid biting were bled through the ear vein using 5 ml and 21G needle as attempts via the cephalic veins proved unsuccessful. The individual blood specimen was dispensed carefully into labelled Biorapid Lithium Heparin anticoagulant sample bottles. The blood specimens were put in cold boxes to prevent deterioration and examined in a conducive location in Dogon Kurmi health centre and a section of the Village leader's house at Ija Gwari; as the case may be. Blood samples were also spotted on whatmann filter paper, air dried, stored at room temperature until use for molecular characterization up to subspecies level. The monkey was finger prinked using lancet and blood collected into heparinised capillary tube. On the spot, examination for the monkey's blood sample was carried out by wet mount (X10, X40 objective lens) and stained thin film (X100 objective lens) and both preparations examined under the microscope.

Clinical examination

All the 460 animals sampled in the study were physically examined under the supervision of veterinary personnel for the manifestation of some key clinical symptoms, such as, pale mucous membrane, ocular discharge, nasal discharge, emaciation, rough hair coat and general appearance of body condition in terms of weakness. Two cattle owners complained of seasonal abortions of four (4) cows during the study.

Parasitological examination

A microhaematocrit capillary tube was filled with about 70 µl blood and centrifuged (Microhaematocrit machine-Cole Medical Instrument England) at 11,000 rev. per min for 5 min. Two microhaematocrit capillary tubes were filled for each specimen. The centrifuged blood was also used for packed cell volume (PCV) estimation. The capillary tube was then cut 1mm below the buffy coat to include the uppermost layer of red cells and 2 mm above to include the plasma. The contents of the capillary tube were gently expressed on a clean slide, mixed and covered with a 22x22 mm cover slip. The preparation was examined for trypanosomes in the field using microscopes (Muller Germany, with CCD linker camera) under x40 objective lens. 200 fields were examined after which a negative slide for trypanosome was regarded as negative.

Treatment of animals

Animals with respiratory tract infections, external wound and general body weakness and the four cows with seasonal abortions, were given oxytetracycline long acting (LA) at a dosage

of 1 ml/10 kg body weight. Similarly, animals found infected with trypanosomes and the four cows with history of seasonal abortions were treated with diminazene aceturate at 3.5 mg/kg body weight. All treatments were administered intramuscularly.

Data analysis

Data entry was carried out and group mean ± SEM was calculated for each analyte and between mean evaluated by analysis of variance (ANOVA); post-test analysis was done using Newman Kelil's comparison. Values of $p<0.05$ were considered as statistically significant. All statistically analysis was performed using Graph Pad Prism software package (Version 5).

RESULTS

Clinical diagnosis

Four hundred and sixty (460) animals were examined and 16 (3.47 %) were sick and emaciated. Respiratory problems with sneezing and ocular discharge were noticed.

Parasitological diagnosis

Blood samples from a total of 460 animals made up of 177 white Fulani/Zebu cattle, 209 Yankasa sheep, 61 red Sokoto goats, 12 local dogs and 1 monkey were examined individually for the presence of pathogenic trypanosomes using the buffy coat method. A total of ten animals were positive for trypanosomes with an overall prevalence of 2.17 %. The morphological identification indicated the presence of *T. brucei*, *T. vivax* and a mixed infection of *T. brucei* and *T. congolense* (Figure 1a &1b). Details by animal species showed 3 cattle (1.69 %), 6 sheep (3.02 %) and 1 goat (1.64 %) infected with trypanosomes. Neither the dogs nor the monkey was positive for trypanosomes. Prevalence across study sites in cattle was higher in Sabo Wuse (3.03 %) compared with Ija Gwari (1.24 %). In respect of sheep, Dogon Kurmi recorded the highest prevalence of 20 % followed by Sabon Wuse (6.67 %), Garam (2.04 %) and Ija Gwari (2.02 %). For goats, only Garam had a prevalence of 2.56 % (Table 1).

Analysis of trypanosomiasis by age and sex

The age and sex distribution of trypanosomiasis are shown in Table.2. Trypanosome infections were observed in age groups 0-6 months and 7 months – 4 years. The age group 7 months - 4years had a higher infection compared with the 0 - 6months group (1 of four months with massive trypanosomes and 2 of six month old), though trypanosomiasis either in young or adult animals is a serious concern given its devastating socio-economic effects. In terms of sex, the female animals were more infected than the males probably because of their preponderance (Table 2).

Analysis of packed cell volume

In order to assess the level of anaemia in the study sites, we measured the animals packed cell volume. The mean PCV values for the study animals showed infected cattle with values of 29.00±4.72 SEM as against 28.77±0.37SEM for uninfected; followed by infected sheep with 26.00±1.92SEM as against 25.88±0.29SEM for the uninfected and finally the one infected goat had a value of 19.00±0.0 compared with 24.07±0.65SEM in relation to its non-infected counterpart. The mean PCV values of infected and uninfected animals were within the normal range (https://oursesononline. iasri.res.in/veterinary physiology/normal PCV values in domestic animals). Further analysis by location and age groups revealed no significant difference ($P>0.05$) in the mean PCV of cattle in all the communities (SW, DK, AB, GR and IG) as their values were within the normal PCV range It is worthy to note that AZ had impressive mean PCV values compared with other communities. In addition, the mean PCV for goats did not differ significantly ($P>0.05$) in all communities and age groups. However, we observed for sheep a significant decrease ($P<0.05$) in the mean PCV values within the 7months-4years age groups in IG compared with SW.

Questionnaire administration

A total of 29 heads of households of which 14 (48.3 %) had medium to large animals (≥31) and 15 (51.7 %) had small size (≤10) animals was interviewed in order to obtain information on knowledge of tsetse, trypanosomiasis, other endemic diseases and management practices. Of those interviewed, 24 (82.8 %) admitted knowledge of tsetse and could relate it to trypanosomiasis (samore) which they termed bush disease and said humans could be affected but mostly animals. Among those that knew the harmful effects of tsetse, some listed body swelling and irritation, emaciation, loss of hair, and ocular discharge. On where tsetse can be found in their communities, they said 'No' but around water points, (rivers/streams) and IG dam and can be seen easily during the rains. The remaining 17.2 % said they cannot recognize tsetse but know that flies were around the rivers especially during the rains but could not tell the harm it could cause.

In regard to other diseases sought, almost all the respondents knew about worms/helminthiasis, Food and mouth disease which they said overcrowding whereby bringing infected animals to stay with others in one place was a major contributory factor. On liver flukes, they knew and called it 'Hanta'. One respondent said one of his animals was down with it; brucellosis knowledge was high too which they attributed presence in slow flowing rivers and drinking stagnant water. In respect of black quarter they said they did not know it but as for rinderpest, they said it no longer was of relevance; heard about it 30 years ago.

On management practices, free grazing was commonly practiced and feeding supplemented with corn starch, legumes from groundnut and beans during the dry season. In the wet season, animals fed only grass and potash. Concerning frequency of disease control, treatment for trypanosomiasis was regular for only animals perceived to be sick and lacked appetite to eat food. Such animals were treated with samorin mixed in water or in feed; but generally treatment was irregular –twice a year. Further the respondents provided regular treatment using Long acting (LA), tylosin, terramycin and procaine against other diseases. They informed too that they dewormed their animals sporadically against worms and practiced programmed movement from 9-10 am daily for animal grazing to guard against liver fluke.

DISCUSSION

The study investigated livestock for presence of *T. b. gambiense* the causative agent of HAT which is targeted for 100 % elimination and zero transmission by the year 2030. The findings demonstrated the presence of *T. brucei, T. congolense, T. vivax*

and a mixed infection of *T. brucei* and *T. congolense*. The *T. brucei* seen in livestock in the study area is of epidemiological significance to HAT in regard to harbouring *T. brucei gambense* and plans are underway for molecular studies to clarify the sub species involved in Part II of our study. The present Part I paper has demonstrated the presence of pathogenic trypanosomes in livestock which suggests that animal trypanosomiasis still portend a great risk to animal health and well-being given its established constraints and losses in livestock production and productivity. The overall prevalence of 2.17 % obtained in the study was lower than the 13.08 % reported by Quadeer *et al*. (2008) in Plateau State, Nigeria; 6.3 % (Adama *et al.,* 2010) in Niger State, Nigeria; 3.8 % (Enwezor *et al*., 2012) in Benue State, Nigeria and 5.1 % (Obaloto *et al*., 2013) in Bauchi State, Nigeria, but higher than 1.9 % in Abia State, Nigeria recorded by Ohaeri (2010). Trypanocidal treatment administered on animals prior to the survey may have reduced the parasite population and consequently the infection rate. For instance, most of the respondents (82.8 %) as revealed from the results of questionnaire are well aware of trypanosomiasis which they described as 'bush disease'; its symptoms can be linked to tsetse and water points. Also administering samorin twice yearly as informed by majority of the respondents could confer appreciable resistance to trypanosomal infection in the cattle such that the accumulated treatment may exert prophylactic effects.

In this study, four cases of seasonal abortions in cow were reported and linked to households with serious trypanosomiasis problem from the questionnaire. These reports were not surprising as evidence exists of natural and experimentally induced trypanosome abortions in livestock (Ikede and Losos 1975; Elhassan *et al*., 1987; Silva *et al*,. 2013; Jaiswal *et al*., 2015; Srikijkasemwat and Kaewhom 2019). Among the 3 infected cattle in the age group 0 -6 months, (one 4 months with massive parasitaemia and two 6 months old) was of serious concern due to their age and reduced chances of exposure. Their infections could be through the trans-placental route which has been well described (Ikede and Losos, 1975, Camprigotto *et al*., 2015). Besides, it could also be either fly bites in the homesteads since the distance 1 -2 km from pens to water sources fall within flying abilities of a tsetse to enable it leave its habitat, pick up a blood meal from any of the animals and return to base; or at watering points when taken by their tenders alongside other animals to their watering places. Evidence for tsetse presence in the study area has been provided through interviews and scientifically by Orji *et al*. *(*2015) and Shaida *et al*. (2018). Further, age related trypanosome infection in this study indicated that younger animals were more infected than adults. Similarly, a higher prevalence was found in sheep compared to cattle which appeared unusual given that cattle were potentially more exposed to fly bites owing to forage across long distances in search of pasture. These findings contradict Sam Wobo *et al*. (2010) and Fasami *et al*. (2014) report that adult animals were more prone to trypanosome infection. However, absence of trypanosome infection in the 5 - 8years age group as observed in this study may be ascribed to occasional treatment of matured animals. There is likelihood that the livestock owners paid more attention to the treatment of their cattle than sheep considering their greater economic and cultural importance.

In relation to sex, more female animals were found to habour trypanosome parasites than the male animals probably because of their preponderance. Likely explanations could be the habit of livestock owners to keep more of their female animals for production and dairy products and market mostly the males for meat purposes and also the practice of having fewer males in herds in order to reduce male competition during mating.

In addition, results of haematological analysis in terms of PCV measurement were generally good even including the one infected goat except in sheep aged 7 months – 4 years where there was a significant decrease ($P<0.05$) suggesting anaemia in the two study locations of IG and SW. This appears to support the assertion of preferential treatment of cattle over sheep. Despite this observation, the overall mean PCV was moderate and AZ gave the most impressive PCV profile. The reason for this seemed to be that the animals studied in that community were farm or semi-sedentary and coupled with the high level of drug use by livestock owners yielded a better performance in terms of calving rate within the locations.

From the foregoing, the study has indicated the presence of *T. brucei* and other pathogenic trypanosomes in the livestock studied. The role of livestock as reservoir of *T. b. gambiense in* HAT epidemiology had been well documented and need be further addressed in the light of the present results. The presence of tsetse flies in the area as reported shows that the transmission cycle is yet to be broken. Therefore the interplay with vector, pathogenic trypanosomes and livestock will continue to create trypanosomiasis problem in the study areas. In the light of this the study recommends effective integrated methods of control (chemotherapy and vector) using the one health approach as a better way to actualize HAT elimination and animal trypanosomiasis problems. The idea of one way approach has been strongly advanced by Simo and Rayaisse (2015). The study also recommends intensive livestock rearing in ranches and grazing reserves together with provision of enabling environment to encourage settlement and limit contact of animals in the bush in order to completely avoid trypanosomiasis problems. The measures if taken into practice will boost agricultural productivity, increase job creation, food security and poverty reduction.

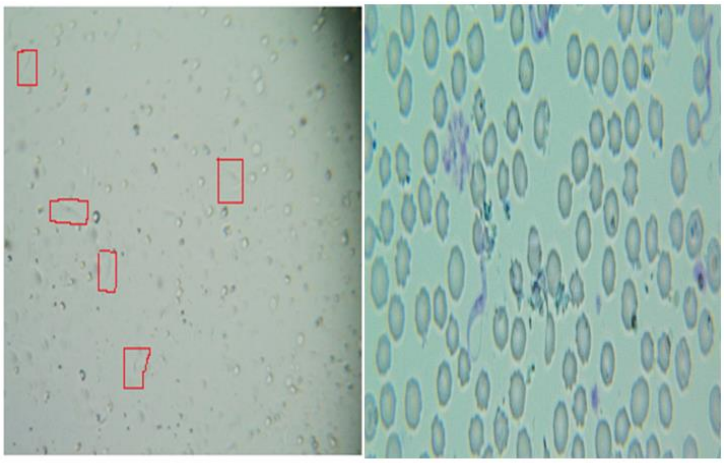

Figure 1: (a) Wet mount slide of buffy coat
(b) Giesma stain film of trypanosome

Table 1: Overall prevalence of trypanosomiasis in domesticated animals in some selected communities of Tafa LGA, Niger State

Overall prevalence = 2.17%				
Cattle = 1.69%	Sheep = 3.02%	Goat = 1.64%	Dog = 0%	Monkey = 0%
SW	**DK**	**AB**	**GR**	**IG**
Cattle = 3.03%	Cattle = 0%	Cattle = 0%	Sheep = 2.04%	Cattle = 1.24%
Sheep = 6.67%	Sheep = 20%	Sheep = 0%	Goat = 0%	Sheep = 2.02%
	Goat = 2.56%	Goat = 0%	Dog = 0%	Goat = 0%
				Dog = 0%
				Monkey = 0%

Keys: SW = Sabon Wuse, DK = Dogon Kurmi, AB = Azhi Bichi, GR = Garam, IG = Ija Gwari

Table 2: Prevalence of trypanosomiasis in domesticated animals in relation to age and sex

	Age and Sex		
Animals	**0 - 6months**	**7months - 4years**	**5years - 8years**
Cattle	1 (0.57%) Female	2 (1.13%) Female	0
Sheep	2 (0.96%) Female	4 (1.91%) 1 (Male), 3 (Female)	0
Goat		1 (1.64%) Female	0

Table 3: Average packed cell volume (Mean ± SEM) of animals in relation to location and age groups.

	Cattle			Sheep			Goat		
	0 - 6months	7months - 4years	5years - 8years	0 - 6months	7months - 4years	5years - 8years	0 - 6months	7months - 4years	5years - 8years
SW	29.21 ± 1.411[a]	26.94 ± 1.126[a]	27.10 ± 0.85[a]	27.71 ± 1.997[a]	29.09 ± 1.019[ab]	0	0	0	0
DK	0	29.94 ± 0.532[a]	27.50 ± 1.765[a]	0	28.33 ± 1.202[a]	0	25.36 ± 1.525[a]	24.07 ± 0.907[a]	0
AB	32.67 ± 1.856[a]	30.50 ± 1.991[a]	31.40 ± 1.030[a]	26.50 ± 2.029[a]	26.65 ± 0.859[a]	0	0	28.50 ± 1.500[a]	0
GR	0	0	0	24.20 ± 1.020[a]	26.88 ± 0.771[a]	0	19.00 ± 3.786[a]	24.50 ± 1.936[a]	0
IG	29.00 ± 0.842[a]	29.64 ± 0.809[a]	28.50 ± 0.723[a]	26.13 ± 0.934[a]	25.10 ± 0.360[ac]	0	21.00 ± 1.155[a]	26.50 ± 6.436[a]	0

Note: Mean ± SEM with different superscript (alphabets) within column indicates significant difference (*P<0.05*)

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