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Trypanosoma evansi: prevalence in naturally infected cattle in Borno and Gombe state, Nigeria

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ABSTRACT

***Introduction*:** *Trypanosoma evansi* the cause of Surra is an important haemoprotozoan disease of economic importance that affect wide range of host. The study aimed at assessing the prevalence of *T. evansi* in cattle in Borno and Gombe states, Nigeria.
***Methods*:** Blood samples were collected from 455 apparently healthy cattle in the study areas and examined for *T. evansi* using thin stained blood smears and haematocrit centrifuge techniques.
***Results*:** An overall prevalence rate of 11.8 % and 9.0%, were detected in Gombe and Borno States, respectively. Yamaltu Deba local government area recorded higher prevalence of 6.9% than 2.0% in Gombe (LGA) of Gombe State, while Konduga LGA recorded higher prevalence of 4.2% than the 1.6% in Jere LGA of Borno State. The White Fulani breed had higher prevalence of 11.1% compared to 0.7% for Bokoloji in Gombe State. While in Borno State, the Red Bororo recorded higher prevalence of 6.1% than 2.9% in Wadara. Based on age, the adult recorded higher prevalence of 8.4% and 7.6% in Borno and Gombe States, respectively, compared to the younger ones. Moreover, the prevalence of *T. evansi* based on sex of cattle revealed higher prevalence rate in male (15.2%) compared to the female (9.0%) in Gombe State, while higher in female (12.4%) compared to male cattle (6.3%) in Borno State. The association between breed, age, sex and the prevalence of infection was not significant ($p > 0.05$).
Significance: This study will assist in providing reference data on the prevalence of *T. evansi* in cattle in the study area.

Introduction

The role of livestock in food security and nutrition is largely by providing meat, milk, draught power, manure and fiber to man (FAO, 2015). In many rural communities, livestock is the only asset of the poor, but it is highly vulnerable to several factors including disease (Thornton *et al.*, 2007; IFAD, 2010). Among the diseases that hinder livestock production, *Trypanosoma evansi* the cause of Surra which is zoonotic in nature, constitute the major threat to Africa's struggle against improved agriculture, economy and poverty alleviation (FAO, 2007; OIE, 2016; Idehen *et al*., 2018). The disease is also among the neglected tropical disease affecting human across 36 Sub-Saharan African countries including Nigeria (Ruberto *et al.*, 2013). *Trypanosoma congolense, T. brucei brucei, T. vivax* and *T. evansi* are the most important species that infect livestock in Nigeria. These trypanosomes are found where its biological vector (tsetse fly) and other biting flies (*Tabanus*, *Stomoxys*, *Haematopota*, *Lyperosia*, and *Chrysops spp*) exist (Gangwar *et al.*, 2019). The host preferences of each trypanosome species vary but *T. evansi*, is known to infect wide host range including camel, cattle, buffalo, horses and donkeys as they can be transmitted both cyclically by tsetse flies and mechanically by other biting flies (Chandu *et al.*, 2021). The wide range of parasite host contribute to it geographical distribution beyond the "tsetse fly belt". The mechanical transmission of *T. evansi*, transmission can occur through the contamination of a wound with infected animal blood (Ereqat *et al.*, 2020). The WHO (2014) reported cases of human infective trypanosomes (*Trypanosoma brucei gambiense*) isolated from domestic animals. These poses a public health risk to livestock rearers, hunters, rural dwellers, farmers, and veterinarians who are often in close contact with these animals and its biological vectors. The economic losses caused by surra in the tropics is mainly due to chronic form of the disease in cattle resulting in severe weakness, abortion, infertility, reduced milk yield, weight loss and reduced drought capabilities that later lead to neuropathy and immune suppression coupled with anaemia and eventually death of the animal (Fesseha *et al.*, 2022). Diagnoses for surra are based on four types of examination; (i) microscopic examination (Mbaya

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e*t al.,* 2012), (ii) DNA detection by polymerase chain reaction (PCR-RoTat 1.2) (Claes *et al*., 2004), (iii) Card agglutination test (CATT/*T. evansi*) (Kyari *et al.*, 2021), and (iv) Enzyme linked immunosorbent assay (ELISA) *T. evansi* (Desquesnes *et al*., 2013). Data on the prevalence of *T. evansi* using any of these techniques is limited in cattle in the studied areas. Therefore, this study was aimed at determining the prevalence of *T. evansi* in cattle in Borno and Gombe State, Nigeria using the microscopic examination technique.

Materials and methods

Study Area

This study was carried out in Borno and Gombe States of North-eastern, Nigeria. Borno State lies between longitude 120^{O} 8E and latitude 10^{O} 23N and the climate, ecology and vegetation are Sahelian, Semi-arid, and Savannah with flooded pastures towards Lake Chad and mountainous areas in the Southeast. The State shares international borders with Niger, Cameroun and Chad Republics, which have similar relative humidity during the dry season and rainy season (Ododo, 2005). However, Gombe State is located between latitude 9^{O} 30$^{'}$ and 12^{O} 3$^{'}$ N and longitude 8^{O} 45' and 11^{O} 45$^{'}$ E (Dabara *et al.,* 2019). Gombe state shares boundaries with Bauchi, Taraba, Adamawa, Yobe, and Borno States. Gombe State has a mean annual rainfall of 818.5mm, with a mean temperature range of 12 ^{O}C – 37 ^{O}C. The hottest months are March – May (40 ^{O}C).

Study Period

This study was conducted from the month of May to October 2018. A Non-probability convenience sampling method was used with emphasis on areas with large populations of cattle.

Sampling Locations

Rural and urban areas within three (3) out of the twenty-seven (27) Local Government Areas of Borno State were visited for sample collection *viz:* Konduga, Jere, and Maiduguri Metropolitan council (MMC). Also, three (3) out of the Eleven (11) Local Government Areas of Gombe state were visited for sample collection *viz:* Yamaltu Deba, Dukku, and Gombe Local Government Areas.

Study Design

This study was carried out via microscopic examination of stained thin blood smears and using centrifuges techniques for the presence of *T. evansi*.

Study Animals and Sample Collection

A total of four hundred and fifty-five (455) blood samples were collected from the jugular vein of apparently healthy cattle from selected livestock markets and farms. Blood samples were collected using vacutainers and dispensed into sample bottles containing EDTA, stored in ice packs for onward transportation to the Haematology Research Laboratory at the Department of Veterinary Parasitology and Entomology, University of Maiduguri, Nigeria. The information noted and recorded during sample collections included location, sex, age group, and breed of the cattle.

Blood Sample Analyses

Microscopic Detection of T. evansi

A drop of blood was put at the edge of a cleaned grease free glass slide. A second slide was applied on top at an angle of 45^{o} such that the blood running at the edge will touch the first. The slide was then pulled away along the first slide toward the opposite edges producing a thin film of blood which reduced the thickness and increased the distance of the initial drop as described by Bennett (1970). Two thin blood smears were made from each blood sample and were left for few minutes to allow drying in air and then labeled appropriately. The slides were fixed with methanol for five (5) minutes and allowed to air dry, later stained with diluted 10% Giemsa stain, pH 7.4 according to the standard procedures described by Thrall (2004), Ribeiro *et al.* (2005) and Zajac and Conboy (2012). The slides were later viewed at high magnification of x400 using light microscope (Olympus, Japan) under oil immersion for 10–15 min for the presence of intracellular blood parasites as previously described by Valkiūnas *et al.* (2008) and Akinpelu (2008). For the microscopic detection of extracellular blood borne parasites, each blood sample was collected into heparinized micro-haematocrit capillary tube and centrifuged at 1,500-g to concentrate the organisms in the buffy coat. The micro-haematocrit tube was cut just below the buffy coat above the packed RBCs. The buffy coat was expressed from the cut end with a small amount of plasma to make a suspension, and then a thin smear was made on a clean dry glass slide, allowed to air dry. Fixed with methanol, labelled accordingly and stained. Stained buffy coat smears on slides were later viewed at magnification of x400 using microscope under oil immersion for 10–15 min for the presence of extracellular blood parasites as previously described by Valkiūnas *et al.* (2005) and Valkiūnas *et al.* (2008). Sample was considered negative when no parasite was detected after examining 100 microscopic fields. All detected parasites were photographed using digital camera at 4 Pixel. Blood sample of cattle from the study areas were considered positive for *T. evansi* when stained blood smears revealed the presence of Trypanosoma organisms having medium sized fusiform shaped body (20–32 µm), centrally placed nucleus, well developed undulating membrane, free flagellum and subterminal kinetoplast placed at posterior end according to Reddy *et al.* (2016).

Data Analysis

Prevalence, chi-square, odd ratio and Student T - test were used for the analyses. The data were analyzed using JMP version 11

software (SAS institute Inc. Cary, NC). Results from the analyses were considered significant at a *p*-value of less than 0.05.

Results

The results of the prevalence of *Trypanosoma evansi* in cattle based on the study areas are presented in Table 1. The prevalence of *T. evansi* was found to be higher in Gombe (11.8%) compared to Borno State (9.0%). The results of the prevalence of *T. evansi* based on the LGAs from samples in Gombe State showed higher prevalence in Yamaltu Deba (6.9%) followed by Dukku (2.8%) and Gombe (2.1%). The association between the statuses of infection in the LGAs of Gombe State was not significant. However, the result of prevalence of *T. evansi* based on the LGAs in Borno State showed higher prevalence rate in Konduga (4.2%) followed by MMC (3.2%) and Jere (1.6%) LGAs. The association between the statuses of infection in the local government areas of Borno State was also not significant.

The result of the prevalence, association and odd ratio in cattle infected with *Trypanosoma evansi* in Gombe and Borno State based on breed of cattle is presented in Table 2. The prevalence of *T. evansi* based on the breed of cattle examined in Gombe State showed higher prevalence in White Fulani (11.1%) compared to Bokoloji (0.7%). The association between the breed of cattle in Gombe State and the prevalence of *T. evansi* was not significant. Likewise, considering the odd ratio of Bokoloji breed of cattle were less expected to be infected with *T. evansi* compared with the White Fulani breed of cattle and the infectivity was not significant. Moreover, Table 2 also shows the results of the prevalence of *T. evansi* based on the breed of cattle examined from Borno State. The prevalence of *T. evansi* was found to be higher in Red Bororo (6.1%) compared to Wadara (2.9%). The association between the statuses of infection and the breeds of cattle was not significant.

The result of the prevalence, association and odd ratio in cattle infected with *Trypanosoma evansi* in Gombe and Borno State based on Age is summarized in Table 3. The prevalence of *T. evansi* based on the age group of cattle examined in Gombe State showed higher prevalence in adult (7.6%) compared to young (4.2%). Similarly, considering the odd ratio, the young cattle were less likely to be infected with *T. evansi* compared with the adult cattle and the infectivity was not significant. The association between the age group of cattle in Gombe State and the prevalence of *T. evansi* was not significant. Table 3 also shows the results of the prevalence of *T. evansi* based on age group of cattle examined from Borno State. The prevalence of *T. evansi* was also found to be higher in adult (8.4%) compared to the young (0.6%) cattle. The association between the statuses of infection and the age group of cattle was not significant. Considering the odd ratio, the young cattle were also less likely to be infected with *T. evansi* compared with the adult cattle in Borno State and the infectivity was not significant.

The result of the present study also considered the prevalence, association and odd ratio in cattle infected with *Trypanosoma evansi* in Gombe and Borno State based on sex of cattle which is summarized in Table 4. The prevalence of *T. evansi* based on the sex of cattle examined in Gombe State showed higher prevalence rates in male (6.9%) compared to female (4.9%) cattle. Considering the Odd ratio, the female cattle were less likely to be infected with *T. evansi* compared with the male cattle and the infectivity was not significant. The association between the sex of cattle in Gombe State and the prevalence of *T. evansi* was not significant. The prevalence of *T. evansi* among cattle examined in Borno and Gombe States was found to be higher in female (5.5%) compared to male (3.5%) cattle. The association between the prevalence of *T. evansi* infection and the sex of cattle was not significant. Also, seeing the odd ratio, the female cattle were highly foreseeable to be infected with *T. evansi* compared with the male cattle and the infectivity was not significant.

Discussion

Out of the four hundred and fifty-five (455) blood samples collected from apparently healthy cattle in different Local Government areas, the results of present study revealed an overall prevalence of *T. evansi* to be 11.8% in Gombe State and 9.0% Borno State. This is very likely to be due to the availability of suitable vectors capable of transmitting the haemoparasites to cattle in the two study areas. The present observations also concur with reports by Laha and Sasmal, (2009), Bal *et al*. (2014) and Birhanu *et al*. (2014) who reported 9.4%, 6.9% and 37.3% prevalence rates of *T. evansi*, in eastern region of India, Jakandhar, Punjab India and Tigay and Afar region of India, respectively, in cattle. However, the prevalence of 6.9% was lower than value reported in the present study while, 9.4% was comparable to the prevalence rate recorded in Borno State but lower than the prevalence rate from Gombe State. Also, the prevalence rate of 37.3% reported by Birhanu *et al*. (2014) was higher compared to the values reported in the present study. These variations in the prevalence rates might be associated with differences in method of diagnosis, variation in ecological and geographical factors, season of sample collection, and the abundance of arthropod vectors.

Based on LGAs, the prevalence rate was found to be higher in Yamaltu Deba (6.9%) compared to Dukku (2.8%) and least in Gombe (2.1%) LGAs of Gombe State, whereas in Borno State, the prevalence of *T. evansi* in cattle was found to be higher in Konduga (4.2%) compared to MMC (3.2%) and least prevalent in Jere (1.6%) LGAs. However, the association between the status of infection in the LGAs of both States was not significant. This finding might be associated with free movement of cattle within the two study areas due to the continuous migration of nomadic cattle perhaps in connection with the Boko haram insurgency within the study areas as well as the availability and effectiveness of transmission of suitable arthropod vectors. This observation is consistent with the reports of Bouyer *et al.* (2013) who have stated the complex interaction between environmental, political, socio-cultural, entomological and livestock management influencing the transmission of arthropod borne

trypanosomosis among migratory cattle in Jabi Tehenan district, Ethiopia.

Table 1. Prevalence of *Trypanosoma evansi* in cattle based on Study Areas in Gombe and Borno States, Nigeria

Study Areas	LGA	No. of cattle examined	No. of cattle infected (%)	Prevalence (%) 95% CI (LL - UL)	χ^2	P - value	Odd Ratio
Gombe	Dukku	44	4 (9.1)	2.8 1.09 – 6.93	2.385	0.3034	1.8333
	Yamaltu Deba	60	10 (16.7)	6.9 3.81 – 12.31			
	Gombe	40	3 (7.5)	2.1 0.71 – 5.94			2.2222
Total		144	17 (11.8)	11.8 (0.08 – 0.18)			
Borno	Jere	103	5 (4.9)	1.6 0.69 – 3.71	3.556	0.1689	2.50
	Konduga	107	13 (12.1)	4.2 2.46 – 7.02			
	MMC	101	10 (9.9)	3.2 1.76 – 5.82			1.23
Total		311	28 (9.0)	9.0 (0.06 – 0.13)			
Grand Total		455	45 (9.9)	9.9 (7.5 – 13.0)			

CI = Confidence Interval; LL = Lower limit; UL = Upper limit; χ^2 = Chi-Square
LGA = Local Government Area

Table 2. Prevalence of *Trypanosoma evansi infection* based on Breed of cattle in Gombe and Borno States, Nigeria

Study Area	Breed of cattle	No of cattle Examined	No of cattle infected (%)	Prevalence (%) 95% CI (LL - UL)	χ^2	p- value	Odd Ratio
Gombe	Bokoloji	7	1 (14.3)	0.7 (0.12 – 3.82)	0.043	0.8354	0.8175
	White Fulani	137	16 (11.7)	11.1 (6.96 – 17.29)			
Borno	Red Bororo	218	19 (8.7)	6.1 (0.0395 – 0.0934)	0.073	0.7865	0.9006
	Wadara	93	9 (9.7)	2.9 (0.0153 – 0.054)			

CI = Confidence Interval; LL = Lower limit; UL = Upper limit; χ^2 = Chi-Square

Table 3. Prevalence of *Trypanosoma evansi* based on Age of cattle examined in Gombe and Borno States, Nigeria

Study Area	Age of cattle	No of cattle Examined	No of cattle infected (%)	Prevalence (%) 95% CI (LL - UL)	χ^2	p- value	Odd Ratio
Gombe	Adult (> 18 months)	105	11 (10.5)	7.6 0.0432 – 0.1316	0.653	0.4189	0.6810
	Young (≤ 18 months)	39	6 (15.4)	4.2 (0.0193 – 0.088)			
Borno	Adult (> 18 months)	304	26 (8.6)	8.4 (0.0577 – 0.1197)	3.336	0.0678	0.2993
	Young (≤ 18 months)	7	2 (28.6)	0.6 (0.0018 – 0.0231)			

CI = Confidence Interval; LL = Lower limit; UL = Upper limit; χ^2 = Chi-Square

Table 4. Prevalence of *Trypanosoma evansi* infection based on Sex of cattle examined in Gombe and Borno States, Nigeria

Study Area	Sex of cattle	No of cattle Examined	No of cattle infected (%)	Prevalence (%) 95% CI (LL - UL)	χ^2	p- value	Odd Ratio
Gombe	Male	66	10 (15.2)	6.9 (0.0381 – 0.1231)	1.301	0.2540	1.6883
	Female	78	7 (9.0)	4.9 (0.0237 – 0.0969)			
Borno	Male	174	11 (6.3)	3.5 (0.0199 – 0.0623)	3.455	0.0631	1.9628
	Female	137	17 (12.4)	5.5 (0.0344 – 0.0858)			

CI = Confidence Interval; LL = Lower limit; UL = Upper limit; χ^2 = Chi-Square

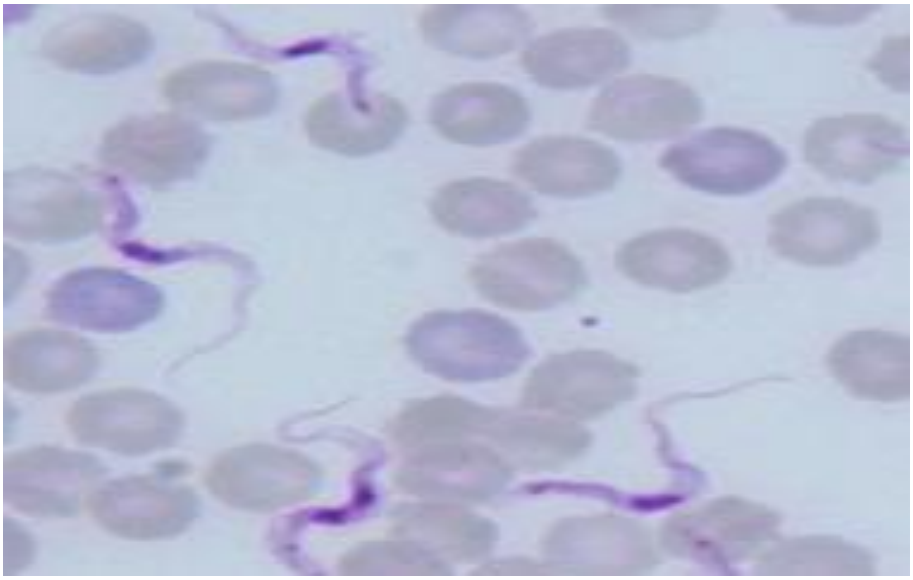

Plate 1: Giemsa-stained blood smear showing the central nucleus (black arrow), subterminal kinetoplast (blue arrow) and free flagellum (red arrow) of *Trypanosoma evansi* in cattle blood (x 400).

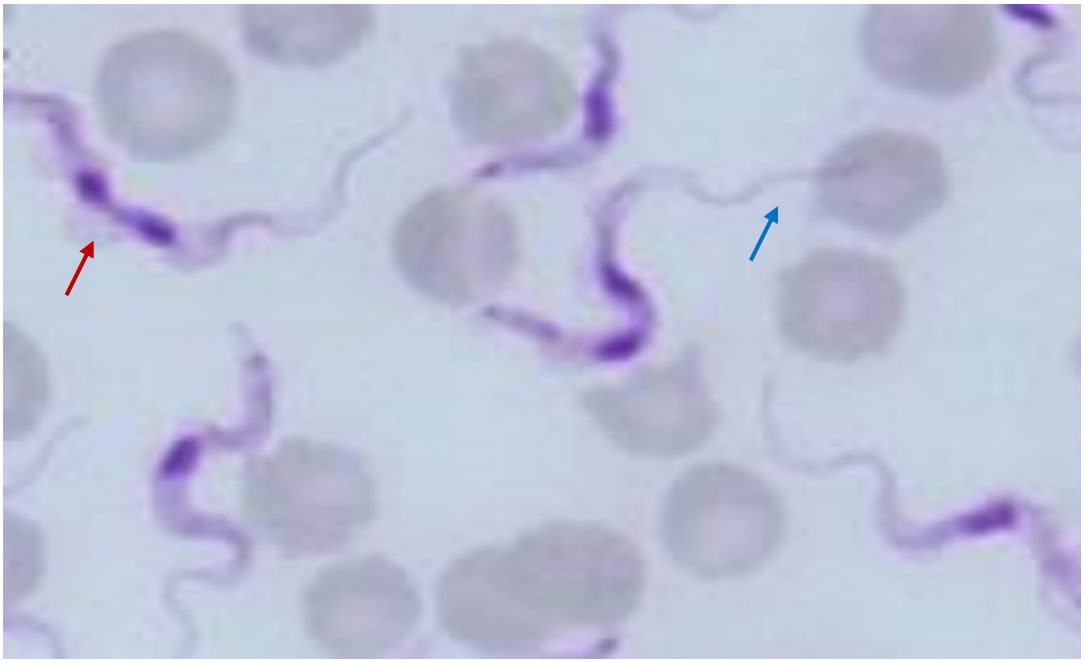

Plate II: Giemsa-stained blood smear (x 400); Showing the Morphological features of *Trypanosoma evansi* in cattle blood, typical morphology with thin posterior extremity (blue arrow) and large undulating membrane (red arrow).

Discussion contd.

The finding of the present study when comparing the prevalence of *T. evansi* and its association with the breed of cattle in the two study areas revealed that, the prevalence of *Trypanosoma evansi* was found to be higher in White Fulani (11.1%) compared to Bokoloji (0.7%) in Gombe State, as well as higher in Red Bororo (6.1%) compared to Wadara (2.9%) in Borno State. Despite these differences, there was no significant correlation between the prevalence of *T. evansi* and cattle breeds in the two study areas. This finding indicated that cattle breeds may not play a significant role in establishing *Trypanosoma evansi* infection in cattle in the two study areas (Gombe and Borno State). Furthermore, it could be inferred that these cattle breeds shared equal opportunities of infection and the presence of suitable vectors in the present study. However, amongst the breeds of cattle in Nigeria, the White Fulani and Red Bororo have been reported to be relatively trypanotolerant (Meghen *et al*., 1999). The results reported in this study are consistent with Zubairu *et al*. (2016) who have reported 12.0% of trypanosomosis in White Fulani and 13.2% in Red Bororo.

The prevalence of *T. evansi* was found to be relatively higher in adults compared to young cattle in Gombe and Borno States. The correlation between *T. evansi* prevalence and age in Gombe and Borno States was also not significant, although the odd ratio showed that the younger cattle were less infected with *T. evansi* compared to the adults in Gombe and Borno States. The observation may be because young cattle are usually sedentary and rarely move during trans-human migration into infected areas and that could be why the infection was less in them. The adults move a lot during the dry season trans-human migration into infected areas in search of dry season fodders and hence the possibility of been bitten by the mechanical vectors (*Tabanus* and *Stomoxys* flies). The observation further corroborates with Zubairu *et al*. (2016), who also reported higher prevalence of Trypanosomosis in adults (16.0%) compared to young cattle (12.0%).

The present study also reported the prevalence of *T. evansi* and its association with sex of cattle in the two study areas. The prevalence of *T. evansi* was found to be relatively higher in male compared to female cattle in Gombe State. In contrast, the prevalence of *T. evansi* was found to be higher in female than in the male cattle in Borno State. The finding reported in Gombe State may be linked to the fact that bulls are typically subjected to strenuous activities, such as those used as drought animals in agriculture, transportation of goods, which could have contributed to stressors that could affect their immunity, thus predisposing them to the infection. This observation is supported by previous reports by Magona *et al*. (2008) and Ataro *et al*. (2016) who reported that male cattle have a significantly higher prevalence of trypanosomosis than female cattle. Nonetheless, the finding of a higher prevalence in female compared with male cattle in Borno State is consistent with the findings of Zubairu *et al*. (2016) who also reported a higher prevalence of trypanosomosis in females than in male cattle. Perhaps, it might suggest that female cattle are more vulnerable to trypanosomosis in pregnancy, parturition, lactation and those in low plane of nutrition. Moreover, the female cattle are generally herded much longer for the purpose of breeding and milk production, thereby

prolonging their exposure to vectors and other challenges of disease agents.

Conclusion

In conclusion, this study showed that; the prevalence rates of 11.8% and 9.0% for *T. evansi* infection was recorded in Gombe and Borno States, respectively. Also, the prevalence of *T. evansi* and statistical association between infection and breed, age as well as sex of cattle were not significant in the two states.

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Conflict of interest

The authors have no conflict of interest

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