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PREVALENCE OF TRYPANOSOMES IN TRADE *BOS INDICUS* (CATTLE) AT KANO, NIGERIA.

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ABSTRACT:
Trypanosomosis has remained a constraint to cattle productivity with serious economic consequences. This study was aimed at investigating the prevalence of Trypanosomes in 560 cattle brought to Kano Abattoir from January to December, 2017, using standard trypanosome detection methods. The result revealed an overall prevalence of 22 (3.93%) with cattle above 3 years of age having higher prevalence, 14 (2.50%, $\chi2 = 6.284$, df = 3, p = 0.0122) compared to other age groups. Similarly, prevalence of bulls was higher, 18 (3.22%, $\chi2 = 0.024$, df = 1, p = 0.877), than that of cows with Sokoto Gudali breeds having higher prevalence, 12 (2. 14%, $\chi2 = 0.086$, df = 1, p = 0.769) than White Fulani breeds. Cattle with poor BCS had higher prevalence, 18 (3.2%) compared to cattle with good BCS, 4 (0.71%) with those scoring II (very thin) having the highest prevalence, 8 (1.43%, $\chi2 = 11.279$, df = 8, p = 0.0008). Moreover, the average Packed Cell Volume (PCV) was reduced in parasitaemic cattle compared to aparasitaemic cattle ($p<0.05$). Similarly, cattle brought from Wudil had higher prevalence, 8 (1.00%, $\chi2 = 11.34$, df = 7, $p <0.0001$) compared to other sources. Conclusively, the prevalence reported in this study was lower compared to earlier reports but recorded a higher number of *T. vivax* in trade cattle which could be an indication that its transmission is highly favoured by the cyclical vector or that the other species respond better to trypanocidal drugs. More epidemiological surveillance is therefore recommended.

***Keywords**: Cattle, Kano, Prevalence, Trypanosomosis*

INTRODUCTION

Trypanosomosis is an infectious disease caused by haemoprotozoan of the genus *Trypanosoma* responsible for a range of complications in infected herds, including loss in productivity, high morbidity and sometimes mortality (N'Djetchi *et al.,* 2017). *Trypanosoma brucei*, *T. congolense* and *T. vivax* are the major pathogenic trypanosomes responsible for the disease in cattle (Dabo and Maigari, 2017; Simwango *et al.*, 2017).

In Nigeria, diagnosis of Trypanosomosis largely depends on parasitological, immunological and molecular methods (Abenga *et al*., 2004; Ezeani *et al.*, 2008; Ezebuiro *et al*., 2009; Fajinmi *et al*., 2011; Gumel *et al*., 2011; Maigari *et al*., 2015; Sidi, 2017). However, Immunological techniques (such as Enzyme Linked Immunosorbent Assays, fluorescent antibody tests and card agglutination tests) may be good for large scale epidemiological studies, but not sensitive enough to detect and differentiate between previous exposure and current infections (Ezeani *et al.*, 2008; Bittar *et al.*, 2016) leading to false positive results. Similarly, molecular techniques such as Polymerase Chain Reaction (PCR) has been shown to be more sensitive and specific than both parasitological and serological methods (Majekodunmi *et al*., 2013; Nhamitambo *et al*., 2017; Tehseen *et al*., 2017). However, this technique is very expensive and relatively new (Vanchau *et al*., 2016) requiring expertise. Therefore, although the use of PCR as a better diagnostic tool to ascertain the incidence and prevalence of Trypanosomosis has been advocated, the technique has not yet been standardized especially in large scale disease surveillance. Hence, the use of classical parasitological method to investigate prevalence of Trypanosomosis in cattle is still relevant.

In addition to that, a pilot study conducted in Kano abattoir prior to the commencement of this study, revealed that livestock are brought from all the local government areas of Kano State, neighbouring states and other West African countries. Consequently, because livestock marketers travel from high – risk areas with limited short – term prospects for therapeutic advances, the continued importation of the disease to non-endemic areas can be expected. Similarly, the diminished investment in previously successful programs on the disease may contribute tothe resurgence of the disease after near – elimination in many parts of the

country.Therefore, the present study was aimed at determining the prevalence of Trypanosomosis of trade cattle brought for slaughter at Kano abattoir from January to December, 2017.

MATERIALS AND METHODS

Sample Size

A total of 560 cattle were screened from January to December, 2017. To estimate slaughter capacity per day, the abattoir was visited daily for 7 daysl and the obtained figures were pooled and averaged to obtain the sample size frame, using the procedure of Shun *et al.* (2009).Thereafter, every nth unit in the population is systematically sampled. The sampling interval was determined by use of the formula K = N/n (where N= total number of animals available & n = the number required in the sample). A random number (r) falling between 1 and K is then chosen by the use of card shuffling physical randomization technique according to Suresh (2011).

Determination of Body Condition Scores (BCS), Breeds and Age of Cattle

The BCS of sampled animals was assessed, *ante mortem*, according to Pruitt (1994) as modified by Glenn (2016) which entails the use of US scoring system to assign scores on some important anatomical features. Similarly, the breeds of cattle (*Bos indicus*) were determined according to the descriptions of Blench (1999) while the approximate age of cattle was determined by examining the teeth and noting the time of appearance and the degree of wear on the temporary and permanent teeth according to Food Safety Inspection Services, FSIS (2013).

Sample Collection and Microscopy

Five ml (5 ml) of blood was collected from the jugular vein of each animal at the point of slaughter using sample bottles containing Ethylene Diamine Tetra Acetic Acid (EDTA) dispensed as 1mg powder per ml of blood. The samples were then conveyed in cold boxes with ice packs to the laboratory of the Nigerian Institute for Trypanosomiasis Research, Kano Liaison Office, located at the State Epidemiology Unit, Infectious Diseases Hospital, Kano, for analysis.

Standard trypanosome detection methods were carried out according to Woo (1970) as described by Nakayima (2016). Wet mount was prepared and microscopically examined under high power (40×) for parasites motility. Some blood was placed in capillary tubes, sealed, centrifuged at 12000 rpm for 5 minutes and microscopically examined for motile parasites. Thereafter, the Packed Cell Volume (PCV)of each sample was determined by using Microhaematocrit reader where the denser layer of each tube, which contains red blood cells, was used in estimating the PCV in percentages (%). Similarly, the buffy coat content, located at the middle layer of the tube, was expressed onto clean slides and microscopically examined under high power (40×). The presence or absence of trypanosomes was elucidated by observing movements and shapes of flagellated parasites. The positive samples were used to prepare thin film smears, fixed using 100% methanol, stained using Giemsa solution and microscopically examined under high power (40×) and oil immersion (100×).

Differential Diagnosis

Differential diagnosis was carried out by observing the motility of bloodstream trypomastigotes within the buffy coat and morphological features from Giemsa-stained thin films according to the descriptions of Woo (1970). Differential parameters including presence/absence of a free flagellum, size and position of kinetoplast, degree of development of undulating membrane, shape of the parasites posterior part and locomotion were observed.

T. congolense was detected as trypanosome that lacks free flagellum with centralized nucleus and marginal-sub terminal kinetoplast of medium size and an inconspicuous undulating membrane (Woo, 1970). *T. vivax* was detected as trypanosome possessing free flagellum with large and terminal kinetoplast, inconspicuous undulating membrane and a swollen, blunt extremity (Woo, 1970). Similarly, *T. Brucei* was detected as a polymorphic trypanosome, existing as long slender (with free flagellum), intermediate forms (that are usually flagellated) or, short stumpy (without free flagellum), sub-terminal kinetoplast, conspicuous undulating membrane and pointed posterior part (Woo, 1970). However, the presence of trypanosomes of different appearance in wet mount and stained preparations was considered as mixed infection (Maigari *et al*., 2015).

Data Analyses

To determine significant differences between infected and uninfected cattle,2x2 contingency tables (with Yates correction factor) and $\chi 2$ – square test of independence were used. Similarly, Kruskal – Wallis test was used for pairwise comparisons on the estimated values of PCV between infected and uninfected cattle brought from different sources. The analysis was performed using Max Stat (Version 3.0), and in all the analyses, a threshold value of $p < 0.05$ was considered as significant.

RESULTS

Prevalence of Trypanosomosis and Association between Age/Sex/Breeds

An overall prevalence of 22 (3.93%) was recorded in this study, where cattle above 3 years of age had significantly higher infection rates, 14 (2.50%, $\chi 2= 6.284$, df = 3, p =

0.0122) compared to other age groups (Table 1). Similarly, infection rates of bulls exceeded,18 (3.22%, $\chi 2$= 0.024, df = 1, p = 0.877), that of cows. Moreover, Sokoto Gudali breeds of cattle had higher infection rates, 12 (2. 14%, $\chi 2$=0.086, df = 1, p = 0.769) than White Fulani breeds of cattle (Table 1).

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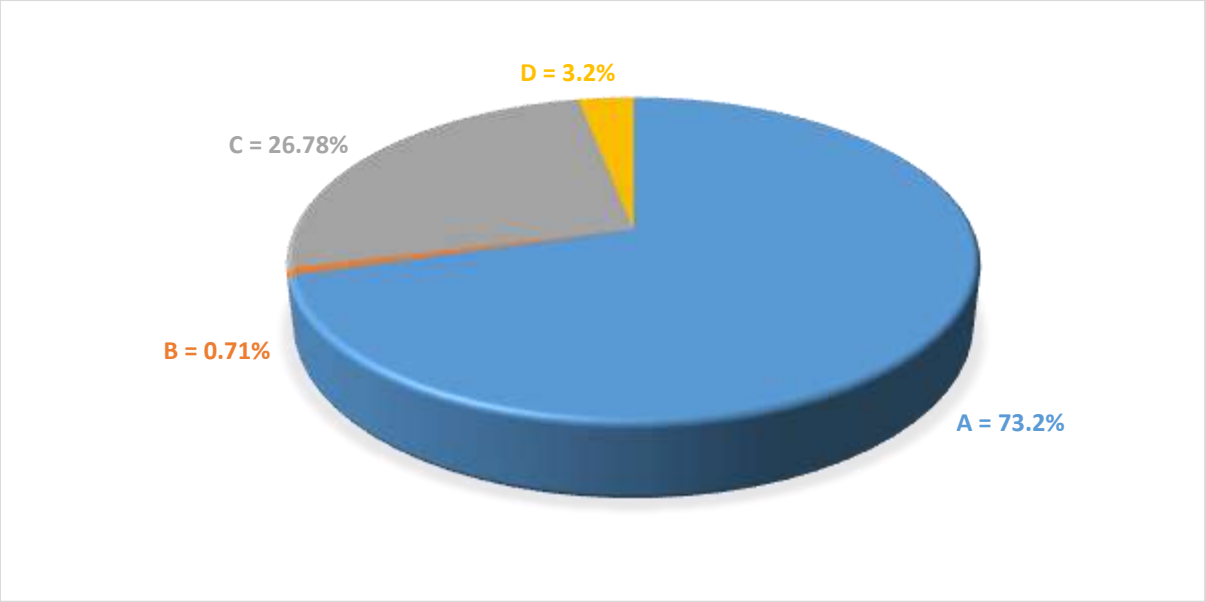
Table 1: Prevalence of Trypanosomes in Cattle at Kano Abattoir by Age, Sex and Breeds (Jan. – Dec., 2017)

Age (year)	No. Examined	Infection rates	*Tb*	*Tc*	*Tv*	M	$\chi 2$	P – value
≤ 1	7	0					6.284	0.0122
1.1 – 2	33	0						
2.1 - 3	101	8 (1.43%)[a]	0	1	7	0		
> 3	419	14 (2.50%)[b]	1	3	8	2		
Overall	560	22 (3. 93 %)	1	4	15	2		
Sex	No. Examined	Infection rates	*Tb*	*Tc*	*Tv*	M	$\chi 2$	P – value
bulls	438	18 (3.22%)[c]	1	3	12	2	0.024	0.877
cows	122	4 (0. 71%)[c]	0	1	3	0		
Overall	560	22 (3.93%)	1	4	15	2		
Breeds	No. Examined	Infection rates	*Tb*	*Tc*	*Tv*	M	$\chi 2$	P – value
Sokoto Gudali	335	12 (2. 14%)[d]	1	2	8	1	0.086	0.769
White Fulani	225	10 (1.79%)[d]	0	2	7	1		
Overall	560	22 (3.93 %)	1	4	15	2		

Tb: *T. brucei*
Tc: *T. congolense*
Tv: *T. vivax*
M: Mixed Infection involving *T. congolense* and *T. vivax*
In each parameter of variables, superscript with different letters indicate significant difference between values at $p < 0.05$, while those with the same letters indicate non-significant difference between values at $p < 0.05$

Prevalence of Trypanosomes and Frequency of BCS in Cattle:
The result of prevalence in relation to the BCS in cattle at Kano abattoir is presented in Figure 1 and Table 2. Generally, cattle with poor BCS had higher infection rates, 18 (3.2%), compared to cattle with good BCS, 4 (0.71%), (Figure 1). Similarly, cattle scoring II (very thin) had higher infection rate, 8 (1.43%, $\chi^2 = 11.279$, df = 8, p= 0.0008) compared to other categories (Table 2).



Legend:
A = Cattle with Good BCS
B = Infected cattle with Good BCS
C = Cattle with Poor BCS
D = Infected cattle with Poor BCS

Figure 1: Prevalence of Trypanosomosis and Overall BCS of Cattle at Kano Abattoir (January – December, 2017)

Table 2: Frequency of the 9 – points BCS and Distribution of Trypanosomes in Cattle (Jan. – Dec., 2017)

BCS	No. Examined	Infection rates	Species	$\chi 2$	P – value
I – Emaciated	21	1 (0.18%)*	*Tv*	11.279	0.0008
II – Very Thin	67	8 (1.43%)*	*Tb*, *Tc*, *Tv* &M		
III – Thin	131	5 (0.89%)*	*Tc* & *Tv*		
IV – Underweight	81	4 (0.71%)*	*Tc* & *Tv*		
V – Moderate	75	3 (0. 54%)*	*Tv*		
VI – Good	95	1 (0.18%)*	*Tv*		
VII - Very Good	49	0			
VIII – Obese	34	0			
IX - Very Obese	07	0			

* Significantly different
Tb: *T. brucei*
Tc: *T. congolense*
Tv: *T. vivax*
M: Mixed Infection involving *T. congolense* and *T. vivax*

Prevalence and Average PCV of Cattle at Kano Abattoir According to their Sources:
The average PCV was significantly reduced in parasitaemic cattle compared to aparasitaemic cattle ($p<0.05$) (Table 3). Similarly, irrespective of trypanosome status, mean PCV was significantly higher ($p<0.05$) in cattle from Garko and Dambatta compared to other sources.

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Table 3: Prevalence of Trypanosomosis and Average Haematocrit Values of Cattle at Kano Abattoir by their Sources (Jan. – Dec., 2017)

Sources	No. Examined	Infected	Average PCV of Infected ± STD	Uninfected	Average PCV of Uninfected ± STD	P – value
Dambatta	139	6 (1.07%)	$23^a \pm 0.05$	133 (23.75%)	$35^b \pm 0.06$	< 0.0001
Gaidam	3	0	0	3	30 ± 0.05	
Garko	8	0	0	8	36 ± 0.05	
Getso	1	0	0	1	30 ± 0.00	
K/Hausa	3	0	0	3	26 ± 0.09	
Mai Adua	144	3 (0.54%)	$26^a \pm 0.09$	174 (31.07%)	$33^b \pm 0.08$	0.0020
Maigatari	177	8 (1.43%)	$21^a \pm 0.09$	136 (24.28%)	$34^b \pm 0.07$	< 0.0001
Wudil	85	5 (0.89%)	$22^a \pm 0.11$	80 (14.29%)	$34^b \pm 0.07$	< 0.0001

Superscript with different letters indicate significant difference between values at $p < 0.05$

Distribution of Trypanosomes in Cattle at Kano Abattoir According to their Sources:

Animals brought from Wudil and Dambatta had significantly higher prevalence of trypanosomes ($\chi 2 = 11.34$, df = 7, $p < 0.0001$) compared to other sources with prevalence of 8 (1.00%) and6 (0.75%), respectively (Table 4).

Table 4: Distribution of Trypanosomosis in Cattle at Kano Abattoir According to their Sources (Jan. – Dec., 2017)

Sources	No. Examined	Infection Rates	Species	$\chi 2$	P – value
Dambatta	139	6 (0.75%)[a]	*T. congolense* & *T. Vivax*	11.34	< 0.0001
Gaidam	3	0	-		
Garko	8	0	-		
Getso	1	0	-		
K/ Hausa	3	0	-		
Maigatari	144	3 (0.38%)[b]	*T. congolense* & *T. vivax*		
Mai'Adua	177	8 (1.00%)[c]	*T. congolense* & *T. vivax*		
Wudil	85	5 (0.63%)[d]	*T. brucei*, *T. congolense* & *T. Vivax*		

In each parameter of variables, superscript with different letters indicate significant difference between values at $p < 0.05$

DISCUSSION

The overall prevalence of trypanosomes reported in this study, 22 (3.93%), was found to be higher than earlier reports by Fajinmi *et al*. (2011) where a prevalence of 1.8% was reported in a similar study at Sokoto Abattoir. However, this point prevalence is lower than previous report by Abenga *et al*. (2004) and Ezebuiro *et al*. (2009) where a prevalence of 6.0% and 5.0%, were reported, respectively. Similarly, a recent study by Sidi (2017) established a higher prevalence of 5.3% in cattle herds under husbandry in some parts of Southern Kaduna. Another study by Gumel (2017) reported a higher prevalence of 8.1% in Jigawa state.

This disparity in between the result of the present and earlier works might be due to the variation among the geographical location of the research area, method of detection and sample size. Similarly, the relatively higher infection rates reported by the previous studies might be associated with the non-random sampling of the studied animals where the sampling in the previous study was simply purposeful (Gumel, 2017; Sidi, 2017).

However, the plausible explanation for the higher prevalence of Trypanosomosis in bulls might be associated with the fact that, bulls, particularly oxen are kept longer and hence have more chances of contracting diseases than cows (Katale *et al*., 2013). Similarly, the differences might be associated to the movement of bulls in search of suitable female partners in which they traversed large areas of land thereby increasing their chances of being bitten by tsetse flies.

Result from this study shows that Sokoto Gudali breeds of cattle had higher infection rates than White Fulani breeds of cattle. However, this variations in the infection rates among the different breeds of cattle screened was found to be insignificant, justifying the fact that both breeds of cattle are trypanosensitive. Therefore, the differences could be attributed to the higher number of Sokoto Gudali cattle brought to the Kano abattoir during the period of sampling.

The higher prevalence of *T. vivax* agrees with the findings of Ezebuiro *et al*. (2009) where *T. vivax* was reported to be the most prevalent in cattle. However, the finding contradicts with the work of Ezeani *et al*. (2008) who reported that *T. brucei* and *T. Congolense* were the prevalent trypanosomes in cattle. The result also depicts a contrary view to that found by Omotainse *et al*. (2004) who reported mixed infection due to *T. brucei* and *T. congolense* as most prevalent in cattle. However, these contrary observations could be attributed to the differences in diagnostic methods used. For instance, the

inability to detect *T. brucei* by microscopy could be due to chronic enzootic nature of the parasites in which the level of parasitaemia is usually below the level of detection by simple microscopy (Salim *et al*., 2011). Similarly, failure to detect mixed infections of the three species by the parasitological method might be due to fluctuating parasitaemic behaviour of trypanosomes whereby only the species with highest proportions are likely to be diagnosed whereas the species which are fewer might not be identified (Ezeani *et al*., 2008).

Iatrogenic transmission is another important point to be considered as it directly affects the transmission of *T. vivax* trypomastigotes (Bittar *et al*., 2016). For instance, Bittar *et al*. (2016) reasoned that reusing the same needle in several animals for oxytocin application pre-milking may be a major factor in the transmission of *T. evansi* because the blood forms of some trypanosomes are directly transferred from one animal to another through needles contaminated with infected blood. Hence, needles that are not properly changed can be a potential disseminator of *T. vivax*.

The result also shows higher prevalence of trypanosomes in cattle with poor BCS. This finding supports previous findings by Tehseen *et al.* (2017) who reported a similar trend in which animals with poor BCS had significantly higher infection rates than those with good BCS, suggesting that cattle with poor BCS may be more susceptible to the disease due to stress, poor nutrition, workload, other infections, and, hence, compromised immunity. In contrast, quality and plentiful pastures and water sources may contribute in making infected cattle to look healthier and thus possess good BCS as evinced in this study by few infected animal. Similarly, cattle with poor BCS that tested negative might be associated to the drought condition and long trekking distance, where animals move long distances in search of pastures and water sources (Katale *et al.*, 2013) which may allow the entry of infected animals and, hence, the onset of the disease.

The fact that several factors may act individually or synergistically justifies the variation encountered in the level of PCV recorded in this study, since many diseases, conditions, and other factors can cause anaemia. For example, anaemia may occur during pregnancy if the body of an animal cannot meet its increased need for RBCs. Similarly, certain autoimmune disorders and other conditions may cause animals' body to make proteins that destroy their RBCs, which can lead to anaemia. Equally, heavy internal or external bleeding (for instance, injuries sustained during transportation and rough handling by livestock conveyers to Abattoir) may lead to loss of blood and consequently causes anaemia.

The higher infection rates recorded in cattle brought from Dambatta and Wudil might be associated with proximity of herd pens to watering point distances, suggesting that hydrological networks played important part in Trypanosomosis transmission. The result also shows that, *T. congolense* and *T. vivax* were the predominant trypanosome species while *T. brucei* was the least encountered trypanosome species occurring only in cattle brought from Wudil market. However, this variation in the prevalence of Trypanosomes with respect to their sources might be attributed to the transhumant activities of herdsmen. This however, concords assertions by Majekodunmi *et al*. (2013) who noted that migration could easily influence risk for Trypanosomosis.

The generally lower prevalence recorded in the present study could be associated to the poor sensitivity of the classical parasitological approaches under field conditions where the peripheral parasitaemia of naturally infected animal may be very low (Nhamitambo *et al.*, 2017; Simwango *et al.*, 2017). Moreover, changes in the methods of transporting livestock to markets, ecological changes in northern Nigeria (Gumel *et al*., 2011), success in previous intensive tsetse eradication programs as well as increased awareness and application of preventive measures by herdsmen might also be the reason for the low prevalence encountered.

CONCLUSION AND RECOMMENDATIONS

The prevalence reported in this study was lower compared to earlier reports which range from 5.0% (Ezebuiro *et al*. 2009) to 8.1 % (Gumel, 2017). However, the higher number of *T. vivax* as well as the mixed infections of *T. vivax* with *T. congolense* in the study is of significance concern which could be an indication that its transmission is highly favoured by the obligate cyclical vector or that the *T. congolense* and *T. brucei* respond better to trypanocidal drugs. More epidemiological surveillance is therefore recommended. Similarly, while our study suggests that the standard trypanosome detection method using parasitological technique is less sensitive, it is able to detect trypanosomes in even cattle with good BCS. Hence, it can still be a better choice of test for large scale epidemiological study of animal Trypanosomosis.

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