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ASSOCIATION BETWEEN ANAEMIA AND BOVINE TRYPANOSOMIASIS IN CATTLE SLAUGHTERED AT SELECTED ABATTOIRS IN KADUNA, NORTH-WESTERN NIGERIA

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ABSTRACT

This study aimed to assess the prevalence of bovine trypanosomiasis among slaughtered cattle in specific abattoirs in Kaduna State, Nigeria. It also sought to explore the potential impact of associated risk factors on anaemia. Sample size was determined using standard epidemiological formula, anticipating a prevalence of 24%. Standard Trypanosome Detection Methods (STDM) among which Hematocrit Centrifugation Technique (HCT) and Buffy Coat Method (BCM) were employed for detecting trypanosomes. Packed cell volume (PCV) was determined as described by Cheesbrough. Epidemiological variables such as source, age, sex, and breed were assessed and used the Chi-square test to analyze the correlation between the prevalence of anemia and trypanosomiasis. Among the 500 examined cattle, 2.6% were infected with trypanosomes while 97.4% tested negative for the infection. Trypanosome-positive cattle had a mean PCV of 25.08 ± 8.78%, whereas trypanosome-negative ones had a mean PCV of 34.34 ± 9.49%. Cattle with PCV levels indicative of anaemia showed the highest incidence of parasitemia at 14.7%. Additionally, there was no statistically significant association between the prevalence of trypanosomiasis and breed, age, sex or district of the animals. It is concluded that bovine trypanosomiasis remains a persistent challenge in Kaduna State, posing a threat to cattle production and potentially leading to economic losses for residents.

Keywords: Anaemia, Cattle, STDM, Trypanosomes
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INTRODUCTION

Trypanosomes are protozoan flagellates that causes human African trypanosomiasis (HAT) and African animal trypanosomiasis (AAT). AAT is caused by a number of trypanosome species, including *T. brucei brucei, T. evansi, T. vivax, T. congolense, T. godfreyi and T. simiae* [1]. If neglected, trypanosomiasis could have serious health implications for both humans and animals. The subsequent financial demands to cure trypanosomiasis and the economic effect on livestock production as a result of AAT cannot be overemphasized [1]. It is therefore important to determine the prevalence of trypanosomes in cattle in order to establish the risk posed to the population. The disease can cause significant economic damage mainly due to reduced milk yields, decreased animal market values and annual mortality rates affecting thousands of animals [2, 3]. The clinical manifestations of trypanosomiasis in animals are influenced by the host and Trypanosoma species. In general, the disease is fatal unless treated and can cause a wide range of symptoms in different animals [4].

The major characteristics of the disease includes cachexia, edema, reproductive disorders and severe anaemia that may lead to death if neglected [5]. African animal trypanosomiasis is the main parasitological constraints to livestock production in many sub-Saharan African countries infested with tsetse flies [6], and it is responsible for about 3 million livestock deaths annually [7].

The trypanosomes that causes this disease are extracellular protozoan parasites that have developed efficient immune escape mechanisms to manipulate the entire host immune response to allow parasite survival and transmission [8]. Anaemia is a cardinal sign of trypanosome infections [8], and its mechanism due to trypanosomiasis is complex and multifactorial in origin [9]. The rate at which anaemia develops is influenced by energy intakes and protein gain [10].

The interplay of several factors acting either individually or synergistically contributes to the development of haemolytic anaemia in animal trypanosomiasis [11]. Most common among these factors are increased intravascular red blood cells destruction caused by lashing action of trypanosome flagella, undulating pyrexia, platelet aggregation, toxins and metabolites from trypanosomes, lipid peroxidation and malnutrition. Packed cell volume (PCV) usually gives indication of anaemia and disease status of

trypanosome-infected animals and is correlated with animal production and reproduction performance [12].

MATERIALS AND METHODS

Study area

The study was conducted in Kaduna State, Nigeria. The state is situated in North-West geopolitical zone of the country. It has an area of 923,768 sq. km with 23 local Government areas in three senatorial zones. It is located on a latitude of 6° and 11° north and longitude 7° and 44° east. It has a population of 6,113,503 according to National Population Commission [13]. The abattoirs involved in the study were the Zango abattoir, Zango Road, Tudun wada, Kaduna South Local Government area, which lies on the latitude of $10^0 36´33.54´´$ N and longitude $7^0 25´46.21´´$E [14], and the Zango abattoir which lies between latitude $11^0 08´13.7´´$N and Longitude $07^0 40´03´´$E in Samaru Road Zaria, Kaduna State.

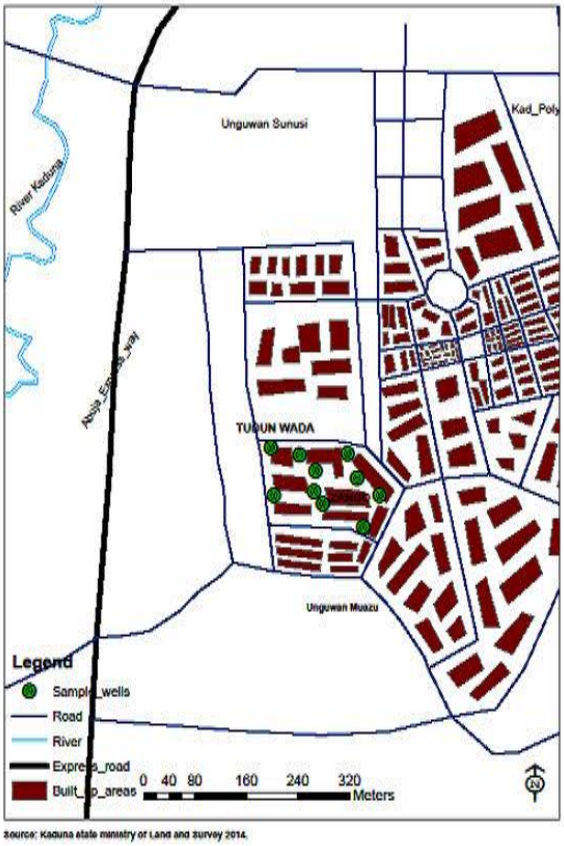

Figure 1: Map of Zango Abattoir Tudun Wada Kaduna. Adopted from Abdullahi [15].

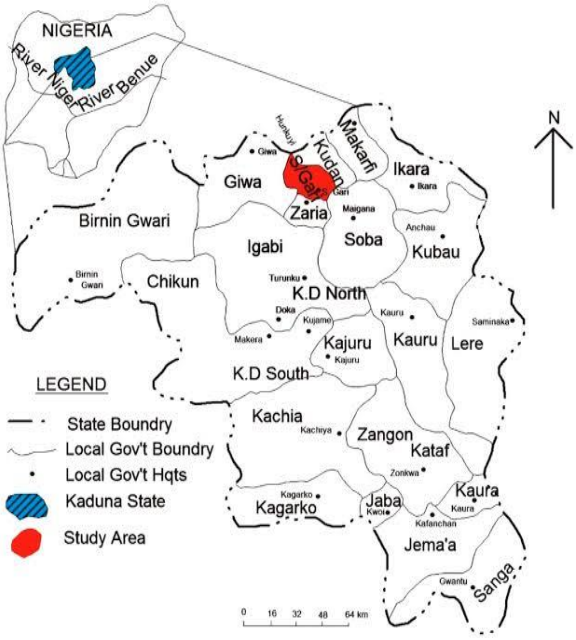

Figure 2: Map of Zango Abattoir in Samaru Zaria. Adopted from Kabiru *et al.* [16]

Study population

The study population included cattle slaughtered at Zango Abattoir Zaria and at Zango Abattoir in Tudun wada, Kaduna with over 20 and 50 cattle slaughtered daily respectively. The study population also included all cattle in the abattoir irrespective of their epidemiological status (age, sex, breed and source).

Sample size

The sample size was determined using a formula described by Naing et al. [17].

$$n = \frac{Z^2 x(P) \times (1-P)}{d^2}$$

$$n = \frac{1.96^2 \times (0.24) \times (1-0.24)}{0.05^2}$$

$$n = 280$$

Where n = Sample Size
Z = Standard normal distribution (1.96 for 95% confidence level)
P = Percentage expressed as decimal (0.5 used for sample needed)
d = Precision, confidence interval at 5% expressed as decimal (0.05)
Using a reported mean prevalence rate of 24% by Majekodunmi *et al.* [18]
A sample size of 500 was used in the study

Collection of blood samples

The blood samples were collected in sterile Ethylene Diamine Tetra acetic acid (EDTA) bottles, each sample was identified appropriately and transported in a cooler with ice packs from Zango Abattoir, Kaduna to Nigerian Institute for Trypanosomiasis Research (NITR) Kaduna, for parasitological analyses immediately. Samples collected from Zango Abattoir Zaria, were analyzed at the Department of Parasitology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

Parasitological analyses

Wet preparation: A drop of blood was placed on a clean glass slide and covered with a coverslip to spread the blood at a mono layer of cells. This was examined using microscope of x40 magnification to detect motile trypanosomes seen either directly, moving between the blood cells, or indirectly, as they cause the blood cells to move. The lower the magnification, the larger the field observed, and the faster the examination [19].

Thick film: A drop of blood was placed on a clean slide and allowed to dry. It was fixed with absolute methanol and stained with 5% Giemsa stain and was examined microscopically using x100 magnification.

Thin film: A drop of blood was thinly smeared on a clean glass slide, and the slide was allowed to air dry before dropping it in Giemsa stain for 10minutes, the stain was washed away and dried. Stained slides were viewed under x100 magnification.

PCV determination: A heparinized capillary tube was filled with blood to a three-quarter level, then sealed using modeling clay sealant and centrifuged in a micro haematocrit rotor at 3,000rpm for 3 minutes and PCV was determined using a micro- hematocrit reader [19].

Concentration method: A heparinized capillary tube was filled with blood to a three-quarter level, then sealed using modeling clay sealant and centrifuged in a micro-hematocrit rotor for 3 minutes at 3,000rpm. It was seen that most of the white cells settled at a slower rate than the red cells as their specific gravity is a little less than that of the red cells. The capillary tube was placed on a glass slide and viewed at x40 magnification for the presence of parasites while the buffy coat that formed between the much thicker red cell layer and the column of plasma was viewed [20].

Statistical analysis: All statistical analyses were performed using SPSS software version 29. Chi-square ($\chi 2$) test was used to determine association between infection and epidemiological variables while student's t-test was used to determine significant difference between mean PCV of infected and non-infected animals. The prevalence was calculated for all data as the number of infected individuals divided by the number of individuals examined and multiplied by 100. A statistically significant association between variables was said to exist if the value of $p \leq 0.05$ at 95% confidence level.

RESULTS

Demography of the cattle: The study includes diverse group of cattle, with five indigenous breeds and their crossbreeds, as well as a foreign breed. The majority of the sampled cattle were White Fulani (71.6%) followed by Sokoto Gudali (11.8%), and the rest accounted for smaller percentages. The ages of the sampled cattle ranged from 9 months to 12 years old, with the highest number falling between 3.5 and 6 years old (63.3%). Female cattle made up 82% of the sample, while males accounted for 18%. Most slaughtered cattle came from Kaduna, Kano, and Katsina. The overall prevalence of trypanosomes in slaughtered cattle was found to be 2.6%, with no statistically significant association between breed or age and infection prevalence observed. Male cattle showed a slightly higher infection rate than females but without statistical significance. Cattle sourced from Bauchi had the highest prevalence of trypanosome infection at 13%, followed by those from Katsina and Kaduna with lower rates, while no infections were found in those from Sokoto, Chad or Farm house (Table 1).

Packed cell volume: There was significant difference (t = 3.747; $p = 0.003$) in the mean packed cell volumes between infected and uninfected cattle (Table 2).

Prevalence of trypanosome infection based on PCV range: There was no significant difference ($\chi 2 = 1.871$; $p = 0.967$) in the prevalence of trypanosomes among cattle based on their PCV ranges. The highest prevalence of trypanosome infection (14.7%) was recorded in cattle with PCVs between 10 and 20%. However, parasitemia was not detected amongst cattle with PCV greater than 40% (Table 3).

PCV based on age range of cattle: There was significant difference ($p < 0.001$) in the mean PCV values of cattle based on their age categories. The mean PCV of cattle between age of 1 month and 1 year was 38.09 ± 7.95%. While the mean PCV between ages 5 and 6 years was 32.56 ± 10.6%; for cattle above 7 years were 33.50 ± 11.7%, respectively (Table 4).

PCV in relation to breed of cattle: There was no statistically significant difference ($p = 0.481$) in the mean PCV values amongst different breeds in the study. The mean PCV of White Fulani and cross breed were 33.63 ± 9.37 and 35.74% ±9.92 respectively (Table 5).

PCV in relation to sex of cattle: In the male, the mean PCV was 36.77 ± 8.92% while in the female it was 33.52

± 9.64%. There was statistically significant difference ($p < 0.05$) in the mean PCV between male and female cattle (Table 6).

Parasitemia based on different trypanosome detection methods: There was significant difference ($\chi 2 = 27.984$; $p < 0.001$) in the number of trypanosome positive cases identified using different basic laboratory methods of trypanosome detection (STDM). The Hematocrit Centrifugation Technique (HCT) and the Buffy Coat methods were most sensitive detection methodologies with corresponding infection prevalence of 2.6% each (Table 7).

Association of trypanosomes infection and occurrence of anaemia: There was a statistically significant difference ($p = 0.004$) between the mean number of cattle that are anemic and non- anemic irrespective of infection status (Table 8).

Table 1: Prevalence of trypanosomiasis and associated risk factors

Demographic factors	No. examined (%)	No. positive (%)	χ^2-value	*p*-value
Breed of cattle				
Sokoto Gudali	59 (11.8)	1 (1.7)	0.900	0.989
White Fulani	358 (71.6)	10 (2.8)		
Red Bororo	10 (2.0)	0 (0.0)		
Kuri	1 (0.2)	0 (0.0)		
Cross breed	62 (12.4)	2 (3.2)		
Keteku	9 (1.8)	0 (0.0)		
Foreign	1 (0.2)	0 (0.0)		
Age (years)				
0 – 2	81 (16.2)	2 (2.5)	0.383	0.944
3 – 5	269 (53.8)	8 (3.0)		
6 – 8	149 (29.8)	3 (2.0)		
9 and above	1 (0.2)	0 (0.0)		
Sex				
Male	90 (18.0)	3 (3.3)	0.233	0.629
Female	410 (82.0)	10 (2.4)		
Source of cattle				
Sokoto	3 (0.6)	0 (0.0)	11.51	0.074
Kaduna	273 (54.6)	6 (2.2)		
Katsina	93 (18.6)	3 (3.2)		
Bauchi	23 (4.6)	3 (13.0)		
Kano	106 (21.2)	1 (0.9)		
Chad	1 (0.2)	0 (0.0)		
Farm House	1 (0.2)	0 (0.0)		

Table 2: Mean packed cell volume of slaughtered cattle

Parameter	Frequency (%)	Mean PCV (%)
Infected	13 (2.6)	25.08 ± 8.78
Uninfected	487 (97.4)	34.34 ± 9.49
***p* –value**	< 0.001	0.003

Table 3: Prevalence of trypanosome infection based on PCV ranges of the cattle

PCV range (%)	Frequency (%)	No. positive (%)	χ^2-value	*p*-value
10 – 20	34 (6.8)	5 (14.7)	1.871	0.967
21 – 30	152 (30.4)	3 (2.0)		
31 – 40	203 (40.6)	5 (2.5)		
41 – 50	96 (19.2)	0 (0.0)		
51 – 60	12 (2.4)	0 (0.0)		
>60	3 (0.6)	0 (0.0)		

Table 4: PCV in relation to age of cattle

Age (year)	Number examined	Packed cell volume (%)
1 Month – 1	22	38.09 ± 7.95
1 – 2	47	35.52 ± 8.59
2 – 3	70	34.46 ± 8.41
3 – 4	97	36.01 ± 8.37
4 – 5	99	34.50 ± 10.4
5 – 6	112	32.56 ± 10.6
6 – 7	34	27.40 ± 7.54[a]
>7	6	33.50 ± 11.7

$p < 0.001$; PCV value with superscript ´a´ differs statistically

Table 5: PCV in relation to breed of cattle

Breed	Number Examined	Packed cell volume (%)
Sokoto Gudali	59	34.68 ± 11.3
White Fulani	358	33.63 ± 9.37
Red Bororo	10	34.60 ± 6.31
Cross breed	62	35.74 ± 9.92
Keteku	9	36.56 ± 6.54

p = (0.481)

Table 6: PCV in relation to sex of cattle

Sex	Number examined	Packed cell volume (%)
Male	90	36.77 ± 8.92
Female	410	33.52 ± 9.64
***p* value**		0.003

Table 7: Determining parasitemia using various trypanosome detection techniques

Method of screening	No. examined (%)	No. positive (%)	χ^2-value	*p*-value
Wet mount	500	5 (1.0)	27.894	< 0.001
HCT	500	13 (2.6)		
Buffy coat	500	13 (2.6)		
Thick film	500	0 (0.0)		
Thin film	500	0 (0.0)		

Table 8: Association of trypanosomes infection and occurrence of anaemia

Status	Infected (%)	Uninfected (%)	Total (%)
Anemic (PCV< 24%)	5 (7.9)	58 (92.1)	63 (12.6)
Non- anemic (PCV ≥24%)	8 (1.8)	429 (98.2)	437 (87.4)
Total (%)	13 (2.6)	487 (97.4)	500

Chi Square ($\chi 2$) = 8.106; $p = 0.004$; Odd ratio: 0.216 (95% CI: 0.068 – 0.684)

DISCUSSION

Trypanosomiasis prevalence was not statistically significant ($p > 0.005$) in association to breed, age, sex and districts (source) of animals which is in conformity with the report of Lelisa *et al.* [21] in Western Ethiopia who reported that there was no significant variation in Trypanosomiasis prevalence between sex and study location. Breeds of cattle were considered, and the highest prevalence was observed in cross breeds, this agrees with the findings of Simwango *et al.* [22] which may be due to random assortment of genes with higher susceptibility to disease contraction. Age related prevalence revealed that juvenile cattle were relatively more infected than adults and the finding agrees with the study of Simwango *et al.* [22] that reported an association between age and prevalence of trypanosome infection. It however contradicts claims of Sam-Wobo *et al.* [23] and Fasanmi *et al.* [24] that says adult cattle were more prone to trypanosome infection than the juveniles. These current findings may be due to equal chances of exposure to tsetse fly bite as well as easy penetration of the tsetse fly proboscis through the developing coat possessed by the juvenile cattle. Sex prevalence of animal trypanosomiasis was also higher in the male cattle compared with female cattle, and may be probably because the males are more often in the fore front of the herd while grazing hence exposing them more to bites by flies than the females. This report is consistent with Sam-Wobo *et al.* [23] assertion that trypanosome prevalence is higher in male than in female animals but differs from the reported findings in Idehen *et al.* [25] that females are more prone to trypanosome infection than males. Bauchi had the highest prevalence which is in agreement with the report of a survey by Weber *et al.* [26] that showed the area to be more favorable and conducive for the survival of the vector. It is also in conformity with the report of Lelisa *et al.* [21] in Western Ethiopia.

Among the 500 cattle examined, 2.6% were infected with trypanosomes whereas 97.4% were negative. These finding shows persistent trypanosome challenge to the area and consequent reason why trypanosomiasis has remained a threat and constraint for cattle production in Kaduna state which could create economic loss to the people in the area. The prevalence of 2.6% from the study is higher than 0.8% reported in Kwara by Habeeb *et al.,* [27]; 1.5% reported in Obudu cattle ranch in Cross Rivers by Anene *et al.,* [28] and 1.8% reported from Sokoto state by Fajinmi *et al.,* [29], all in Nigeria. The lower prevalence reported in those areas is not surprising because they were earlier designated as tsetse and trypanosomiasis free zones [27], and it could also be because of improper report of survey or lack of thorough surveillance carried out. However, Idehen *et al.* [25] reported 4.8% in Jos. Bello *et al.* [30] also reported an overall prevalence rate of 11.8% and 9.0% in Gombe and Borno States, respectively. In another study by Chibuogwu *et al.* [31] a prevalence of 5.66% is reported in Asaba, Delta state. Lelisa and Meharenet [11] reported an overall prevalence of bovine trypanosomiasis of 7.81% in West Gojjam zone, Northwest Ethiopia. In another study by Mamoudou *et al.* [32], a prevalence of 9% was reported from Cameroon. The higher level of trypanosomes detection could be due to the sensitive molecular based technique employed in their studies. Another source of such variations might be associated with differences in agro-climates, distributions and density of vectors, season, and vectors control applications that may hinder the epidemiology of trypanosomiasis.

The Standard Trypanosome Detection Method (STDM) used in the study is to avoid misdiagnosis of the infection as it's a combination of different techniques. The buffy coat and haematocrit centrifugation methods detected more of the infection (2.6%) than other methods. This could be as a result of the samples been in concentrated forms hence the higher chance of detecting the parasites, which concur with the findings of Fajinmi *et al.* [29]. The positive association between *Trypanosoma* infection and anaemia could be as a result of trypanosomes being haemoflagellates as such red blood cells are destroyed by their infection.

Lower PCV (11-20%) was recorded among the samples with the highest occurrence of the parasites (14.7%) and this could be an indication of anaemia [33]. The mean PCV in trypanosome positive and negative cattle was 25.08 ± 8.78% and 34.34 ± 9.49% respectively. Relatively Similar values were reported by different authors. Idehen *et al.* [25], reported a mean PCV of 21.93 ± 5.50 and 27.02± 4.39 in Bassa, Plateau Nigeria. It is also reported by Dagnachew *et al.* [34] a mean PCV of 24.29% in trypanosome positive and 27.46% in Northwest Ethiopia. The average PCV value was notably lower in cattle infected with trypanosomes compared to those not infected. Furthermore, as the onset of anaemia is a key indicator of trypanosomiasis, the decrease in PCV is associated with the increasing prevalence of the disease. The severity of trypanosomiasis and its impact on production performance in susceptible or infected animals can be assessed by the intensity of anaemia indicated by declining PCV values. Nevertheless, there were anaemic cattle that were not infected with trypanosomes that would be attributed to limitations of the detection methods utilized. Other factors may induce anaemia in absence of trypanosomiasis which includes diseases that cause erythrocyte haemolysis or specific nutritional deficiencies, gastrointestinal and blood parasites, may all result in occurrence of anaemia [35]. Positive animals with PCV levels above 24% may have recently been infected and could be tolerating parasitemia without

displaying anaemia. There were no previous reports on multifactorial anaemia in the study area, so the possibility of its contribution cannot be dismissed. The study highlighted that PCV alone is not sufficient for diagnosing trypanosomiasis, aligning with the findings in a report by Lelisa and Meharenet [11], who emphasized that measuring PCV alone may not confirm a diagnosis of trypanosomiasis.

CONCLUSION

The study shows that Trypanosomiasis prevalence was not statistically significant ($p > 0.005$) in association to breed, age, sex and source of animals. Trypanosome infection rate of 14.7% was found to be associated with cattle having lower PCV values and also infection rate was observed to decrease with increasing PCV. Infected cattle had significantly lower mean PCV (25.08%) compared to uninfected cattle (34.34%), this aligns with expectations as trypanosomiasis causes anaemia. These findings reveal PCV can be used as screening index for trypanosomiasis in cattle and PCV <30% could be targeted for screening to detect infections early.

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