International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2019; 4(4): 07-11

www.veterinarypaper.com
Received: 04-05-2019
Accepted: 06-06-2019

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Haemoparasitic infection and haematological indices of cattle slaughtered for sale in Calabar, Nigeria

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Abstract
Due to economic losses to livestock producers caused by haemoparasitic infection, this study was conducted to determine the prevalence of haemoparasites and some haematological parameters of slaughtered trade cattle in Calabar abattoir. A total of 180 blood samples were randomly collected from cattle between May and October 2018. The samples were screened for haemoparasites by examining Giemsa stained thin blood films. Packed cell volume, haemoglobin concentration, total red blood cell counts and total white blood cell counts were determined using standard methods. An overall prevalence of 14(7.78%) was recorded for *Anaplasma* (2.22%) and *Trypanosoma* sp. (5.56%). Older cattle (10%) were more infected than younger Cattle (5%). There was significant difference (p<0.05) in mean packed cell volume (PCV) and mean corpuscular haemoglobin concentration (MCHC) counts between infected and un-infected slaughtered cattle. Haemoparasites are endemic in cattle populations in Calabar and the prevalence of haemoparasites may be associated with changes in PCV.

Keywords: Haemoparasites, haematological indices, anaemia, cattle, Nigeria

1. Introduction
Cattle in sub-Saharan Africa may be infected with wide varieties of parasites such as the Rikettsiae: *Anaplasma* and *Ehrlichia* (cowdria) and also the protozoan parasites, *Babesia*, *Theileria* and *Trypanosoma* (Bell-Sakyi, *et al*., 2004; Okaryeto *et al*., 2008) [6,20]. These haemoparasites have generally been shown to cause destruction of the red blood cell leading to anaemia, jaundice, anorexia, weight loss and infertility (Akande *et al.*, 2010; Okorafor and Nzeako, 2014) [3,21].

Parasitic diseases have debilitating effects on animal health worldwide, particularly in developing countries (Ellies *et al*., 2003) [10]. The direct losses caused by haemoparasites are linked to acute illness and death, premature slaughter and rejection of some body part at meat inspection. Indirect losses includes, reduction in production potential, such as decreased growth rate, weight loss in young growing animals and late maturity of slaughter stock (Hansen and Perry, 1994) [12]. Farmers may not appreciate the effect of these haemoparasites on animals perhaps due to subclinical and chronic nature of disease presentation in affected animals (Jatau *et al*., 2011) [14].

Most cattle owners graze their cattle under pastoral husbandry system. In this system cattle are extensively grazed on pasture and forest, and may be exposed to various arthropods vectors of haemoparasite such as ticks and tsetse fly (Obadiah and Shekaro, 2012) [19]. Ticks and tsetse flies transmit haemoparasites of genera *Babesia*, *Anaplasma*, *Theileria* and *Trypanosoma* respectively. Their impact on cattle production and productivity accounts for economic losses to livestock producers in the tropics and subtropics (Soulby, 1982; FAO, 1984) [24, 11]. They are also responsible for destruction of red blood cell leading to anaemia, jaundice, anorexia, weight loss and infertility in livestock (Akande *et al*., 2010) [3].

Animal production in many Africa countries such as Botswana, Mauritana and Namibia, contributes 20 to 30 percent agricultural Gross Domestic Products (Anon, 2004) [5], in countries. In Nigeria, livestock account for one third of agricultural gross domestic production (GDP), providing income, employment, food, farm energy, manure, fuel and transport. They are also a major source of government revenue.

Animal Agriculture is an indispensable perquisite towards the sustainability of human development (Oluwafemi, *et al*., 2001) [22].

Health issues of livestock caused by haemoparasites may be accompanied by a decrease in some blood parameters including blood trace elements, mineral levels and other biochemical parameters. A reduction in haematocrit and haematological values are reported in infested cattle as compared to those cattle which were free from tick infestation (Zawua, *et al.,* 2015) [27]. An increase in number of eosinophils and lymphocytes in ticks infested cattle has also been reported (Delpreet *et. al.,* 2017) [9].

This study aims at evaluating some haematological indices and their correlation with haemoparasitic infection of cattle slaughtered for sale in Calabar. The study also determines the prevalence of haemoparasites, the single, mixed infection and haematological indices based on sex and age distribution of the haemoparasites and also the association between haematological indices of infected and non-infected cattle.

2. Materials and Methods

2.1 Study area

The study was carried out in Calabar in three (3) different locations which include Ultramodern Abattoir Ikot Eneobong, Nassarawa Abattoir and Amalgamated butcher union Abattoir Anantigha.

Calabar is a capital city in Cross River State in South Southern Nigeria with total area of 406km^2 (157 sq mi) with elevation of 32m (105 ft), population of 371,002 in 2006 census and density of 910/Km^2 (2400/sq mi). Calabar is divided into Calabar Municipal and Calabar South Local Government Areas. Calabar is located around Latitude 4^{o}57'0"N and longitude 8^o 19'30" E.

2.2 Study Design

The study was conducted between May, 2018 and October, 2018. A total of 180 trade cattle presented for slaughter in the three (3) selected Abattoir in Calabar was selected for the study; 60 in Ultramodern Abattoir Ikot Eneobong, 60 in Amalgamated butcher union Abattoir Anantigha and 60 in Nassarawa Abattoir.

Characteristics of the population such as sex and age was observed and recorded for each sample throughout the study. Sex differentiation was based on the appearance of external genitals, while ageing was determined by rostral dentition as described by (Lasisi *et al*., 2002) [17]. Only male cattle were available during the study. Cattle less than three (3) year old was categorized as young while older ones was considered as adult.

2.3 Collection of Blood Sample

5ml of blood was collected from the jugular vein of the animals at the point of slaughter into EDTA (Ethyl-Diamine-Tetra Acetic acid) bottles. Sample bottles were labeled appropriately and placed in ice packs in a bucket for onward delivery to the Department of Zoology and Environmental Biology Laboratory, University of Calabar where further analysis was carried out.

2.4 Parasitological examination

Thin and thick blood films was prepared as described by Soulby (1982) [24] for haemoparasites examination. Also, Buffy Coat concentration method was used for the detection of Trypanosomes in the blood (Cheesebrough, 2005) [8].

2.5 Determination of Haematological of Parasites

2.5.1 Microhaematocrit Centrifuge Techniques

Packed Cell Volume (PCV) was determined using microhaematocrit centrifuge techniques as described by Cheesebrough (2005) [8].

2.5.2 Sahli method

Sahli acid haematin method was used to determine the haemoglobin count using the haemometer. Blood was mixed with N/10HCl which will result in conversion of haemoglobin to acid haematin which is brown in color. The solution was diluted till its color matches with the brown colored glass of the comparator box. The concentration of haemoglobin was read directly from the graduation in the calibration tube.

2.5.3 Neubauer haemocytometer

Neubauer haemocytometer was used in determining the red and white blood cell counts. This method was used as described by Juarez, *et al.,* 2002 [15].

2.5.4 Determination of other Blood Cell Indices

The other blood indices used in the study include Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC). These indices were calculated using the following standard formulas

$$\text{MCV (fL)} = \frac{PCV}{RBC_s\ counts} \text{ X } 10$$

$$\text{MCH (pg)} = \frac{Hb}{RBC_s\ counts} \text{ X } 10$$

$$\text{MCHC (g/dL)} = \frac{Hb}{PCV} \text{ X } 100$$

2.6 Data Analysis

The prevalence rate among the different categories were expressed as percentage of the total animals sampled. The *Chi*-square test was used to test for significant differences in haemoparasitic infection among sexes and age groups. Haematological indices was analyzed using ANOVA and P-values < 0.05 was considered significant. All analysis was done using SPSS, version 22.

3. Result

3.1 Prevalence of Haemoparasitic Infection

The distribution of haemoparasites in slaughtered trade Cattles as shown in Table 1 indicated that out of 180 samples, an overall prevalence of 14 (7.78%) was recorded while 166 (92.22%) were negative. The highest prevalence was recorded in Nassarawa abattoir 6(3.33%), followed by Ikot Eneobong abattoir 5(8.33%) and Anantigha 3(5%), although there was no significant difference (p>0.05) (Table 1).

Trypanosoma sp. had the highest infection (5.56%) while *Anaplasma* was recorded in (2.22%) of the sample examined. Also, there was higher prevalence of *Trypanosoma* sp. in Ikot Eneobong abattoir (8.33%) and Anantigha abattoir (5%) compared with Nasarawa abattoir (3.33%) while the incidence of *Anaplasma* was recorded in Nasarawa abattoir (6.67%) only (Table 1).

Table 2 shows the haemoparasites distribution based on age. Older cattle (10%) were more infected than younger Cattle (5%) although this was not significant (P>0.05).

3.2 Haematological Parameters

Some haematological parameters of infected and uninfected cattle as shown in Table 3 indicate that there was a significant difference (p<0.05) in Packed cell volume and Mean corpuscular haemoglobin concentration between infected and uninfected cattle slaughtered for sale in Calabar.

Based on location as shown in Table 4, there was significant difference (p<0.05) in Packed cell volume, White blood cell count, Mean cell volume and Mean corpuscular haemoglobin levels between infected and uninfected cattle in Ikot Eneobong while haematological parameters varied insignificantly in infected and uninfected Cattles examined in Anantigha and Nasarawa abattoirs.

With respect to age as shown in Table 5, the packed cell volume of infected young Cattles was lower than the normal range of 30% - 45% (Merck 2012) [18] while Mean corpuscular haemoglobin concentration of young Cattles was higher than the normal range of 30% to 36% (Merck 2012). Other parameters remained under the normal range. However, Packed cell volume and Mean corpuscular haemoglobin concentration levels varied significantly in both infected and uninfected younger and older Cattles.

The number of infected and anaemic 7(3.89%) with uninfected and anemic cattle 2(1.11%) slaughtered for sale (Table 6) using a standardize PCV of 30% to 45% as normal value (Merck 2012) [18] showed no significant difference (P>0.05) between them.

Table 1: Prevalence of haemoparasites in the three study locations

Sample Location/Abattoir	No. Examined	No. Positive (%)	No. Negative (%)	No. +ve for *Trypansome* (%)	No. +ve for *Anaplasma* (%)
Ikot Eneobong	60	5(8.33%)	55(91.67%)	5(8.33%)	_
Anantigha	60	3(5%)	57(95%)	3(5%)	_
Nasarawa	60	6(10%)	54(90%)	2(3.33%)	4(6.67%)
Total	180	14(7.78%)	166(92.22%)	10(5.56%)	4(2.22%)

$X^2 = 2.273$; $P > 0.05$

Table 2: Age distribution of haemoparasites

Age	No. Examined	Ikot Eneobong No. +Ve (%)	Anantigha No. +Ve (%)	Nasarawa No. +Ve (%)	Total No. +Ve	X^2	p-value
Younger	80	1(1.25)	2(2.50)	1(1.25)	4(5.00)		
Older	100	4(4.00)	1(1.00)	5(5.00)	10(10.0)	0.167	0.061

Table 3: Haematological parameters of infected and uninfected slaughtered trade cattle in Calabar

	Mean± Se (Range)	
Haemtological Indices	**Positive (N=14)**	**Negative (N=166)**
PCV (%)	27.35 ± 2.14(40-18)^a	34.12 ± 0.48(44 -21)^b
WBC($\times10^3$/mm^3)	5.3 ± 0.52(9.3-1.6)^a	5.61±0.16 (10.5 -1.4)^a
RBC($\times10^6$/mm^3)	6.03 ± 0.43 (8.4-3)^a	6.61±0.13 (9.9 -3)^a
Hb(g/dl)	10.20 ± 0.37 (13.6-8.2)^a	10.02±0.17 (14.6- 5)^a
MCV(fl)	49.27 ± 4.76(76.9-28.1)^a	53.28±1.26 (91-29.3)^a
MCH(pg)	18.67 ± 2.17(36.6-9.7)^a	15.81±0.44 (37-9.2)^a
MCHC(g/dl)	40.62 ± 3.63 (59.5-21.7)^a	30.25±0.67 (52.3-11.9)^b

PCV= Packed cell Volume, WBC= White Blood Count, RBC=Red Blood Count Hb= Haemoglobin, MCV=Mean Corpuscular volume, MCH= Mean Corpuscular Haemoglobin, and Mean Corpuscular Haemoglobin Concentration values represented in Mean ±SE (Range), row means with unmatched superscripts (^{ab}) differ significantly at P<0.05.

Table 4: Haematological parameters of infected and uninfected slaughtered trade cattle according to sampling locations

	Ikot Eneobong		Nasarawa		Anantigha	
Haemtological Indices	**Positive (N=5)**	**Negative (N=55)**	**Positive (N=6)**	**Negative (N=54)**	**Positive (N=3)**	**Negative (N=57)**
PCV (%)	21.4±1.07 (24-18)^a	34.84±0.81 (44-21)^b	32.33±3.04 (40-23)^a	34.5±0.89 (41-25)^a	27.33±5.89 (39-20)^a	33.00±0.76 (40-22)^a
WBC ($\times10^3$/mm^3)	3.88±0.81 (36.3-1.6)^a	5.08±0.28 (8.8-1.4)^b	6.05±0.42 (7.6-4.7)^a	5.92±0.23 (8.3-4.2)^a	6.16±1.58 (9.3-4.3)^a	6.04 ±0.25 (10.5-4.2)^a
RBC ($\times10^6$/mm^3)	4.54±0.67 (6.5-3)^a	6.22±0.20 (9.2-3)^b	6.96±0.46 (8.4-5.2)^a	6.92±0.24 (9.3-4.1)^a	6.66±0.33 (7.1-6)^a	6.89 ±0.23 (9.9-4.7)^a
HB (g/dl)	11.18±0.63 (13.6-10)^a	9.92±13.6 (13.6-5)^a	9.41±0.36 (10.3-8.2)^a	9.73 ± 0.25 (12-7.1)^a	10.16±0.86 (11.-99.3)^a	10.34 ±0.34 (14.6-6)^a
MCV (fl)	51.42±7.80 (73-34.6)^a	58.25±91 (91-35.7)^b	53.3±7.84 (76.9-32.3)^a	49.77±2.27 (82.9-32.9)^a	37.63±9.43 (56.5-28.1)^a	49.52±1.74 (66.6-29.3)^a
MCH (pg)	26.54±3.99 (36.6-19.2)^a	17.14±37 (37-9.4)^b	13.86±1.27 (19.2-9.7)^a	14.05 ±0.49 (19-9.7)^a	15.2±0.96 (16.7-13.4)^a	15.32 ±0.59 (27-9.2)^a
MCHC (g/dl)	52.22±2.34 (56.6-44)^a	29.55±52.3 (52.3-11.9)^a	30.65±3.60 (44.7-21.7)^a	29.15 ± 1.12 (44-22.9)^a	41.23±10.31 (59.5-23.8)^a	31.80±1.23 (51.818.7)^a

Values are represented in Mean ±SE (Range), row means with unmatched superscripts ($^{a\ b}$) differ significantly at P<0.05.

Table 5: Haematological parameters of infected and uninfected young and older slaughtered trade cattle in Calabar

	Young Cattle		Older Cattle	
Haemtological Indices	**Positive (N=4)**	**Negative (N=76)**	**Positive (N=10)**	**Negative (N=90)**
PCV (%)	23.75±3.25 (33-18)[a]	36.15±1.00 (44-26)[b]	26.1±2.23 (40-18)[a]	34.06±0.5 4(44-22)[b]
WBC($\times10^3$/mm^3)	4.62±1.05 (6.3-1.8)[a]	4.78±0.39 (7.6-1.4)[a]	4.89±0.56 (7.6-1.6)[a]	5.66±0.18 (10.5-1.4)[a]
RBC($\times10^6$/mm^3)	5.65±1.11 (8.4-3)[a]	6.49±0.27 (9.2-4.6)[a]	5.93±0.58 (8.4-3)[a]	6.81±0.15 (9.9-4.1)[a]
Hb(g/dl)	9.62±0.58 (11.-8.2)[a]	10.10±0.42 (13.6-5)[a]	10.24±0.47 (13.6-8.2)[a]	10.12±0.21 (14.6-5)[a]
MCV(fl)	50.4±11.01 (68.7-28.3)[a]	58.05±3.00 (91-35.7)[a]	50±5.18 (73-32.3)[a]	51.4±1.39 (91-29.3)[a]
MCH(pg)	20.25 ± 5.79 (36.6-9.7)[a]	16.90±1.17 (37-9.4)[b]	19.67±2.99 (36.6-9.7)[a]	15.40±0.48 (37-9.2)[b]
MCHC(g/dl)	43.25±6.95 (55.5-24.8)[a]	28.71±1.37 (39.9-11.9)[b]	42 ± 4.09 (56.6-21.7)[a]	30.40±0.77 (51.8-11.9)[b]

Values are represented in Mean ±SE (Range), row means with unmatched superscripts ([a] [b]) differ significantly at $P<0.0$.

Table 6: Distribution of infected and anemic cattle and uninfected anemic slaughtered trade cattle in Calabar metropolis

Location	Total Examined	No. +Ve (%)	No. -Ve (%)	Infected and Anemic (%)	Uninfected and Anemic (%)
Ikot Eneobong	60	5(8.33%)	55(91.67%)	4 (6.67%)	1 (1.67%)
Anantigha	60	3(5%)	57(95%)	2 (3.33%)	1 (1.67%)
Nasarawa	60	6(10%)	54(90%)	1 (1.67%)	0 (0%)
Total	180	14(7.78%)	166(92.22%)	7 (3.89%)	2 (1.11%)
				$X^2=2.000$, $P>0.05$	

4. Discussion

The overall prevalence (11.66%) gotten in this study is lower than that recorded by Kamani *et al*., (2010) [16] who reported the overall higher prevalence of 25.7% for haemoparasites in North-central, Nigeria and also Zawua *et al.,* 2015 [27] who recorded an overall prevalence of 28.9% in slaughtered cattle from Gboko, Benue State. James – Rugu, (2006) [13] and Tambuwal *et al.,* 2009 [25] also reported higher prevalence of haemoparasites in cattle from Plateau and Sokoto states respectively in Nigeria. However, Okorafor and Nzeako (2014) [21] and Ademola and Onyiche (2013) [1] reported lower prevalence of haemoparasitic species, 6.7% and 5% in Oyo State. This high disparity in prevalence values could be attributed to local differences in prevalence of haemoparasites due to variations in geographical location (Velusamy *et al.,* 2014) [26] which arbitrates the distribution of the arthropod vectors of the parasites (Agbede, 2013) [2]. The prevalence of 2.22% recorded for Anaplasmosis in this study is lower than Paul, *et al.,* 2016 [23] who reported an overall prevalence of 5.8% for bovine Anaplasmosis in slaughtered trade cattle from Maiduguri, Nigeria and also Zawua *et al*., 2015 [27] who recorded 9.9% for *Anaplasma sp* in slaughtered cattle from Gboko, Benue State. The low prevalence of *Anaplasma* could be due to the improvement in the husbandry system, better veterinary care and climate change (Ademola *et al.,* 2013) [1]. In contrast, the prevalence of *Trypanosoma* sp. (5.56%) is higher than prevalence reported by both Zawua *et al.,* 2015 [27] and Paul *et al.,* 2016 [23].

The effects of age on prevalence of haemoparasites has been previously reported (Kamani *et al.,* 2010; Alim *et al.,* 2011; Ademola and Onyiche, 2013; Okorafor and Nzeako, 2014) [16, 4, 1, 21]. Older cattle had a higher prevalence of haemoparasitic infection compared to their younger counterparts which is in contrast with Ademola *et al.,* (2013) [1] who observed that prevalence of haemoparasites in ruminants decreased with increasing age. This could be as a result of immunity acquired from previous infection by the adult cattle. Kamani *et al.,* (2013) [16] however reported higher prevalence in older Cattle, and stated that this could be due to fact that adult are readily susceptible to haemoparasites than younger ones because of longer period of exposure to the arthropod vectors. Sex wise only male cattle were available during the period of the study. Kamani *et al*., (2010) [16] also reported that adult animals are more readily susceptible to trypanosomiasis than the younger ones.

The mean pack cell volume of infected cattle was 23.35 while uninfected cattle was 34.12. A similar trend was reported by Zawua *et al.,* 2015 [27] who stated that the reason for the low PCV in infected cattle as against the high PCV of uninfected cattle was probably due to the biochemical activity of the parasites to destroy red blood cells leading to anaemia. Berhanu *et al.,* 2010, Paul *et al.,* 2016 and Kamani *et al.,* 2010 [7, 23, 16] also reported similar findings.

Some uninfected cattle were found to be anaemic although reasons for this were not investigated. It is thought that this could be as a result of poor nutrition or injury during grazing. Cattle which were infected but not anaemic may have active immune systems or haemoparasitic infection may be at the early stage and so there was no significant effect on PCV levels.

5. Conclusion

The result obtained in this study indicates that haemoparasites are endemic in cattle populations within Calabar and its environments, even though the overall prevalence is low. Also, their occurrence maybe be linked with changes in some haematological parameters. The routine screening of animals is therefore recommended to reduce the prevalence of haemoparasites in the study area.

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