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Research Article

Haemobiochemical Changes in Nigerian Indigenous Cattle with Paramphistomosis in Jos Abattoir

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Abstract

Ruminants are infected by diverse gastrointestinal parasites such as trematodes, cestodes and nematodes. *Paramphistomum*, a trematode also known as rumen fluke affects domestic and wild ruminants resulting in significant economic losses within the dairy industry and meat production sector. Forty blood samples were collected from slaughtered cattle in the Jos Abattoir to assess the haematobiochemical parameters of those infected with paramphistomosis. From the 40 cattle examined, 23(57.50%) were positive for paramphistomosis. The packed cell volume (PCV) of cattle positive with *Paramphistomum* infection recorded a lower packed cell volume (32.96 ± 1.62%), which showed a statistically significant ($p < 0.05$) variation to cattle without *Paramphistomum*. There was no statistically significant difference ($p > 0.05$) in the total white blood cell count (TWBC) of cattle without the *Paramphistomum* infection ($7.48x10^3 \pm 4.85x10^2$) compared to those positive ($6.90x10^3 \pm 4.23x10^2$). A total of 13 analytes were evaluated in the serum chemistry and nine of the analytes were observed to be higher in non-infected cattle, while four (4) analytes were higher in *Paramphistomum*-infected cattle. Creatinine, alanine transaminase (ALT), triglyceride and Low density lipoprotein (LDL) cholesterol were found to be higher in infected cattle with mean values of 65.22 ± 2.44 mcmol/L, 44.17 ± 3.16 U/L, 0.32 ± 0.02 mmol/L and 1.41 ± 0.14 mmol/L respectively as compared with the mean values in the non-infected which were 62.35 ± 3.69 mcmol/L, 43.29 ± 5.50 U/L, 0.29 ± 0.02 mmol/L, 1.29 ± 0.17 mmol/L respectively. The haematological findings showed that the disease is an acute infection associated with anaemia. The vast serum analytes evaluated in cattle infected with *Paramphistomum* have provided useful information that will aid clinicians with diagnosis.

Keywords: Abattoir; Anaemia; Cattle; Haematological; Paramphistomosis; Serum Chemistry

Abbreviations

ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ANOVA: Analysis of Variance; DLC: Differential Leucocyte Count; EDTA: Ethylenediaminetetraacetic Acid; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein; PCV: Packed Cell Volume; RPM: Revolutions per minute; TG: Triglyceride; TWBC: Total White Blood Cell.

Introduction

Parasitic diseases are global problems and are considered major obstacles to the health and productive performance of animals [1]. Decreased meat and milk quantity as well as quality and reduced skin quality, reproductive failure and mortality are associated problems [2-5].

Ruminants are infected by diverse gastrointestinal parasites such as trematodes, cestodes and nematodes [6]. In view of the aforementioned, *Paramphistomum* a trematode responsible for *paramphistomosis* also known as rumen fluke disease or amphistomosis affects domestic and wild ruminants. *Paramphistomosis*, a pathogenic ailment affecting domesticated ruminants, results in significant economic losses within the dairy industry and meat production sector. The disease is caused by several genera (Calicophoron, Cotylophoron, Explanatum, Gigantocotyle), among which is *Paramphistomum* [7,8] belonging to the Paramphistomatidea superfamily. There are several species of *Paramphistomum* in cattle, which include *Paramphistomum microbothium*, *P. ichikawai* [9]. As a neglected tropical disease, it exhibits prevalence across tropical and subtropical regions, notably in Africa, Asia, Europe, and Australia, with a broad geographical distribution observed in specific areas such as Thailand, Ethiopia [10], and Nigeria [11]. Calves and lambs are most susceptible especially weaners. Adult animals can also be affected, particularly if they have had no prior exposure to the parasite or are immunosuppressed by other factors such as nutritional imbalance, disease etc.

Prevalence and severity of the disease vary according to countries, climate and humidity. Several factors determine the epidemiology of *Paramphistomum* which is centred around parasite-host environment interaction. The major factor is the rate at which pastures are infected by the larvae. Climatic requirements for hatching of eggs, development and survival of larvae in pastures are also important factors [12].

The impact of Paramphistomosis is more intense in extreme climates like those in the tropics and subtropical regions than in temperate regions [13]. Paramphistome burdens also vary with the number or population of infected host snails. In Nigeria, *Paramphistomosis* has been reported in some states like Sokoto, Imo, Edo, Ebonyi, and Kwara States by [14-18], respectively.

Clinical manifestations of the disease range from digestive irregularities (severe watery diarrhoea) to polydipsia and unthriftiness, anorexia, submandibular oedema and rough hair coat. Symptoms including bloating and loss of condition have been reported in infected cattle [19-21].

The morbidity and mortality associated with the disease in cattle are high [22-24]. Negative impacts on milk production and growth have also been reported [25,26].

In cattle, paramphistomes grow larger, migrate more rapidly, mature sooner, live and produce eggs for a longer period, and survive migration in greater numbers than occurs in either sheep or goats and consequently cause the disease. In view of the pathology and attendant economic losses associated with paramphistomosis in ruminants, it is pertinent to understand the haemobiochemical changes associated with the disease in cattle slaughtered in Jos Abattoir. Knowledge of this would greatly help clinicians with diagnosis and provide information about the danger posed by the infection and the need for strategic deworming to avert the deleterious effects.

Materials and Methods

Study area

Jos is the capital city of Plateau State. The area is located in the North-Central geo-political zone of Nigeria and shares geographic boundaries with Bauchi, Kaduna, Nasarawa, and Taraba States at its North-Eastern, North-Western, South-Western and South-Eastern boundaries, respectively. The State is situated within the Guinea Savannah region of Nigeria, and it is made up of seventeen Local Government Areas, covering an area of about 30,913 km^2. The average temperature ranges between 18°C and 22°C. There are two seasons within the year: dry (November-April) and rainy (May-October) seasons. The main occupations of the local indigenes are crop and animal farming. The population of domestic ruminants in Plateau State is estimated to be 1.3million cattle, 1.8million goats and 1.2 million sheep [27].

Sample collection

Blood samples from forty cattle of different ages and breeds slaughtered at the Jos Abattoir were collected and grouped into 2, i.e, control group (GP 1) and infected group (GP 2). Ten samples were used in GP1 and thirty (30) samples in GP2. The criteria for selection in group 1 were apparently healthy animals and

those with no visible *Paramphistomum* attached to their rumen or reticulum while for group 2, cattle with severely pale mucous membranes, emaciation, rough hair coat, diarrhoea and visible *Paramphistomum* on the rumen and reticulum were used. Both the control and the *Paramphistomum*-infected blood samples were collected individually via the jugular vein into a 5ml EDTA bottle and transported to the Clinical Pathology Laboratory of the Veterinary Teaching Hospital, University of Jos, for haematology.

Serum from each cattle was obtained for serum chemistry, as some analytes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatinine, total protein, albumin, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL)) were evaluated.

Experimental design

Hematology

Blood samples were collected in 5ml EDTA bottles after severing the jugular vein. The blood in the sample bottles was collected in capillary tubes and centrifuged at 6000 revolutions per minute (rpm) for 10 minutes according to the method outlined by [28]. After centrifugation, the packed cell volume (PCV) was measured using the microhaematocrit reader.

For differential leucocyte count (DLC), a drop of blood was spread on a grease-free slide to form the head, body, and tail of the smear. The smear was then fixed with methanol and stained with Wright-Giemsa stain, and the smear was further viewed microscopically at low and high magnification x1,000 magnification. One hundred cells were counted, and absolute values were further calculated. The total white blood cell count (TWBC) was calculated by counting the white blood cells within the 16 cells at the four corners of the New improved Neubauer counting chamber and multiplied by 50 according to the method outlined by [28].

Serum chemistry

Blood samples were collected in 8.5 ml plain bottles, allowed to clot for 30 minutes, and then centrifuged for 10 minutes at 30 RPM to separate serum from corpuscles. The sera were separated by aspiration using a separate disposable pipette. Serum was stored for biochemical analysis of the various analytes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatinine, total protein, albumin, globulin, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride (TG)) according to the method outlined by [29].

Data analysis

The data from the haematological and serum biochemical analyses were expressed as mean ± standard error of the mean (SEM). Differences among groups were analysed using one-way analysis of variance (ANOVA), Differences were considered statistically significant at $p < 0.05$.

Results

Demography of cattle slaughtered at the Jos Abattoir

From the 40 cattle examined, 23(57.50%) were positive for *Paramphistomum* infection, with *Paramphistomums* visibly attached to the reticular and ruminal walls (Plates 1 and 2). In terms of gender, twenty-five (25) females were sampled, which accounted for a prevalence of 16(64.00%), while out of the fifteen (15) males sampled, 7 (46.67%) were positive. In terms of age, 4-5-year-old cattle were the most examined (20), with a prevalence of 12(60.00%), while 5≥6years recorded the lowest number examined (2), with both samples being positive. On the basis of breed, White Fulani were the highest samples examined (38), with a positive prevalence of 21(55.26%). Sokoto gudali and Red Bororo had only 1 sample each, and both cases were positive for infection (Table 1).



Plate 1: Reticulum of a cow slaughtered in the Jos abattoir showing *Paramphistomums* (arrow).



Plate 2: Rumen of a cow slaughtered in the Jos abattoir showing *Paramphistomums* (arrows).

	Number Examined	Positive (%)	OR (95% confidence interval)	P-values
Gender				
Female	25	16(64)	0.786(0.112-5.490)	0.808
Male	15	7(46.67)		
Age				
2≥3years	4	1(25)	3.201(0.686-14.933)	0.139
3≥4years	14	8(57.14)		
4≥5years	20	12(60)		
5≥6years	2	2(100)		
Breed				
Sokoto gudali	1	1(100)	NA	
Red Bororo	1	1(100)		
White Fulani	38	21(55.26)		
Plasma Colour				
Normal	8	7(87.50)	0.087(0.006-1.236)	0.071
Yellow	32	16(50.00)		
Total	40	23(57.50)		

Table 1: Demography of cattle slaughtered at the Jos abattoir.

Based on plasma colour, most of the samples were observed to have yellow plasma colour (32) which recorded a frequency of 16(50.00%), while the normal plasma coloured samples were 8 which recorded a prevalence of 7(87.50%) that are positive for infection (Table 1).

The variations recorded with respect to all the variables examined were found to be statistically not significant ($p > 0.05$) and the odds ratio recorded were found to be low except for age, which recorded the value of 3.201 (Table 1).

Haematological indices of cattle slaughtered in the Jos Abattoir with *Paramphistomum* Infection

The hemogram (Table 2) shows a variation in the haematological parameters between cattle infected with *Paramphistomum* and those devoid of the parasitic infection. For most of the haematological parameters evaluated, the cattle free of infection had the highest mean values except in total white blood count, segmented and band neutrophils.

Parameters	Positive	Negative	F-ratio	P-value
PCV (%)	7.31 ± 0.53	6.04 ± 0.57	0.523	0.927
TWBC ($x10^3$)	32.96 ± 1.62	37.76 ± 1.24	4.930	0.032
Segmented Neutrophil ($x10^3$)	5.86 ± 0.53	3.79 ± 0.61	5.645	0.018
Band Neutrophil ($x10^3$)	0.61 ± 0.12	0.33 ± 0.12	1.170	0.108
Lymphocyte ($x10^3$)	0.54 ± 0.24	1.40 ± 0.31	7.594	0.039
Monocyte ($x10^3$)	0.23 ± 0.10	3.04 ± 1.08	1.055	0.604
Eosinophil ($x10^3$)	0.08 ± 0.03	0.21 ± 0.07	1.198	0.128

Table 2: Haemogram of cattle slaughtered in Jos abattoir with *Paramphistomum* Infection.

Mean ± SEM of multiple values of each parameter.

$p < 0.05$ is considered statistically significant.

The packed cell volume (PCV) of cattle positive for the infection was lower, showing a statistically significant ($p < 0.05$) decrease compared with cattle without the infection. There was no statistically significant difference ($p > 0.05$) in the total white blood cell count (TWBC) of cattle without the infection compared with those positive for the *Paramphistomum* infection. The variation in terms of segmented neutrophils of cattle with the infection compared to those without the infection was found to be statistically significant ($p < 0.05$) (Table 2).

The lymphocyte count in non-infected cattle was statistically higher ($p < 0.05$) than that recorded in those infected with *Paramphistomum*. The monocyte and eosinophil counts of positive samples recorded lower mean values than those of negative. However, the variation was found to be statistically not significant ($p > 0.05$) in both cases.

Serum chemistry of cattle slaughtered in Jos Abattoir with *Paramphistomum* infection

A total of 13 analytes were evaluated in the serum chemistry of samples collected (Table 3). Nine (9) of the analytes were observed to be higher in non-infected cattle, while four analytes were higher in *Paramphistomum*-infected cattle.

Analytes	SI Unit	Positive	Negative	F-ratio	P-value
Urea	mmol/L	4.94 ± 0.27	4.98 ± 0.28	0.007	0.934
Creatinine	µmol/L	65.22 ± 2.44	62.35 ± 3.69	0.454	0.505
ALT	IU/L	44.17 ± 3.16	43.29 ± 5.50	0.022	0.883
AST	IU/L	135.49 ± 26.47	172.51 ± 18.26	1.093	0.303
ALP	IU/L	55.13 ± 5.77	55.62 ± 6.05	0.509	0.480
Total Protein	g/L	74.85 ± 2.17	77.55 ± 2.37	0.693	0.410
Albumin	g/L	33.30 ± 1.57	33.31 ± 1.15	0.000	0.998
Globulin	g/L	41.55 ± 2.58	44.25 ± 2.37	1.7093	0.1950
Total bilirubin	µmol/L	5.46 ± 0.35	6.29 ± 0.64	2.889	0.097
Triglyceride	mmol/L	0.32 ± 0.02	0.29 ± 0.02	1.016	0.320
Total Cholesterol	mmol/L	2.59 ± 0.13	2.72 ± 0.23	0.311	0.580
HDL Cholesterol	mmol/L	1.03 ± 0.09	1.19 ± 0.12	1.155	0.289
LDL Cholesterol	mmol/L	1.41 ± 0.14	1.29 ± 0.17	0.001	0.976

Table 3: Serum chemistry of cattle slaughtered in Jos Abattoir with *Paramphistomum* Infection.
Mean ± SEM of multiple values of each Analyte.
$p < 0.05$ was considered statistically significant.

Creatinine, ALT, triglyceride and LDL cholesterol were found to be higher in infected cattle compared with the non-infected cattle. However, the variation recorded was found to be statistically not significant ($p > 0.05$).

Urea, AST, ALP, albumin, total bilirubin, total cholesterol, HDL cholesterol, globulin and Total protein were all found to be higher in the non-infected group compared with the mean values in the *Paramphistomum*-infected cattle. However, the variation was also found to be statistically not significant ($p > 0.05$) (Table 3).

Discussion

The overall prevalence of paramphistomosis in cattle slaughtered at the Jos Abattoir in this study is 23(57.50%) out of 40 cattle examined. This is higher than the prevalence of 8.95% in cattle in Turkey [12], 51.82% in Ethiopia [30], 18.8% in Spain [31], 36.9% in South-Eastern Iran [32], 20% in France [33] and 56% in Sokoto [14], but lower than the report of [34], which reported a prevalence of 59.1% in Abuja. Climatic variation could be the reason for the differences in prevalence and the intensity or availability of the intermediate host (snail) in these locations.

In this study, female cattle (64.00%) had higher *Paramphistomum* infection than males (46.67%), and this is in accordance with the reports of [34-36] who also revealed higher prevalence of helminths in females, which could be attributed to variation in sample size [37], and immunosuppression during pregnancy, birth and lactation [38] in females. Another reason for the high prevalence in female cattle could be attributed to having more females in the herd and keeping them longer for breeding purposes. Although the variation in prevalence of *Paramphistomum* infections between female and male cattle in this study was not statistically significant ($p > 0.05$), the prevalence of *Paramphistomum* infection by age of cattle examined in this study showed higher prevalence in adult (63.6%) cattle compared to the young ones (50%). This is in accordance with the findings of [34], who reported a higher prevalence of infection in adult (62.0%) cattle than in the young (42.1%). This can be attributed to stress in adult cattle over a period of time, such as stress from hunger and thirst, fatigue, illness or tissue lesions, which could inflict physical constraints on the functioning of the body. Transportation stress associated with the loading of cattle into a lorry and subsequently into the abattoir, where the unfamiliar presence of humans and other animals may lead to a disruption, often in their social rearing group due to separation, could also serve as another cause of stress [39].

The packed cell volume (PCV) of *Paramphistomum*-infected cattle from this study was significantly lower when compared to that of the non-infected cattle and this is in accordance with the findings of previous authors who recorded a lower PCV in infected animals when compared to the non-infected [40-50]. The lower PCV could be attributed to the loss of blood from severe haemorrhage and ulceration in rumen mucosa and intestinal tissue due to migration of young immature flukes via the intestine to their predilection site in the rumen or extensive migration of the young fluke through the hepatic parenchyma and from blood-sucking activity of adult fluke [47,51,52].

Leucogram of infected cattle from this study revealed leucocytosis occasioned by neutrophilia with regenerative left shift and lymphopenia. There was monocytopenia and eosinopenia in the infected cattle from this study, but they were not statistically significant. The leucocytosis observed in the present study is similar to the significant increase in total leucocyte count reported by [48] in buffaloes suffering from paramphistomosis compared to normal buffaloes. This study revealed neutrophilia, which is not different from the findings of [46,48], who reported significant neutrophilia in buffaloes infected with paramphistomes compared to non-infected buffaloes, and that of [50]. The lymphopenia in this study is similar with the reports of [43,46,48] who reported lymphopenia in *Paramphistomum* infected cattle but were not statistically significant on the other hand. Monocytopenia reported in this study is similar to the findings of [46], which reported monocytopenia that was not statistically significant. The eosinophilia from this study is similar to the findings of previous authors [40-48,50].

The neutrophilia with regenerative left shift, lymphopenia and monocytopenia from this study indicate an acute infection. Lymphocytes are the second line of cellular defence, and their low number indicates acute infection, as there is usually high demand for lymphocytes in chronic infection [53]. Lymphopenia and eosinopenia reported in this present study indicate stress.

Serum analytes examined in this study showed a decrease in urea, AST, ALP, TP, albumin, globulin, total bilirubin, total cholesterol and HDL cholesterol, with an increase in creatinine, ALT, triglyceride and LDL cholesterol levels, which were not statistically significant. The decrease in ALP, TP and increase in ALT from this current study is in consonance with the findings of [43,49,54], who observed reduction in total protein in infected cattle with significant increment in serum ALT. The decrease in AST in this study is different from the findings of significantly elevated serum AST levels reported by [43,54], and the decreased serum ALP in this study is different from the elevated serum ALP reported by [55] in infected buffaloes. The findings of [45,47,55-58] were similar to the present study wherein these authors also recorded the reduction in the values of total serum protein. The low total protein observed in the present study could be associated with malnutrition, as most of the cattle examined during the study were very sick animals.

A study carried out by [56] revealed that serum cholesterol values decreased and were more severe as the infection time of the parasite increased, which we also observed in the present study.

Albumin and globulin concentrations observed from this study were low in the infected cattle when compared to the non-infected group. This finding is similar to the report of [2] in an experiment

carried out in sheep with paramphistomosis, which revealed that infected sheep showed a marked decline in serum albumin 2 weeks post-infection. The authors attributed the severe decrease in serum albumin to be the major cause of the reduction in serum protein. However, our finding of decreased albumin and globulin concentrations in infected cattle is different from the finding of [47], who reported a higher albumin: globulin ratio in cattle, sheep and goats infected with immature flukes of *Paramphistomum* than in the same animals infected with just the adult flukes and also in the control groups of the same animals.

The high serum creatinine level in cattle infected in this study is different from the findings of [56], who reported a marked decrease in serum creatinine values in cattle suffering from paramphistomosis. This has been shown to be more evident in cattle with medium and high infection rates than in cattle with low infection rates when all groups were compared with the control group. The high creatinine level observed in this study could be attributed to dehydration, as most of the cattle in the infected group had severe diarrhoea.

Conclusion

This study has established that there is a high prevalence of paramphistomosis in cattle slaughtered at the Jos Abattoir. The haematological findings showed that the disease is an acute infection associated with anaemia. The various serum analytes evaluated in cattle infected with *Paramphistomum* have provided useful information that will aid clinicians in making diagnoses and offer informed advice on strategic deworming of cattle.

Acknowledgements

We sincerely want to appreciate the Veterinary Teaching Hospital, University of Jos, Plateau State, Nigeria for allowing us to use the Veterinary Clinical Pathology Laboratory for analysing our samples. Our gratitude also goes to Mr Inusa Sunday Arangu for assisting with the haematological aspect of this work and Mr Dishon Charibu for the statistical analysis of the data collected during the period of this work. Our immense gratitude also goes to Dr Lucius Imoh and Miss Mercy Ozue for the biochemical analyses of this work, which were carried out at Lushcare Medical Laboratory and Research Services Limited, Jos, Plateau State.

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Conflict of Interest

There is no conflict of interest.

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