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Paramphistomes in Matebeleland South Province Zimbabwe and their effect on aspects of blood plasma composition in infected cattle.

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Abstract: *Cattle originating from various localities in Matabeleland South Province were examined for adult Paramphistome prevalence. Of the three thousand 27% were infected with paramphistomes. For identification of paramphistomes, the structures and measurements of diagnostic features were made in median sagittal sections. Analysis of the structures of the acetabulum, pharynx and genital atrium revealed the following parasites in the area: Calicophoron microbothrium, Calicophoron clavula, Calicophoron calicophorum, Calicophoron raja, and Gigantocotyle symmeri in 20 %, 2 %, 5%, 2 % and 2% of cattle examined respectively. The visible damage by adult parasites on the hosts' tissue were as a result of their sucking the reticulum and rumen mucosa into the acetabulum, which eventually nipped off, leading to slightly hardened areas devoid of rugae due to necrosis. In a few severe cases the papillae was damaged and catarrhal pus like exudate was noticed on the mucosa. Fifty coded Nguni cattle aged three years, infected with paramphistomes had their final carcass mass and blood chemistry recorded. Only total protein and cholesterol showed significant difference between the infected and the controls $p<0.05$. The final carcass mass was significantly different between cattle harboring more than 500 parasites per animal and the controls $P<0.05$.*

Key words: *Calicophoron, Gigantocotyle, plasma, median section, Cattle.*

I. Introduction

Many Dams constructed to alleviate water shortage, have resulted in the increase in the population of aquatic snails among which are Lymnea spp. Bulinus spp. Biomphalaria spp. and Melanoides spp which are intermediate hosts for paramphistomes (1, 2, 3, 4, 5).

Cotylophoron cotylophorum, Carmyerius spotiosus and Carmyerius bubalis were recovered from Alcelaphus spp, Tragelaphus spekei and Tragelaphus stresiceros in Zimbabwe(6). Calicophoron raja was recovered from Connochaeters taurinus while Paramphistomum microbothrium (Syn.Calicophoron microbothrium Eduardo 1983) was recovered from Aepyceros melampus, Kobus leche and Taurotragus oryx in Zimbabwe(7). These animals share the same niche with cattle and are susceptible to many parasitic diseases found in cattle (6, 8, 9). P. microbothrium was also recovered from cattle in Mashonaland(9). The distribution of paramphistomes is worldwide (7, 9,10, 11, 12, 13). Adult parasites are not normally associated with clinical disease, however, occasional outbreaks of parasite gastroenteritis in susceptible livestock is associated with migration of immature parasites through the upper small intestine. Generally cattle not previously exposed are susceptible (14, 15, 16, 17). Whereas domestic ruminants seldom die of adult paramphistome infections, secondary clostridial infection of damaged alimentary canal lesions may cause death in animals. The most important losses due to paramphistome infection are not usually associated with paramphistome infection by farmers, these include reduced fertility of the brood cow herd, lighter calves at weaning, slower growth of replacement heifers, light weight cull cows, poor hides, reduction in milk production in dairy cattle, and dairy replacement heifers that take long to reach breeding age (5, 18). Stress from parasites could affect the blood picture; in case of malabsorbtion and wasting syndrome the result could be decreased total cholesterol an indication of anemia. Aneroxia could result in increased total protein an indication of dehydration whereas malnutrition could result in decreased total protein an indication of low albumin (8, 19, 20). Decreased glucose could indicate conditions interfering with absorption of glucose. Sodium and potassium decreases can result from severe diarrhea (21). Creatinine increases are associated with impaired renal function and other maladies

The aim of this study was to determine the identity and prevalence of paramphistomes that occur in cattle and how they affect the final carcass mass and blood composition in infected cattle.

II. Materials And Methods

Examination for parasites prevalence was done on the intestinal walls and stomachs inner walls of 3000 cattle slaughtered in Bulawayo Cold Storage Commission Abattoir. The cattle under study originated from different parts of Matebeleland South province, namely, BeitBridge, Esigodini, Fort Rixon, Figtree, Gwanda, Insiza, Kezi, Matopo, Marula, Plumtree, Shangani, Umzingwane and West Nicholson. The parasites were handpicked into normal saline solution. In the laboratory the uteri of some parasites were teased for recovery and measurements of egg size. Some parmphistome specimens were flattened dorsoventrally between microscope slides held by rubber bands to facilitate examining diagnostic features like vitelline glands, positioning of testes, esophagus, nature of caeca and uterus. Some were preserved in formol saline or 70% ethanol for median sagittal sectioning in order to determine species using the keys previously established(7, 22, 23). The prevalence and degree of infection with parasites were recorded. The number of cattle infected was expressed as a percentage of the total number of cattle examined in each area.

For the blood picture and final carcass mass fifty coded Nguni cattle aged three years, infected with paramphistomes had their final carcass mass recorded using an Avery Scale to the nearest 5 grams The animals had neck venous blood collected before slaughter into heparinized bottles for analysis of the following parameters, cholesterol, glucose, creatinine, total plasma proteins, sodium and potassium using Cobas mira blood analyser. To exclude animals infected with other intestinal parasites their rectal contents were examined for eggs as indicators of the presence of such parasites, any one with other intestinal parasites other than paramphistomes was excluded from the study sample. The livers of all animals were examined to also exclude those infected with liver flukes.

Another fifty coded Nguni cattle aged three years, not infected with paramphistomes or other intestinal parasites had their final carcass mass recorded using an Avery Scale to the nearest 5 grams. These were used as the control group. The control animals also had neck venous blood collected before slaughter into heparinized bottles for analysis as was done for infected animals.

III. Results

On average 27% of the three thousand cattle examined were infected with paramphistomes. The percentage of infected cattle from each area is shown in Table 2. Five paramphistome species were identified using keys previously established(7, 22, 23). These were Calicophoron microbothrium, Calicophoron clavula, Calicophoron calicophorum, Calicophoron raja and Gigantocotyle symmeri infecting 20 %, 2 %, 5%, 2 % and 2% of cattle examined respectively. Measurements and features used for identification and confirmation of species are given in Table 1. There were no externally observable symptoms distinguishing the infected cattle from those not infected. There were no preferential infections based on breed of cattle or their age. Nests of paramphistome numbering between 20 and 1500 were found in the folds of the rumen and between papillae in the reticulum, where they adhered to knobbed parts of the mucosa. Occasionally the worms nipped off the mucosa sucked into the acetabulum leading to slightly hardened areas devoid of rugae and papillae due to necrosis. In a few severe cases the papillae was damaged and catarrhal pus like exudate was noticed on the mucosa. The geographical locations where the parasites were recovered are shown in Table 2. The species recovered are here after described:

Calicophoron microbothrium Fischoeder, 1901 (Synonym Paramphistomum microbothrium)

Description:

The body is conical. When fresh, the acetabulum and the pharyngeal region are red while the rest of the body is yellowish-white. Measurements of diagnostic structures are shown in Table 1 for sectioned specimens. The acetabulum(Fig. 1B) is of the paramphistomum (sensu Nasmark, 1937)

The pharynx is of the paramphistomum type (sensu Nasmark, 1937) (Fig. 1A). The testes are in tandem and they are deeply lobed (Fig. 1B).

The genital atrium is of the microbothrium type (sensu Nasmark, 1937). It lies close and posterior to the gut bifurcation. The genital papilla lies either behind the ventral atrium or protrudes(Fig. 1C). The sphincter papilla is present and is made of loosely packed tissue. The uterus is wavy and runs dorsal to the testes close to the middle of the body. It opens into the genital papilla through the metraterm. The eggs are filled with evenly scattered granules and are light blue-green.

Calicophoron clavula Nasmark 1937 (Synonym Paramphistomum clavula,).

Description:

The description of this parasite matches that already made for C. microbothrium except for the genital atrium which is of the clavula type (sensu Nasmark, 1937) and course lobed testes(Fig. 1D). The genital papilla has a large sphincter papilla. In the median sagittal sections the vesicula seminalis appears as a solid mass with an indistinct lumen. The acetabulum is shown in (Fig.1E).

Calicophoron calicophorum Fischoeder, 1901
Description

Body pea-shaped, may bend slightly ventrally, distinctly broader in acetabulum region. The surface has papillae densely arranged around oral opening and extending ventrally to about midbody, except for an oval area surrounded by a ridge around the genital pore region, which is free of papillae. There are much smaller papillae also present randomly arranged around acetabulum opening.

The acetabulum (Fig. 1J) is sub terminal, of the calicophoron type (sensu Nasmark, 1937) in median sagittal section. The pharynx is of the calicophoron type (sensu Nasmark, 1937) with the internal surface lined by small papillae. The testes are deeply lobed (Fig. 1H), obliquely tandem or sometimes nearly side-by-side in middle of body.

The genital pore is at the level of the posterior to oesophageal bifurcation and is surrounded by an oval area bounded by a ridge free of tegumental papilla. The terminal genital atrium is of the calicophoron type (sensu Nasmark, 1937), when inverted it forms a genital pillar with genital pore opening at tip. The excretory vesicles dorsal to acetabulum and the excretory pore opens on the dorsal surface at level of posterior testis and anteriorly to opening of Laurer's canal.

Calicophoron raja Nasmark, 1937
Description

The description of this parasite matches that already made for C.calicophorum except for the genital atrium which is of the raja type (sensu Nasmark, 1937) characterized by presence of a genital pillar or column, which is covered by densely, arranged small tegumental papillae and the acetabulum which is sub terminal, pisum type (sensu Nasmark, 1937) with the (de_2) units fewer and more irregularly spaced(Fig 1K and 1I).

Gigantocotyle symmeri Nasmark 1937
Description:

Body is conical and the dorsal line is semicircular. The acetabulum, is symmeri type (Fig.1F) is well developed. This type is distinguished less developed circular series. The pharynx is of explanatum type (Fig. 1G).

Results on analysis of the blood picture are shown in (Fig. 2). From a t test carried out $p<0.05$ only cholesterol and total protein showed a significant decrease in cattle infected by more than 500 parasites when compared with the controls. Results on final carcass mass are shown in (Fig.3). From a t test carried out $p<0.05$ only cattle infected by more than 500 parasites showed a significant decrease in final carcass mass when compared with the controls.

IV. Discussion

Although the percentage of cattle infected is high the number of parasites per herd was relatively low. It should be noted that the percentage take of paramphistomes is less than 20% (8, 21). These seemingly low figures of less than 2000 in the stomach will have preceded higher numbers in the duodenum where the pH and oxygen tension is low and the parasites are normally buried in the submucosa away from the harsh conditions in the gut lumen. The fluctuation of numbers among individual animals could arise as a result of some cattle drinking from drying water bodies where the levels of cercaria always tend to increase per unit volume of water (14, 21). Development of immunity in cattle is low thus unlikely to affect parasite loads significantly (14, 21). The areas sampled are geographical located such that they reflect the true distribution of the paramphistomes in the provinces. No area showed low levels of parasites.

For effective diagnosis and control of parasitic diseases, it is essential that parasite isolates be accurately identified by method that is simple and reproducible (5). In our study we have positively identified five species using previously constructed identification keys (7, 22, 23). Calicophoron. microbothrium was identified based on the histology of the structures of the acetabulum pharynx and the genital atrium, which are in agreement with previous descriptions Dube et al (2002). It seems that Calicophoron. microbothruim is the most frequent ruminant paramphstome in Africa in view of the fact that it has been reported in all places where studies on ruminant paramphistomes have been made (4, 7, 9, 22, 23, 24). The present study shows it is the most frequently encountered paramphistome in the Province.

Calicophoron. clavula, which resembles Calicophoron .microbothrium in many respects, was identified based on the histological structures of the genital atrium, which are peculiar to this species, which is in agreement with the descriptions by other workers (5, 25).

C. calicophorum was identified on the basis of the genital atrium, which protrudes as a long shaft, well-developed pars prostatica, and deeply lobed testes, which correspond to similar diagnosis for this species in previous literature (7, 22, 23).

C. raja, which resembles C. calicophorum in many respects was identified based on the histological structures tandem testes and of the genital atrium which has tergumental papillae on its protruding shaft both which are in agreement with the descriptions in previous literature (7, 22, 23).

Calicophoron. microbothrium was found in all areas sampled and is known to cause paramphistomiasis even when it occurs in small numbers (8,19). Chronic infections with paramphistomes can result in loss of, weight, milk production and plasma proteins (17, 20). The results of our study point to the fact paramphistome infections should not be ignored as stress due to their presence when in numbers above 500 parasites has a significant effect on weight gain and total plasma proteins. There are suggestions that such decrease could be as a result of protein seepage from lesions caused by the parasites (18).

C. calicophorum was previously reported in wild life in Zimbabwe (6). Very low numbers of this species can also causes serious disease conditions therefore there is need to establish its epidermiology (17, 20).

There has been reported decrease in milk production in cattle infected with paramphistome and other intestinal helminthes which relates closely with blood protein reduction(17). Glucose levels were erratic this was attributed to the fact that response to stress are varied in animals brought under different conditions as the ones in this study. While severe diarrhoea could cause sodium and potassium levels to drop the infected animals did not seem to have been experiencing severe diarrhoea. Cholesterol and creatinine levels had no relationship to number of parmphistomes which is consistent with other observations (20). In another study it was reported that there was reduction in final carcass mass in cattle infected with species of paramphistomes in France(18). In this study such reduction in mass occurred when the number of adult paramphistomes exceeded 500 per animal. Preventive control measures should be taken against paramphistomes in livestock to maximize production efficiency.

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Table 1 Measurements in µm for diagnostic morphological characters in the median section of paramphistomes of cattle from Matebeleland South Province

	C. microbothrium	C. clavula	C.calicophorum	C.raja	G.symmeri
Body length (BL)	8400±867	6900±1150	8100±1520	10350±1250	9000±874
Body Breadth (BB)	3000±46	3750±13	3500±46	5700±132	4200±147
Acetabulum diameter(ad)	1688±65	2700±98	2470±159	2820±54	3000±160
Pharynx length (PL)	713±27	1050±45	1000±27	2250±190	660±23
Anterior testis Breadth	900 ±31	1200±265	1150 ±35	900±35	750±54
Anterior testis length	1770±31	2100±265	2500±35	1800±35	1800±54
Posterior testis Breadth	825 ±31	1200±265	1200 ±35	900±35	750±54
Posterior testis length	1637±31	2100±265	2250±35	1800±35	1800±54
Pars prostatica Breadth	300± 35	420 ±35	300	600±26	90±12
Pars prostatica length	420±10	600±15	450	1200±26	300±12
Egg breadth	85 ±13	60±8	90±8	70±8	95±11
Egg length	158±13	150±17	180±11	150±11	175±11
Ratio of ad/BL	1:3.9	1:2.778	1;4.05	1:3,67	1:3

Table 2 Prevalence of paramphistome species found in Matebeleland South Province

Location	No. cattle examined	of(n)% Cattle infected	species recovered
BeitBridge	414	17.87	A; B
Esigodini	396	19.7	A; E
Fort Rixon	224	12.95	A; B; C; D
Figtree	143	17.48	A; B; D; E
Gwanda	477	23.06	A; B
Insiza	91	25.27	A; E
Kezi	387	18.86	A; B
Matopo	300	26.83	A; B; E
Marula	133	84.96	A; B; C; D; E
Plumtree	301	41.2	A; B; E
Shangani	69	50	A; B
Umzingwane	30	30	A; B; C; D; E
West Nicholson	28	39.44	A; B

KEY
A= C. microbothrium
B= C. clavula
C= C. calicophorum
D=C. raja
E= G. symmeri

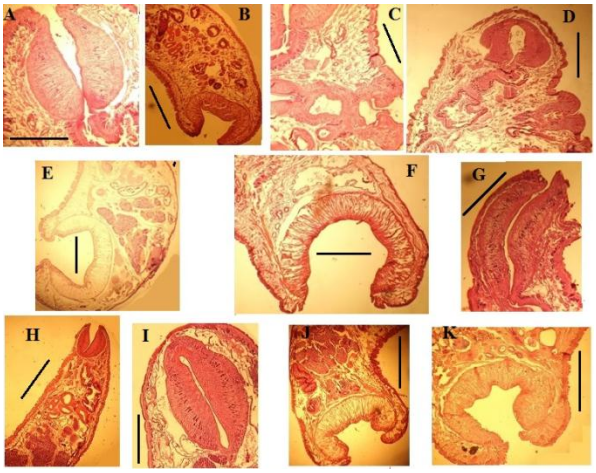

Figure 1. Median sections of the anterior and posterior regions of paramphistomes: A) Calicophoron microbothrium pharynx region. Scale bar =2mm. B) C. microbothrium posterior region acetabulum and lobed posterior testis. Scale bar =2mm. C) C. microbothrium genital atrium showing papilla. Scale bar =1mm. D) Calicophoron clavula anterior region. Scale bar =2mm. E) C. clavula posterior region acetabulum and testis prominent. Scale bar =2mm. F) Gigantocotyle symmeri acetabulum. Scale bar =2mm. G) Gigantocotyle symmeri pharynx. Scale bar =1mm. H) Calicophoron calicophorum anterior showing pharynx. Scale bar =3mm. I) Calicophoron raja anterior showing pharynx. Scale bar =1mm. J) C. calicophorum posterior showing acetabulum and testis. Scale bar =2mm. K) C.raja posterior showing acetabulum. Scale bar =2mm.

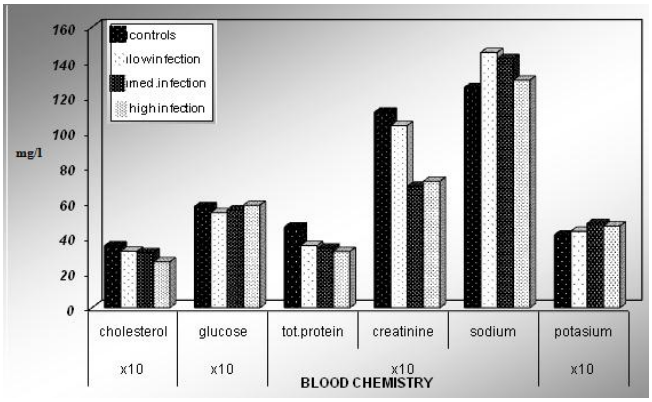

Figure 2. Comparison of blood plasma components in non infected(controls) and those infected with various levels of paramphistomes. For cholesterol, glucose, creatinine, and potassium the values have been multiplied by a factor of 10 to ease comparison with other elements.

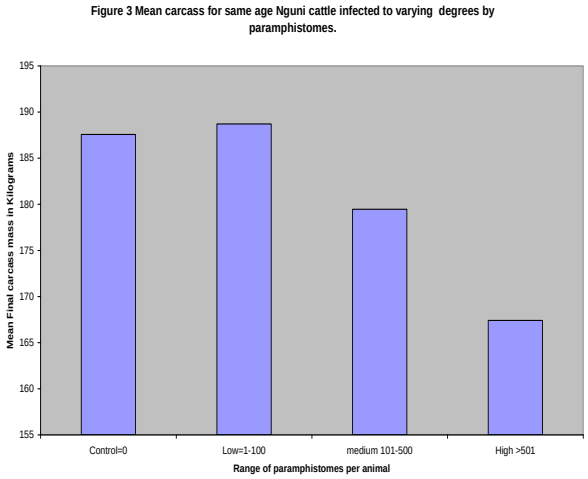

Figure 3. The mean carcass mass for same age Nguni cattle infected to varying degrees with paramphistomes.