

Descriptive studies on Paramphistomes of small domestic ruminants in Southern Nigeria

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Abstract

Goats and sheep slaughtered in Southern Nigeria had their rumens and reticulums examined for the frequency and intensity of infections with paramphistomes. The overall frequency of infected goats and sheep was 30% and 80% respectively. The range of intensity of parasite in goats and sheep was, 2 to 500 and 2 to 700 respectively. From the morphological and histological studies of diagnostic features, which included the acetabulum, genitalium and pharynx, carried out on flattened whole mounts and median sagittal sections the following parasites were identified, *Cotylophoron cotylophorum* infecting 8% of the goats, *Cotylophoron fuelleborni* infecting 20% of the goats, *Carmyerius synethes* infecting 4% of the goats and 70% sheep, and *Calicophoron microbothrium* infecting 20% of the goats and 33%, sheep. Goats were infected with all the four species while sheep infected with only the last two species. The degree of tissue damage on both hosts was mild.

Key words: Paramphistomatidae, *Cotylophoron*, *Carmyerius*, *Calicophoron*, Sheep and Goats

1. INTRODUCTION

Mature members of the family Paramphistomidae Fiscoeder, 1901 are especially prevalent in the reticulum and rumen of ruminants where they rarely produce any clinical symptoms [1,2] immature migrating forms of some species have been reported to cause serious disease and even deaths of their hosts by burying themselves into the sub mucosa of the duodenum and feeding on the epithelial cells of the Brunner's gland resulting in anorexia, polydypsia, profuse foetid diarrhea, drop in plasma protein concentration and anemia

[3,4,5,6,7]. If the animals are not visibly sick farmers tend to ignore the more serious losses due to paramphistomiasis such as reduced fertility, lighter kids at weaning, slower growth, light weight cull animals, poor hides, and replacement stocks that take long to reach breeding age [2,5]. Whereas small domestic ruminants seldom die of fluke infections, secondary clostridial infections of damaged alimentary canal lesions may cause death in animals [8,9]. The taxonomy of paramphistomes has been extensively studied by light and electron microcopy, yet there are still areas where consensus is lacking [10,11,12,13]

Carmyerius gregarius, *Carmyerius spatiosus*, *Cotylophoon cotylophorum* and *Calicophoron microbothrium* have been reported from previous collections in Nigerian sheep and goats [14]. The country's ecosystem has changed due to human resettlements, oil drilling, use of fertilizers, pesticides and dam constructions this necessitates a new investigation to provide the farmers and veterinarians the current status of paramphistomes in goats and sheep. Not all paramphistome species are responsible for disease in goats and sheep, it is therefore important to have accurate information about existing species so that where pathogenic ones occur preventive control measures can

be taken in order to avoid outbreaks [15]. To date we found no literature or information on the prevalence, frequency and species of Paramphistomes of goats and sheep in Southern Nigeria. Data on frequency and prevalence of paramphistomes can be used eventually to construct predictive models of outbreaks of disease [16]. The models of disease are important in implementation of control measures that are effective and economical. The aim of this study was to describe paramphistomes species, determine the prevalence and frequency of those that occur in goats and sheep in Southern Nigeria.

Table 1: Measurements in mm from whole mounts flattened specimens and median sagittal sections of paramphistome species obtained from goats and sheep in Southern Nigeria.

	<i>C. cotylophoron</i>	<i>C. cotylophoron</i>	<i>C. fuelleborni</i>	<i>C. fuelleborni</i>	<i>C. synethes</i>	<i>C. synethes</i>	<i>C. microbothrium</i>	<i>C. microbothrium</i>
	Median section	Flattened	Median section	Flattened	Median section	Flattened	Median section	Flattened
Body length	4.65±0.28	7±1.89	3.58±0.07	4.85±0.45	6.5±1.1	6.59±0.07	5±1.11	11±1.51
Body width	2.51±0.15	4. 0±0.89	1.43±0.27	2.68±0.15	2.13±0.38	2.08±0.47	2.22±0.12	5±0.88
Acetabulum diameter	1.43±0.09	1.85±0.29	1.36±0.16	1.63±0.14	1.25±0.18	1.16±0.16	1.72±0.34	2.68±0.24
Ratio of acetabulum diameter to body length	1 : 3.25	1 : 3.78	1 : 2.63	1 : 2.98	1 : 5.20	1 : 5 .68	1 : 2.91	1 : 4.10
Pharynx length	0.54±0.05	0.62±0.1	0.36±0.12	0.32±0.12	0.45±0.11	0.47±0.09	0.5±0.15	0.9±0.15
Ratio of pharynx length to body length	1 : 8.61	1 :11.29	1 : 9.94	1 : 15.16	1 : 14.44	1 : 14.02	1 :10.00	1 : 12.22
Oesophagus length	0.61±0.12	0.62±0.1	0.32±0.12	0.31±0.04	0.9±0.11	0.81±0.19	0.50±0.14	0.72±0.18
anterior testis length	1.43±0.23	1.25±0.84	0.46±0.05	0.46±0.06	1.9±0.29	0.88±0.22	0.43±0.02	0.54±0.08
anterior testis breadth	0.32±0.04	0.83±0.35	0.17±0.12	0.46±0.06	0.49±0.2	0.49±0.12	0.43±0.02	0.54±0.08
Posterior testis length	1.43±0.23	1.25±0.84	0.46±0.0.12	0.39±0.06	1.9±0.29	0.88±0.22	0.43±0.02	0.54±0.08
Posterior testis breadth	0.32±0.04	0.83±0.35	0.17±0.05	0.47±0.06	0.49±0.20	0.49±0.12	0.43±0.02	0.54±0.08
Genital atrium diameter	0.57±0.04	0.84±0.3	0.43±0.08	0.66±0.04	0.54±0.16	0.12±0.01	0.3±0.03	0.35±0.05
Ovary diameter	0.17±0.1	0.21±0.09	0.47±0.09	*	0.18±0.05	0.28±0.07	0.23±0.03	0.73±0.24

* Data not obtained

2. MATERIALS AND METHODS

Two hundred goats and one hundred and eighty sheep were examined from Benin City, Lagos, Onitsha, Auchu and Sapele in Southern Nigeria. Specimens were recovered from the mucosa of the rumen and reticulum of sheep and goats slaughtered privately. The stomachs were cut open with a sharp knife. Parasites were handpicked and transferred into plastic containers of normal saline and were washed in the same solution. Some fresh whole worms from every sample were flattened dorsoventrally between two slides held together with two rubber bands and were left to fix for twenty-four hours in 10% formal saline. The specimens were washed and then stained

for twenty-four hours in 0.5% Borax carmine. Studies and measurements of diagnostic organs were done under a microscope. Some whole worms were serially sectioned dorsoventrally in the median sagittal plane using a rotary microtome, then stained in Heamatoxylin-Eosin, dehydrated in ethanol series and mounted in Canada Balsam as previously documented [17]. Diagnostic features of sectioned specimens were measured and photographed with a camera mounted on a Wild Heerbrugg microscope. Specimens were identified according to Keys in earlier systems [10,11,13,18]. Some specimens were preserved in sample bottle containers in 10% formal saline or 70% ethanol.

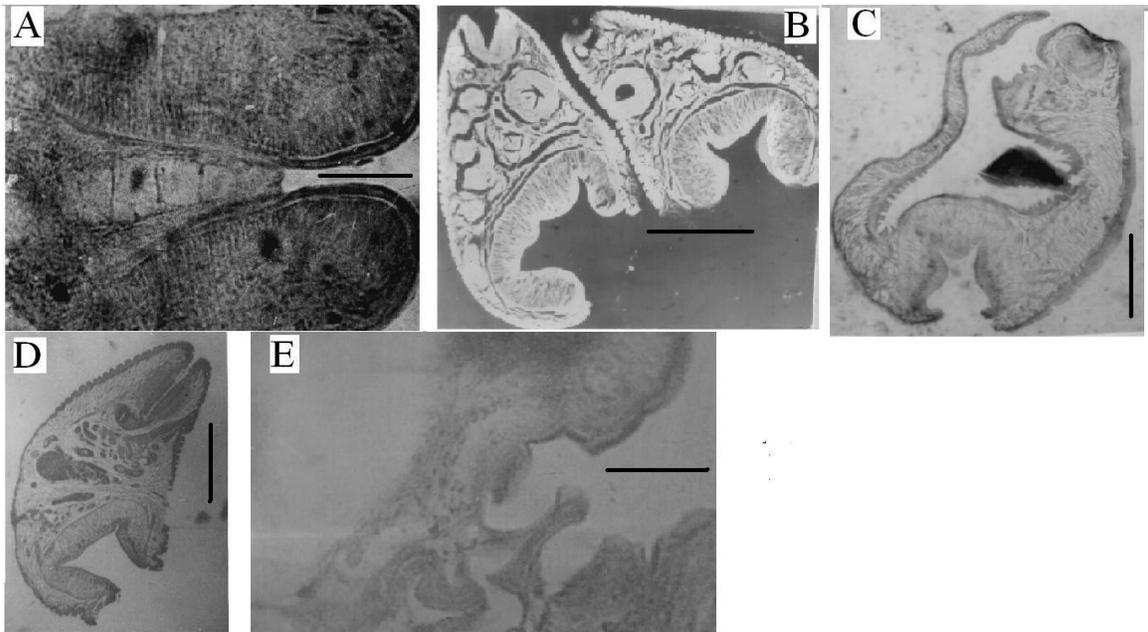


Figure 1: A) Genital atrium *Cotylophoron* type. Scale bar 50µm. B) Section of *Cotylophoron fuelleborni*. Scale Bar 900µm. C) Section of *Carmyerius synthes*. Scale bar 2mm. D) Section of *Calicophoron microbothrium*. Scale bar 2mm. E) Genital atrium of *Calicophoron microbothrium*. Scale bar 500µm.

3. RESULTS

The overall frequency of infected goats and sheep was 30% and 80% respectively. The range of intensity of parasite in goats and sheep was, 2 to 500 and 2 to 700 respectively. Nests of parasites 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.

From the morphological and histological studies of diagnostic features, which included the acetabulum, genitalium and pharynx, carried out on flattened whole mounts and median sagittal sections the following parasites were identified using documented keys [10,12,18]. *Cotylophoron cotylophorum* infected 8% of the goats, *Cotylophoron fuelleborni* infected 20% of the goats, *Carmyerius synethes* infected 4% of the goats and 70% sheep, and *Calicophoron microbothrium* infected 20% of the goats and 33%, sheep. Goats were infected with all the four species while sheep infected with only the last two species. The species recovered are here after described:

Cotylophoron cotylophorum Fiscoeder, 1901.

Description:

Body conical, wrinkled, with small tegument surface papillae. Fresh specimens colour, yellowish brown or pink. Tegument thin, body length to body width ratio moderate. Widest region just above the acetabulum. Morphometric data of sectioned and flattened whole mounts are given in Table 1

Acetabulum sub terminal; of the cotylophoron type (*sensu* Nasmark,

1937), in median sagittal sections circular muscle units numbers are given in Table 2.

Pharynx terminal, of the calicophoron type (*sensu* Dinnik, 1964), in median section the internal surface is lined by small dome shaped papillae. The posterior, anterior and lip-sphincters are absent. Oesophagus with strong muscular posterior bulb; lumen lined by hyaline layer throughout. Caeca in lateral sides of the body, forming about six dorso-ventral bends, reaching level of acetabulum with blind ends directed dorsally

Testes deeply lobed, diagonal and in posterior half of body. Uterus winding forward, dorsal to the testes then ventral to the male ducts; vitellaria in lateral fields extending from the oesophageal bifurcation to the acetabulum. Ovary sub spherical, posterior to testes and dorsal to acetabulum; Mehlis' gland close to the ovary either anterior or level with it. Genital pore opening on ventral surface at the level of oesophageal bifurcation or posterior to it; terminal genitalium (Fig.1A) is of the cotylophoron type (*sensu* Nasmark, 1937). Genital sucker present. Ductus ejaculatorius and metraterm open side by side at the tip of the genital papilla, which tapers on its free end. Pars prostatica cylindrical, short; pars musculosa loops once and then leads to the vesicular seminalis and eventually to posterior and exterior testes. Laurer's canal crossing excretory vesicles and opens on the dorsal surface posterior to the excretory pore. Eggs operculate, light green, with scattered granules in the yolk.

C. fuelleborni Nasmark, 1937.

Description:

Body conical, wrinkled, rounded anteriorly and posteriorly (Fig.1B). Colour of fresh specimens yellowish brown or pink. Tegument thick, surface with papillae, body length to body width

ratio small. Widest region just above the acetabulum. Morphometric data of sectioned and flattened whole mounts are given in Table 1.

Acetabulum sub terminal; of the cotylophoron type (*sensu* Nasmark, 1937), follows the description for *C. cotylophorum*; in median sagittal sections circular muscles units numbers are given in Table 2. The acetabulum diameter is large compared with the short body length.

Pharynx terminal; of the calicophoron type (*sensu* Dinnik, 1964), and follows the description for *C. cotylophorum*. Oesophagus walls thin with no muscular posterior bulb; lumen lined by hyaline layer throughout. Caeca in lateral sides of the body, forming about six dorsoventral bends, reaching level of acetabulum with blind ends directed dorsally

Testes deeply lobed, diagonal and in posterior half of body. Uterus winding forward, dorsal to the testes then ventral to the male ducts; vitellaria in lateral fields extending from the oesophageal bifurcation to the acetabulum. Ovary sub spherical, posterior to testes and dorsal to acetabulum. Mehlis' gland close to the ovary either anterior or level with it. Genital pore opening on ventral surface at the level of oesophageal bifurcation or posterior to it; terminal genitalium of the cotylophoron type (*sensu* Nasmark, 1937). Genital sucker present. Ductus ejaculatorius and metraterm open side by side at the tip of the genital papilla, which tapers on its free end. Pars prostatica cylindrical, short. Pars musculosa loops once and then leads to the vesicula seminalis and eventually to posterior and exterior testes. Laurer's canal crossing excretory vesicles and opens on the dorsal surface posterior to the excretory pore. Eggs operculate, light green, with scattered granules in the yolk.

C. synethes Fischoeder, 1901

Description:

Body slender, cylindrical and tapering anteriorly (Fig.1C). Tegument with small surface papillae but no wrinkles. Morphometric data of sectioned and flattened whole mounts are given in Table 1.

Acetabulum sub terminal; deep; of the gastrothylax type (*sensu* Nasmark, 1937), in median sagittal sections circular muscle units are shown in Table 2.

Pharynx round; basal layer large and removed from the margins; internal surface with small dome-shaped papillae. Oesophagus; long; musculature of wall moderate and vary in thickness, no bulb or posterior sphincter; lumen with two types of lining, the anterior third being lined by a hyaline layer and the posterior two thirds by ciliated epithelium as found lining the caeca. Caeca in lateral sides; almost straight; terminating middle of body with blind ends directed posteriorly.

Genital pore opening on ventral surface inside the ventral pouch above level of and anterior to oesophageal bifurcation. Terminal genitalium is bothriophoron type (*sensu* Nasmark, 1937). Ventral atrium large; small genital papilla. Ductus ejaculators and the metatherm open side by side at the tip of the genital papilla. Pars prostatica; long; cylindrical and joins a straight pars musculosa. Looped vesicula seminalis branches into two vasa differentia which lead to left and right testes. In median sagittal section, excretory vesicle antero-dorsal to acetabulum, ovary and Mehlis' gland: excretory pore opening on dorsal surface at level of testis anteriorly to opening of Laurer's canal.

Ventral pouch (Fig.1C), triangular in cross section, beginning above acetabulum; opens and terminates mid oesophagus. Vitellaria run along the

lateral sides of the body and end at the level of the acetabulum. Ovary and Mehlis' gland lie between side-by-side round testes. Uterus wavy; runs along the middle of the body. Eggs are operculate.

Calicophoron microbothrium

Fischoeder, 1901 syn. *Paramphistomum microbothrium*.

Description:

The body is conical. (Fig 1D). Fresh specimens red anteriorly and posteriorly, yellowish-white in the middle. Surface with papillae around oral opening, around genital pore region and acetabulum opening; papillae on oral end smaller than those on genital pore region and those around the acetabulum opening are fewer, and much smaller and more randomly arranged than the two other groups. Morphometric data of sectioned and flattened whole mounts are given in Table 1.

Acetabulum sub terminal (Fig. 1D), of the paramphistomum type (*sensu* Nasmark, 1937). In median sagittal section the numbers of dorsal exterior circular muscles one (d.e.c1), dorsal exterior circular muscles two (d.e.c2), dorsal interior circular muscles (d.i.c), ventral exterior circular muscle (v.e.c) and ventral interior circular muscle v.i.c. is shown in Table 2.

Pharynx is of the paramphistomum type (*sensu* Nasmark, 1937). In median sagittal section, internal surface with small dome-shaped papillae. Oesophagus, musculature of wall moderate and uniform in thickness, no bulb or posterior sphincter; lumen with two types of lining, the anterior third being lined by a hyaline layer and the posterior two thirds by ciliated epithelium as found lining the caeca. Caeca in lateral sides of the body, forming dorsoventral bends, reaching level of acetabulum with blind ends

directed ventrally.

Genital pore opening on ventral surface at level posterior to oesophageal bifurcation. Terminal genitalium (Fig 1E) of the microbothrium type (*sensu* Nasmark, 1937). In median sagittal section excretory vesicle dorsal to acetabulum ovary and Mehlis' gland: excretory pore opening on dorsal surface at level of posterior testis and anteriorly to opening of Laurer's canal. Pars prostatica small; barrel shaped; opens into the genital papilla through the ductus ejaculators; dorsally connects to pars musculosa then to the vesicula seminalis; vesicula seminalis branches into vasa differentia leading to anterior and posterior testes. Uterus wavy; dorsal to the testes; runs along the middle of the body and opening into the genital papilla through the metatherm. The eggs are filled with evenly scattered granules and are light blue-green.

The intensity and prevalence of paramphistomes in goats and sheep is given in Table 2. In all cases examined, where goats were infected with paramphistomes, damage to the ruminal and reticulum tissues was minimal. In sheep damage to the ruminal and reticulum tissues was minimal except for one case in which there were lesions on the epithelial tissues.

4. DISCUSSION

Two species recovered in our study belong to the genus *Cotylophoron* Stiles et Goldberger, 1910. The body shape, the presence of well-developed genital sucker, position and shape of testes, acetabulum, ovary, and vitelline glands agree with earlier descriptions [10,13,18].

Table2: Distribution, prevalence and acetabulum muscle units of paramphistomes in goats and sheep from Southern Nigeria

Species recovered	Host	Habitat	Prevalence	Intensity of parasites	d.c.e*	d.c.i*	v.c.i*	v.c.e*
<i>C. cotylophorum</i>	Goats	Reticulum	8%	7-10	18	40	41	20
<i>C. fuelleborni</i>	Goats	Reticulum	20%	2-5	13	45	52	17
<i>C. synethes</i>	Goats	Reticulum	4%	5-500	38	33	29	40
	Sheep	Rumen	70%	5-700				
<i>P. microbothrium</i>	Goats	Reticulum	20%	2-7	23+15	54	43	15
	Sheep	Rumen reticulum	33%	2-10				
All species pooled	Goats	Rumen reticulum	30%	2-500				
All species pooled	Sheep	Rumen reticulum	80%	2-700				

KEY:

d.c.e.* - dorsal exterior circular muscles units

d.c.i.* - dorsal interior circular muscles units

v.c.i.* - ventral interior circular muscles units

The identification of *C. cotylophorum* Fiscoeder, 1901 was based on its possession of a well-developed oesophageal bulb, which is characteristic of this species [10,13]. We found the histological structures of the acetabulum, pharynx and genital atrium are similar between *Cotylophoron* species in this study. From closely examining the description of *Cotylophoron guangdongense* Wang, 1979 and comparing with *C. cotylophorum* it has been concluded that the two are synonymous [13]. The genital sucker, which lacks a sphincter and the genital papillae, which tapers towards its free end, were used to establish identification

of this species as earlier proposed [10,13].

C. fuelleborni Nasmark, 1937 was identified on the basis of it lacking an oesophageal bulb, the presence of thin oesophageal wall musculature and the testes are diagonal which correspond to similar diagnosis [10,13]. *Cotylophoron indicum* Nasmark, 1937 and *Cotylophoron noveboracensis* Price & McIntosh, 1953 are synonyms of *C. fuelleborni* [13]. The specimens examined by Eduardo [13] although differing in size, that according to him was not sufficient to assign them to different species. The thick integument, the small body size with a mean of 3.58mm and a large acetabulum with a

diameter of 1.36mm agree with the description for this species [10].

The third described parasite belongs to the genus *Carmyerius* Stiles et Goldberger, 1910. The body shape, the presence of the ventral pouch which is triangular in cross section, symmetrical testes, the position of the uterus, pars musculosa and vesicula seminalis which run in the median field, the position of the vitelline glands and inter-testicular ovary agree with the descriptions for this species [11,18]. The material was identified *C. synethes* because histological features of the acetabulum, pharynx and genital atrium correspond to earlier descriptions and illustrations [11]. The pharynx is of the paramphistomum type (*sensu* Nasmark 1937) and is short and spherical in the median sections and flattened specimens. The genital atrium is of bothriophoron type (*sensu* Nasmark 1937) and has wider aperture than that of other species in this genus, while the acetabulum is horseshoe shaped in the median sagittal sections its similar to that described by other authors [11]. It should be noted that this is the first report of *C. synethes* in Nigerian goats and sheep.

The fourth described parasite belongs to the genus *Calicophoron* Fiscoeder, 1901. The body shape, the ratio of the acetabulum diameter to body length, the position and shape of the acetabulum, ovary, vitelline glands, testes and genital pore agree with the diagnosis for the genus *Calicophoron* [10]. It was assigned to *Calicophoron microbothrium* Fiscoeder, 1901 on the basis of the histology of the acetabulum, pharynx and genital atrium, which agrees with the earlier descriptions and illustrations [10,19,20,21,22,23]. The acetabulum is paramphistomum type (*sensu* Nasmark 1937) with well-

developed exterior and interior circular muscles. The dorsal exterior is divided into (d.c.e 1) and (d.c.e 2) as previously reported [22,23]. The pharynx, which is paramphistomum type (*sensu* Nasmark 1937), shows a well-developed basal layer, interior and exterior circular layers. The genital atrium though showing slight variation of the genital papilla the extent to which the genital sphincter and sphincter papilla are developed is always the same. The turning dorsal of the blind ends of the caeca agrees with similar observations [20,22]. It appears that *C. microbothrium* is the most frequent ruminant paramphistome species in Africa in view of the fact that it has been reported in all places where studies on ruminant paramphistomes have been made [19,24,25,26,27]. The present investigation shows that *C. microbothrium* is also the most frequent ruminant paramphistome in Southern Nigeria. Since it has been responsible for disease in experimentally infected goats and sheep in South Africa [5] we need more studies of its overall effect on the health of goats in the field in Southern Nigeria as this seems not to have been done. The size of the paramphistomes recovered from goats and sheep is generally smaller than of those from other ruminants [5,15]. This was also true in this study.

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