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## ORIGINAL ARTICLE

# *Ceylonocotyle, Bothriophoron*, and *Calicophoron* species Parasitic in some Nigerian Cattle

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**ABSTRACT**
*The following genera Ceylonocotyle, Bothriophoron and Calicophoron were recovered from the inner walls of stomachs of cattle slaughtered in abattoirs in the North and South of Nigeria. Some parasites were flattened and diagnostic features taken while some were sectioned in the median saggital plane from where the histology and morphology of the pharynx, genital atrium, acetabulum and testis were analysed for identification. Ceylonocotyle dicranocoelium. Bothriophoron bothriophoron, Calicophoron calicophorum, and Calicophoron microbothrioides were identified. Photomicrographs of the features used in identifications were made and are here presented. Damage to host tissues of the reticulum and rumen was in the majority of cases severe when Ceylonocotyle dicranocoelium was present but the other species did less damage to rumens and reticulum. Parasite loads ranged between 20 and 200 parasites in the infected animals and frequencies of occurrence in cattle were 10% for Ceylonocotyle dicranocoelium, 2 % for B. bothriophoron, 15% for C. calicophorum, and 5 % for C. microbothrides. All the described parasites are being reported in Nigeria for the first time.*
***Key words**: Ceylonocotyle, Bothriophoron, Calicophoron, cattle, Nigeria*

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## INTRODUCTION

*Ceylonocotyle dicranocoelium, Bothriophoron bothriophoron, Calicophoron calicophorum* and *Calicophoron microbothrioides* are trematodes of the family Paramphistomideae Fischioder 1901and are parasitic in the alimentary canal of many ruminants. Mature parasites of many species are especially prevalent in the reticulum and rumen where they rarely produce any clinical symptoms [1]. Immature migrating amphistomes of certain species have been reported to cause serious disease and even deaths of their hosts by burying themselves into the submucosa of the duodenum and feeding on the epithelial cells of the Brunners gland which results in anorexia, polydypsia, profuse foetid diarrhea, drop in plasma protein concentration and anemia [1, 2, 3, 4, 5, 6]. A few species such as *Cotylophoron cotylophorum, Paramphistomum microbothrium, Paramphistomum ichikawai, Paramphistomum cervi* and *Calicophoron calicophorum* species have been studied for their role in disease [1, 5, 6]. Documentation of prevalence and pathogenicity can only be ascertained for species that have been accurately identified. Owing to the difficulties in identifying members of this group except through the median saggittal sections there has been a mix up in the taxonomy of this group [7, 8, 9, 10, 11, 12]. Material referred to as *Cotylophoron* spp. by earlier workers was in fact found to be *P. microbothrium* [7, 9]. *Carmyerius gregarious, Carmyerius spatiosus, C. cotylophorum* and *P. microbothrium* were present from past collections from Nigerian domestic livestock [13]. Nigerian flora and fauna have changed drastically due to human resettlements, oil drilling, use of fertilisers, pesticides and dam constructions.

The aim of this study was to determine the identity, prevalence, frequency of *Ceylonocotyle dicranocoelium, Bothriophoron bothriophoron, Calicophoron calicophorum* and *Calicophoron microbothrioides* in Nigerian cattle. Not all paramphistomes species are responsible for disease in Livestock, is it therefore important to have accurate information about existing species so that where pathogenic ones occur preventive control measures can be taken instead of waiting for outbreaks [5]. Information on *Cotylophoron, Paramphistomum* and *Gastrothylicinae* has been documented in Nigeria but the described parasites have yet to be documented [14, 15, 16].

## MATERIALS AND METHODS

Specimens were obtained from the inner walls of the rumen and reticulum from cattle, slaughtered at the abattoirs Benin, Maiduguri, Kano and Zaria in Nigeria. The condition of the rumen and reticulum were assessed for damage caused by the parasites. The parasites were hand-picked into plastic containers containing normal saline and were washed in the same solution. Some specimens were teased to obtain eggs; some were flattened dosovetrally between two slides to facilitate examining diagnostic features like vitelline glands, positioning of testes, oesophagus, nature of caeca and uterus. Some specimens were fixed and preserved in formal saline or 70% ethanol for histological characterisation in the median sagittal sections using the systems proposed by earlier authors in whom the acetabulum pharynx and genital atrium were analysed [7, 17, 12]. The specimens were prepared for sectioning by the method of Mahoney [18]. In brief graded alcohol series are used for dehydration of specimens, which were then embedded in wax, sectioned with a rotary microtome and stained using Heamatoxylin/Eosin. The specimens were mounted in Canada balsam. The slides were dried on a hot plate at 60°C for 96 hours. Photographs were taken using a camera mounted on a microscope while diagnostic measurements were taken with calibrated microscope.

## RESULTS AND DISCUSSION

Whereas no damage has been reported to have been caused by adult paramphistomes in this study there was visible physical damage done by *Ceylonocotyle* and *Bothriophoron* species. The physical damage done to rumen tissues that resulted in pus could result in opportunistic bacterial infections and also an increase in stress which could lower the productivity of the animal. Snails occurring in the Nigeria have been documented and among them are those implicated in the transmission of paramphistomes [19, 20]. While these parasite are being reported for the first time in Nigeria, there are three possible reasons why this is so. 1) these parasites have always been there but unnoticed by investigators or overlooked 2) These parasite have only recently arrived in Nigeria from animals from neighbouring countries 3) Or they could have all along been hosted in ruminants whose paramphistomes had not yet received attention but have recently started infecting cattle.

Four distinct species of paramphistomes not previously reported in Nigeria were recovered in the rumens and reticulum of cattle slaughtered in various abattoirs. Nests of parasites numbering between 20 and 2500 were found in the folds of the reticulum and between papillae in the rumen, 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. The first group of parasites recovered was identified as *Ceylonocotyle dicranocoelium infecting10*% of the cattle examined and caused the most serious damage in the infected areas resulting in pus like exudates. The number of parasites infecting each animal ranged from 200-2500.

*Ceylonocotyle dicranocoelim* N ä s m a r k 1937 has the following description, the body is pear-shaped (Fig1 A and B). It is curved towards the ventral side. The integument is without wrinkles. When fresh they are cream in colour. The acetabulum is subterminal and is of the *Streptocoelium* type (*sensu* N ä s m a r k 1937)(Fig.1D). The pharynx is usually subterminal (Fig.1A). The pharynx is of the *Dicranocoelium* type (*sensu* N ä s m a r k 1937). The lip-sphincter is strongly developed and is horse-shoe shaped. It occurs on both sides of the oral opening. The oesophagus in median sagittal section is curved. The caeca run laterally almost straight on either side of the body. They terminate at the level of the acetabulum with blind ends facing the posterior direction. Vitelline glands extend from the level of the gut bifurcation to the acetabulum. They form between 10-15 solid masses, lying between the lateral margins of the body and the caeca. The testes are oval and situated one behind the other. The ductus ejaculatorius unites with the metatherm before getting into the genital papilla (Fig.1C). The pars prostatica is cylindrical. It is connected to the pars musculosa which makes a few loops before joining the vasa deferentia. The vasa deferentia lead to the anterior and posterior testes. The ovary and Mehlis gland lie between the acetabulum and the posterior testis. The uterus is wavy, running dorsal to the testes before reaching the genital papilla through the metatherm. The genital atrium is of the *Streptocoelium type* (*sensu* N ä s m a r k 1937) (Fig.1 C). The eggs are operculate and light-green, with small granules scattered in the yolk. The dimensions of the eggs are given in Table.2. *Ceylenocotyle* N ä s m a r k , 1937 (= *Orthocoelium* Price McIntosh 1953) was identified because of the body shape, position and shape, of the acetabulum, ovary, vitelline glands, testes and genital pore which correspond to earlier descriptions for genus *Ceylenocotyle* and for genus *Orthocoelium*[7, 17]. It is assigned to the species *C. dicranocoelium* N ä s m a r k , 1937 on the basis of histology of acetabulum, pharynx and genital atrium which follow previous descriptions [7, 17]. The acetabulum is of the *Streptocoelium* type. The pharynx is of the *Diacranocoelium* type with a characteristic horse-shoe shaped lip-sphincter. The genital atrium is of the *Streptocoelium* type. The heavily clustered vitelline glands and large rounded testes are very conspicuous in this species. These features are visible in flattened

specimens. Therefore in Nigeria *C. dicranocoelium* can be identified without requiring median sagittal sections.

*Bothriophoron bothriophoron* (Syn, Braun 1892=Paramphisotomum *Bothriophoron* N ä s m a r k *1937*) infected 2% of the cattle examined also caused considerable damage to stomach walls. The number of parasites infecting each animal ranged from 10-500, had the following description, the body is conical. When fresh the body is yellowish-white. The integument is marked by well defined transverse wrinkles. The genital pore is clearly visible large, lying encircled by an oval swelling. It lies about one fifth of the body length from the oral end. Measurements of diagnostic structures are shown in Table 1 for sectioned specimens and for flattened specimens.

**Table** 1. Measurements for flattened specimens and median sagittal sections in millimeters for *Celonocotyle Calicophoron* and *Bothriophoron* species from Nigeria

| | Median section | Flattened | Median section | Median section | Median section |
|---|---|---|---|---|---|
| | *C. dicranocoelium* | *C. dicranocoelium* | *C. calicophoron* | *C. microbothrioides* | *B. bothriophoron* |
| Body length | 4.93±0.16 | 8.1±0.85 | 5.36±0.78 | 4.65 | 5.16 |
| Body breadth | 1.75±0.23 | 3.15±0.34 | 3.76±0.41 | 2.10 | 1.8 |
| Acetabulum diameter | 0.99±0.05 | 1.2±0.36 | 2.01±0.11 | 1.68 | 1.44 |
| Ratio of acetabulum diameter to body length | 1:5 | 1:6.78 | 1:2.66 | 1:2.77 | 1:3.58 |
| Pharynx length | 0.45±0.03 | 0.56±0.01 | 0.78±0.16 | 0.63 | 0.42 |
| Ratio of pharynx length to body length | 1:10.9 | 1:14.72 | 1:6.94 | 1:7.38 | 1:12.29 |
| Oesophagus length | 0.39±0.09 | 0.66±0.18 | 0.68±0.09 | 0.6 | 0.3 |
| anterior testis length | 1.04±0.01 | 2.22±0.3 | 1.21±0.68 | 0.75 | |
| anterior testis breadth | 0.96±0.04 | 2.22±0.3 | 0.81±0.12 | 0.6 | 0.45 |
| Posterior testis length | 1.30±0.06 | 2.26±0.41 | 1.21±0.68 | 0.75 | 0.6 |
| Posterior testis breadth | 1.04±0.01 | 2.26±0.41 | 0.81±0.12 | 0.6 | 0.45 |
| Genital atrium diameter | | 1.35 | | 0.45 | 0.57 |
| Ovary diameter | 0.33±0.03 | 0.67±0.01 | 0.67±0.01 | 0.3 | 0.21 |

The acetabulum is of the *Streptocelium* type (*sensu* N ä s m a r k 1937) (Fig 1E). The pharynx is of the *Streptocelium* type (*sensu* N ä s m a r k 1937). The esophagus has no bulbous expansion and its wall has two thin muscle layers, the inner layer is longitudinal, while the outer layer is circular. Its lumen is lined by a thick integument like layer. The gut caeca makes six identical bends on either side of the body. After the last bend which is on the ventral side, the terminal part of the caeca turns dorsally. Their blind ends lie on each side of the acetabulum. The testes are in tandem and they are deeply lobed.

The excretory bladder consists of gland-like tissue of irregular thickness. It opens through the excretory pore about three tenths of the body length from the posterior end. In flattened specimens the ovary lies between the posterior testis and the inner margin of the acetabulum. The Mehlis gland lies ventral to the ovary in the median sections. The Mehlis gland lies besides the ovary in flattened specimens. Clusters of vitelline glands are conspicuous in flattened specimens. They extend from the posterior margins of the pharynx to the acetabulum. They occupy the space between the lateral margins of the body and the caeca. The genital atrium is of the *Bothriophoron* type (*sensu* N ä s m a r k 1937) (Fig. 1F). It lies close and posterior to the gut bifurcation. The genital papilla lies deep in a canal either behind the ventral atrium or protrudes. The pars prostatica is small in relation to the body size. It is barrel shaped. It opens to the genital papilla through the ductus ejaculators. Dorsally the pars prostatica connects the pars musculosa which is long and makes a few loops and then connects to the vesicula seminalis which makes many closely packed irregular loops. In the median sections the vesicular seminalis appears as a solid mass with an indistinct lumen. In flattened specimens it branches into two vasa deferentia which lead to the interior and posterior tests. 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 metatherm. The operculate eggs are filled with evenly scattered granules and are light blue-green.

*B. bothriophoron* Fischoeder, 1901 was identified on the basis of the histology of the acetabulum, pharynx and genital atrium which agrees with previous descriptions and illustrations [7, 12, 17]. The acetabulum is clearly S*treptocoelium* type N ä s m a r k 1937. The pharynx is *Paramphistomum* type. The genital atrium in a very deep trough that can easily mistaken to the same as the genital sucker of *Cotylophoron* type as has been done by before by previous researchers [7]. The pars prostatica is small in diameter short in length.

For the two parasites in the genus *Calicophoron* N ä s m a r k 1937 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 previous descriptions for this genus [7, 17].

*Calicophoron calicophorum* Fischoeder, 1901 infected 15% of the cattle examined no apparent damage was observed from this infection. The number of parasites infecting each animal ranged from 20-1000 had the following description: The body is conical (Fig.1G and H). The colour is pink for fresh specimens. The integument has wrinkles. The genital pore is visible especially when the genital atrium is everted. It lies about one fifth of the body length from the oral end. Diagnostic measurements are shown in Table 1 for sectioned specimens. The acetabulum is of the *Calicophoron* type (*sensu* N ä s m a r k 1937). The pharynx is of the *Calicophoron* type (*sensu* N ä s m a r k 1937) (Fig 1I). The oesophagus is shorter than the pharynx and is made of two layers, and outer circular layer and an inner longitudinal layer. The caeca makes six bends and after the last bend which is on the dorsal side, the caeca turn towards the ventral direction. The excretory bladder opens through the excretory pore three tenths of the body length from the posterior end. The testes are situated diagonally in the mid-third of the body. They are deeply lobed. The ovary and the Mehlis glad lie adjacent to each other between the posterior testis and the acetabulum towards the dorsal side. Clusters of vitelline glands extend from the pharynx to the acetabulum on the lateral margins of the body. The genital atrium is of the *Calicophoron* type (*sensu* N ä s m a r k 1937) (Fig1 J). The pars prostatica is long and large, opening to exterior through the ductus ejaculatorius. Dorsally the pars prostatica leads to a pars musculosa which leads into the vesicula seminalis with many loops. The vesicula seminalis branches into two vasa deferentia which lead to the posterior and anterior testis. The uterus is wavy extending from the ovary along the midline to the metratherm.

*C. calicophorum* Fischoeder, 1901 was identified on the basis of the histology of the acetabulum, pharynx and genital atrium which correspond with the previous descriptions and illustrations [7, 8, 21, 22, 23]. The acetabulum is of the *Calicophoron* type. The absence of (de) stressed by previous a u t h o r s has since been disproved [7, 8, 21, 22]. The pharynx is of the *Calicophoron* type. Suggestion to merge of this pharynx type with the *Paramphistomum* type has been made but ignored [23].

*Calicophoron microbothirioides* Eduoardo 1983 (Syn. *Paramphistomum microbothrioides* Fischoeder, 1901) infected 5% of the cattle examined had no apparent damage caused. The number of parasites infecting each animal ranged from 20-500 had the following description:

The body is conical. When fresh, the acetabulum and the pharyngeal region are red while the rest of the body is yellowish-white. The integument is marked by well defined transverse wrinkles. The genital pore is clearly visible, lying encircled by an oval swelling. It lies about one fifth of the body length from the oral end. Measurements of diagnostic structures are shown in Table 1 for sectioned specimens and for flattened specimens. The acetabulum is of the *Paramphistomum* (*sensu* N ä s m a r k 1937) (Fig1K). The pharynx is of the *Paramphistomum* type (*sensu* N ä s m a r k 1937). The esophagus has no bulbous expansion and its wall has two thin muscle layers, the inner layer is longitudinal, while the outer layer is circular. Its lumen is lined by a thick integument like layer. The gut caeca makes six identical bends on either side of the body. After the last bend which is on the ventral side, the terminal part of the caeca turns dorsally. Their blind ends lie on each side of the acetabulum. The testes are in tandem and they are deeply lobed. The excretory bladder consists of gland-like tissue of irregular thickness. It opens through the excretory pore about one fifth of the body length from the posterior end. In flattened specimens the ovary lies between the posterior testis and the inner margin of the acetabulum. The Mehlis gland lies ventral to the ovary in the median sections. The Mehlis gland lies besides the ovary in flattened specimens. Clusters of vitelline glands are conspicuous in flattened specimens. They extend from the posterior margins of the pharynx to the acetabulum. They occupy the space between the lateral margins of the body and the caeca. The genital atrium is of the *Microbothrium* type (*sensu* N ä s m a r k 1937). It lies close and posterior to the gut bifurcation. The pars prostatica is very small in relation to the body size. It is barrel shaped. It opens to the genital papilla through the ductus ejaculators. Dorsally the pars prostatica connects the pars musculosa which is long and makes a few loops and then connects to the vesicula seminalis which makes many closely packed irregular loops. In the median sections the vesicular seminalis appears as a solid mass with an indistinct lumen. In flattened specimens it branches into two vasa deferentia which lead to the interior and posterior testis. 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 metatherm. The eggs are filled with evenly scattered granules and are light blue-green.

*C. microbothrioides* Fischoeder, 1901 was identified on the basis of the histology of the acetabulum, pharynx and genital atrium which agrees with previous descriptions and illustrations [7, 12, 17]. The acetabulum is clearly *Paramphistomum* type [7].The pharynx which is *Paramphistomum* type. The turning dorsal of the blind ends of the caeca agrees with previous similar observations [24, 25]. The pars prostatica is small.

All the paramphistomes occurred all year round with no preferential infection based on age or breed of host cattle. As these species are being reported for the first time in Nigerian cattle brief description of the taxonomic features of each has been given.

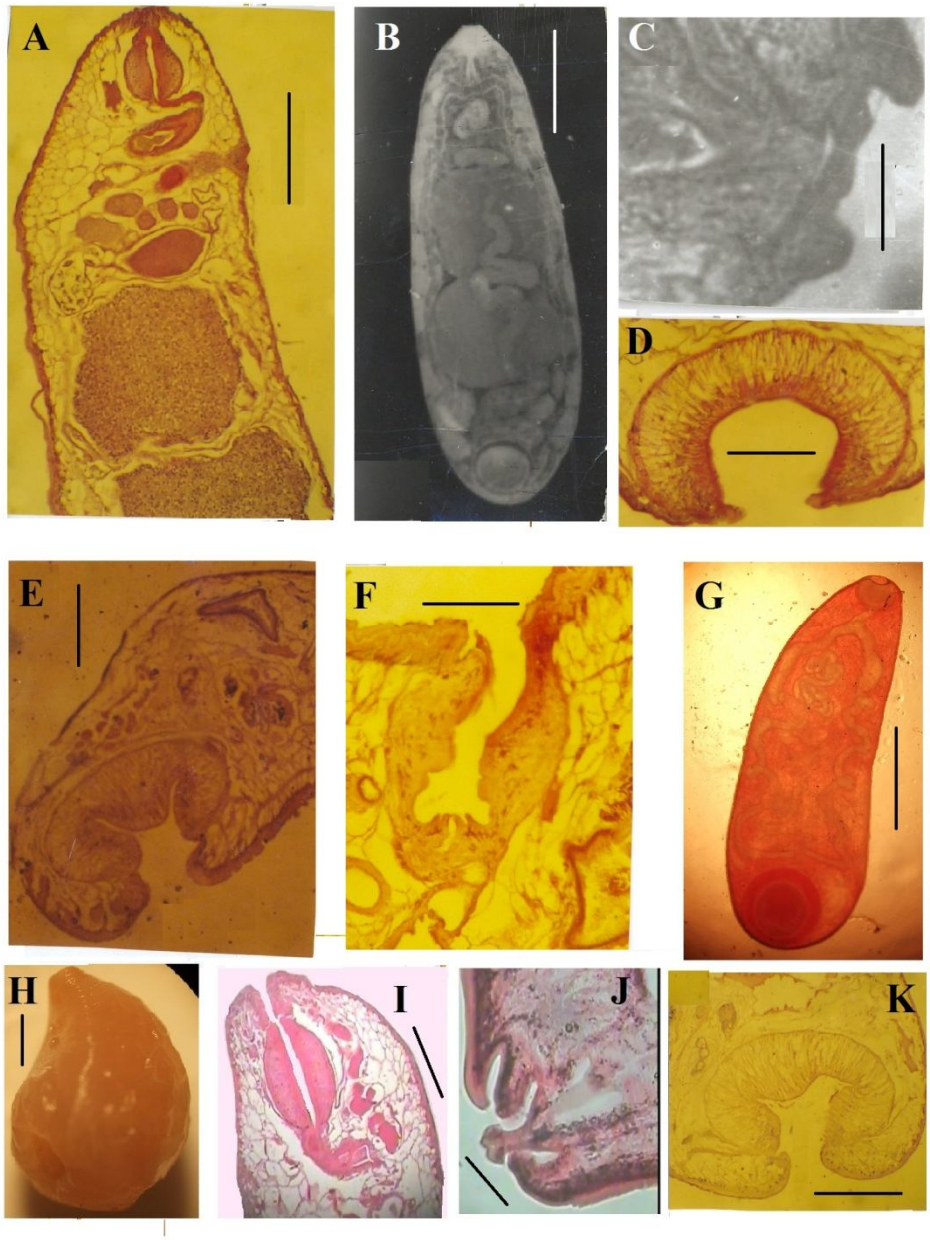


Fig 1 A) *Ceylocotyle dicranocoelium* anterior median section (scale bar =1000µm). B) *Ceylocotyle dicranocoelium* Flattened specimen (scale bar =700µm). C) *Ceylocotyle dicranocoelium* genetilia (scale bar =200µm). D) *Ceylocotyle dicranocoelium* acetabulum (scale bar =1000µm). E) *Bothriophoron bothriophoron* acetabulum (scale bar =1000µm). F) *Bothriophoron bothriophoron* genetilia (scale bar =600µm). G) *Calicophoron calicophorum* Flattened specimen (scale bar =1500µm). H) *Calicophoron calicophorum* whole fresh specimen (scale bar =1000µm). I) *Calicophoron calicophorum* anterior median section (scale bar =1000µm). J) *Calicophoron calicophorum* genetilia (scale bar =200µm). K) *Calicophoron microbothrioides* acetabulum (scale bar =1000µm).

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