Studies on paramphistomes infecting goats and sheep from Gwanda District in Zimbabwe

S. Dube[1], K .E. Masanganise[2] and C. Dube[3]

[1]Department of Applied Biology and Biochemistry
National University of Science and Technology, P. O. Box AC 939 Ascot
BULAWAYO
Zimbabwe
shdube@nust.ac.zw

[2]Veterinary Department, P. O. Box Ry 72, Raylton BULAWAYO, Zimbabwe.

[3] United College of Education Box 1156 BULAWAYO Zimbabwe

Abstract

Paramphistomes were collected from 3000 goats and 1000 sheep from various localities in Gwanda district in Matabeleland South Province Zimbabwe. On slaughter their stomachs, rumens and reticulum were cut open and their inner walls were examined for attached paramphistomes. Identifications were based on the morphological structures and measurements of diagnostic features from flattened and 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* and *Ceylonocotyle dicranocoelium*. The degree of tissue damage on the host was generally slight except for cases where *Ceylonocotyle dicranocoelium* was involved. The percentage of goats infected with paramphistomes was 2% while in sheep it was 6%. The number of parasites in goats ranged from 5 to 500 while in sheep the range was 5 to 1000.

Key words: Paramphistome, *Calicophoron, Ceylonocotyle,* sheep, goat, Zimbabwe.

1. INTRODUCTION

Gwanda district in Zimbabwe lies approximately 21° parallel South and 29° *longitude* East covering an area 2500sq. Km. The annual average temperature is 26°. The rainfall, which comes between mid-November and late March, averages 400mm per annum. Rainfall in this province has been usually below normal compared to the rest of the country. Drought or near-drought conditions are experienced frequently in this region. Some like the severe 1992 drought resulted in the death of many goats and sheep that contributes substantially to the income for people in this area. Drinking water for livestock comes from numerous dams, twelve, which have a capacity above two mega cubic meters. Dams, unfortunately, have resulted in the increase in the population of aquatic snails among which are *Lymnea* spp. and *Bulinus* spp. that are intermediate hosts for paramphistomes, [1,9]. Water ponds and dams are the main sources of drinking water for livestock in this province. *Cotylophoron cotylophorum, Carmyerius spotiosus* and *Carmyerius bubalis* were recovered from *Alcelaphus* spp, *Tragelaphus spekei* and *Tragelaphus stresiceros* in Zimbabwe [19]. *Calicophoron raja* was recovered from

Connochaeters taurinus while *P. microbothrium* (Syn. *Calicophoron microbothrium* Eduardo 1983) was recovered from *Aepyceros melampus*, *Kobus leche* and *Taurotragus oryx* in Zimbabwe [6]. Most of the ruminants listed above occupy the same ecological niche with goats and sheep. Several paramphistome species have been reported world over among small and large ruminants [6, 7, 8, 16, 20, and 26]. 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 and generally animals not previously exposed are susceptible [2,3,17].

The aim of this study was to determine the prevalence, frequency and species of paramphistomes that occur in goats and sheep. Not all paramphistomes species are responsible for disease in goats and sheep, it is therefore important to have information about existing species so that where pathogenic ones occur preventive control measures can be taken instead of waiting for outbreaks [24]. To date we found no literature or information on the prevalence, frequency and species of paramphistomes in goats and sheep from Gwanda Zimbabwe. Such information can be used eventually to construct predictive models of outbreaks of disease [17]. The models of disease are important in implementation of control measures that are effective and economical.

2. MATERIALS AND METHODS

Paramphistomes were obtained from the inner walls of rumens and reticulum from 3000 goats and 1000 sheep brought for slaughter at the Bulawayo Cold Storage Commission abattoir. The goats and sheep were brought from Gwanda district, which has the highest population of goats and sheep in Zimbabwe. The presence of mature paramphistomes and their numbers in the animals examined were recorded. Physical damage to infected areas was noted. The number of goats and sheep infected was expressed as a percentage of the total number of animals examined. During collection days paramphistomes from each animal were temporarily kept in normal saline in separate plastic containers. Some specimens were teased for egg measurements; 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 measurements and histological characterisation in the median sagittal sections using previously documented keys, in which the acetabulum pharynx and genital atrium were analysed [6, 16, and 26]. The specimens were prepared for sectioning by previously documented method of Mahoney in which graded alcohol series are used for dehydration of specimens which were then embedded in wax, sectioned and stained using Heamatoxylin/Eosin[14]. The specimens were mounted in Canada balsam and examined under a light microscope.

3. RESULTS

The percentage of goats and sheep infected 2% and 6% respectively. The number of parasites per infected animal ranged between 5 and 1000. The parasites recovered from goats were generally fewer than those from sheep. In the majority of

Table 1. Measurements in micrometers from median sagittal sections of the identified paramphistomes from sheep and goats in Gwanda.

	Calicophoron microbothrium	*Calicophoron clavula*	*Ceylonocotyle dicranocoelium*
Body Length	8400±212	6900±750	6300±580
Body Breadth	3000±740	1950±525	2100±106
Acetabulum diameter	2100±636	1945±78	1500±99
Pharynx length	660±170	750±45	600±87
Anterior Testis Breadth	1350±150	900±125	900±134
Anterior Testis length	1800±0	1800±350	900±134
Posterior Testis Breadth	1350±150	900±125	900 ±134
Posterior Testis length	1800±0	1800±350	900±134
Pars Prostatica Breadth	360±45	300 ±75	300±75
Pars Prostatica length	450±0	400±25	300±75
Egg Breadth	90±5	70±10	75±10
Egg length	150±5	150±15	150±10

infected animals damage was limited to slight papillae erosions except for cases where *Ceylonocotyle dicranocoelium* was involved in which pus like exudates was noticed. The parasites were either located in the rumen or reticulum. They tend to form nests, with a few sparsely scattered outside the nest. There were no externally observable symptoms distinguishing the infected goats and sheep from those not infected. There were no preferential infections based on breed of goats and sheep or their age. As the parasites attain their maximum size their grip on the walls of the stomach, reticulum and rumen was observably less tight judging by the fact that they could easily be detached from the host with minimum force when hand picking. Measurements and features used for identification and confirmation of species are given in Table 1. The population of goats is five times that of sheep. *Calicophoron microbothrium, Calicophoron clavula* and *Ceylonocotyle dicranocoelium* were identified in goats and sheep and are here after described:

Calicophoron microbothrium Eduardo 1983 syn. ***Paramphistomum microbothrium*** Fischoeder, 1901

Description: The body is conical Fig 1A. When fresh, the acetabulum and the pharyngeal region are red while the rest of the body is yellowish-white. The tergument 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.

The acetabulum is of the *Paramphistomum type* (*sensu* Nasmark, 1937). The dorsal exterior muscles are divided into two parts, the dorsal exterior one (de_1) and the dorsal exterior two (de_2). The (de_1) are larger and strongly developed than (de_2) which are smaller, sparse and more in numbers. The dorsal interior muscle units (di) are well developed and correspond to the ventral interior muscle layer (vi) in development. All the muscle units are of the same size in the (di) layer. In the (vi) the last ten muscles are smaller than the rest. In the ventral muscle layer (ve) the units are developed to the same extent as the (de_1). The muscle units occupying the central position are larger than the rest and give the impression of two muscle units joined together.

The pharynx is of the *Paramphistomum* type (*sensu* Nasmark, 1937). The anterior sphincter is absent and interior circular layer consists of small wall defined units arranged in a single row along the whole length of the pharynx these units are of the same size throughout. The interior longitudinal layer occupies between one quarter and one third of the thickness of the pharynx. The posterior sphincter and lip sphincter are absent. The exterior circular layer is well developed and clearly seen in groups of loosely packed units arranged along the whole length of the pharynx, about one fifth of the width of the pharynx from the exterior margin. The external longitudinal layer is very narrow abut one tenth of the width of the pharynx. The middle circular layer is absent. The basal layer is present and is made of two layers the inner layer is less developed than the out layer.

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 tergument 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 turn 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 third 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. The Laurer's canal crosses the excretory canal and it opens dorsally close to it. 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 Fig 1D (*sensu* Nasmark, 1937). It lies close and posterior to the gut bifurcation. The genital papilla lies either behind the ventral atrium or protrudes. The sphincter papilla is present and is made of loosely packed tissue. The ventral sphincter is absent. The radial musculature of the genital fold is easily seen and well developed. The pars prostatica is

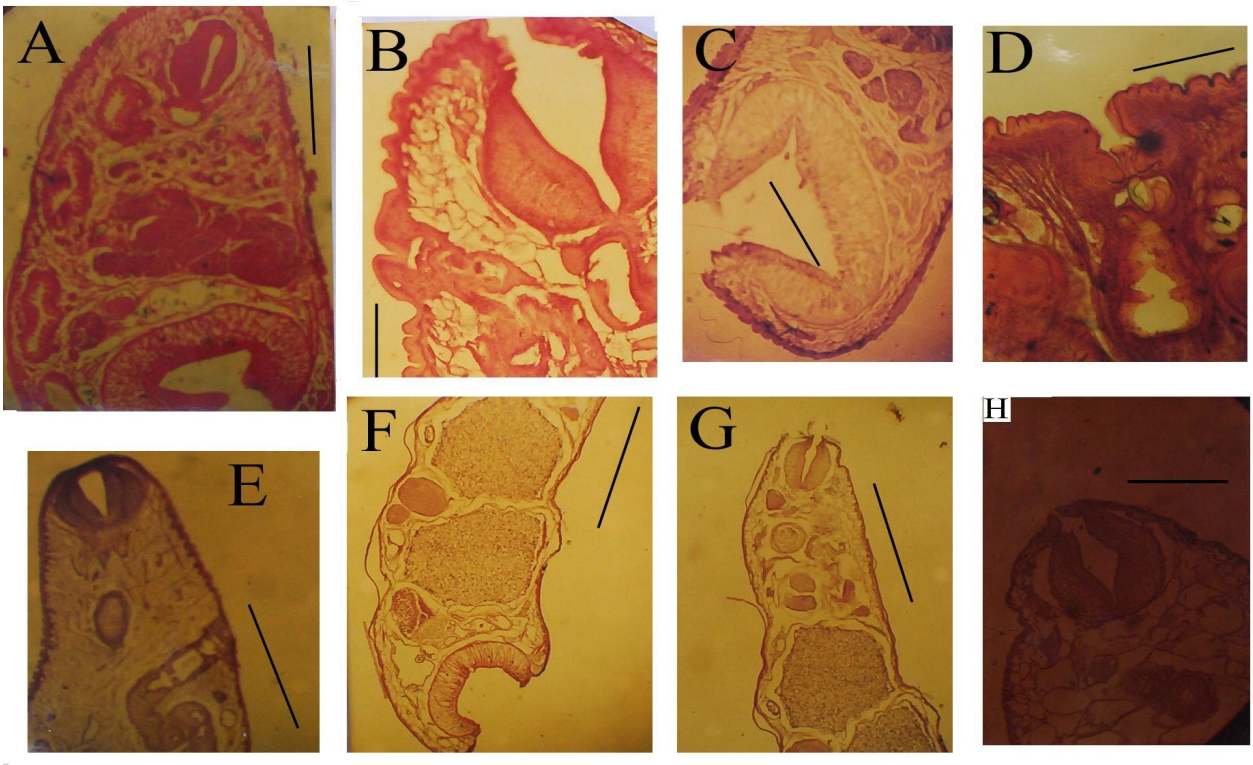

Fig 1: A) Calicophoron microbothrium median section (scale bar =500µm). B) Calicophoron clavula median section anterior region. (scale bar =700µm). C) Calicophoron clavula median section posterior region. (scale bar =500µm) D) Calicophoron microbothrium genitalia median section (scale bar =500µm). E) Calicophoron clavula median section anterior region (scale bar =500µm). F) Ceylonocotyle dicranocoelium median section posterior region. (scale bar =500µm). G) Ceylonocotyle dicranocoelium median section anterior (scale bar =500µm). H) Ceylonocotyle dicranocoelium median section anterior region showing pharynx (scale bar =500µm).

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 vesicular 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 differentia, which lead to the anterior 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 eggs are filled with evenly scattered granules and are light blue-green.

Calicophoron clavula Eduardo 1983 syn.***Paramphistomum clavula*** Nasmark, 1937.

Description: The body is conical. The tegument has transverse wrinkles. The colour of the body is pink when fresh. The genital pore is clearly visible lying flat with the body; there is no swelling on this region as in *C. microbothrium*. It leis about one fifth of the

body length from the oral end. The acetabulum is of the *Paramphistomum* type (*sensu* Nasmark, 1937) Fig 1C. It follows the same description outlined for *C. microbothrium*. The pharynx is of the *Paramphistomum* type *(sensu* Nasmark, 1937) Fig 1E and therefore corresponds to the description for *C microbothrium*. The esophagus resembles that of *C. microbothrium.* The caeca makes six bends on either sides of the body after the last band, which is towards the ventral side; the blind ends of the caeca turn dorsally. The caeca terminate on the lateral sides of the acetabulum. The excretory bladder opens three tenths of the body length from the posterior end. The testes are situated diagonally one behind the other in the mid-third of the body. They are deeply lobed. The ovary lies between the posterior testis and the margin of the acetabulum. The Mehlis glands lies close to the ovary. The Laurer's canal runs directly to the dorsal side of the body and opens close to the excretory pore. Clusters of vitelline glands extend from the pharynx to the acetabulum between the lateral margins of the body and the caeca. The genital atrium lies close and posterior to the gut bifurcation. It is of the *Clavula* type (*sensu* Nasmark, 1937) Fig 1E, the genital papilla has a large sphincter papilla. The genital sphincter is loosely packed and occupies a wide area. The radial muscles are easily seen. The pars prostatica is large and barrel-shaped. It connects to an inflated pars musculosa, which makes a few coils before connecting the vesicula seminalis, which makes many coils and then branches into two vasa differentia, which lead to the anterior and posterior testis. In the median sagittal sections the vesicular seminalis appears as a solid mass with an indistinct lumen. The uterus is wavy and runs in the midline dorsal to the testes. The eggs are operculate and light-green with granule evenly scattered in the yolk.

Ceylonocotyle dicranocoelium Nasmark 1937

Description: The body is pear-shaped. It is curved towards the ventral side. The integument is without wrinkles. The greatest width is in the middle portion of the body. When fresh they are white.

The acetabulum is sub terminal and is of the *Streptocoelium* type (*sensu* Nasmark, 1937) Fig 1F. The ventral and dorsal exterior muscles are slight developed. These units are about equally sized but are smaller at the beginning and at the end of the series. The dorsal exterior muscle units are not divided into two. The ventral and dorsal interior muscles are identical and almost have the same number of units. The interior circular muscles series form an arch-shaped curve having the smallest units at the beginning and end of the series with the largest units about one third way along the series. The fibers of the radial muscles are coarse and run in bundles, being widely spaced form each other. The pharynx is usually sub terminal. The pharynx is of the *Dicranocoelium* type (*sensu* Nasmark, 1937) Fig 1G and Fig 1H. In median sagittal sections, the interior circular muscle units are of moderate in size, running the whole length of the pharynx. The interior longitudinal layer is about one quarter of the breadth of the pharynx. Exteriorly this layer is not distinct. The middle circular is absent. The radial layer is of varying appearance, thickness, closeness and staining capacity. The exterior circular layer is quite developed and its units are clustered. The muscle fibers are some-what separated. The exterior longitudinal layer is present but small. The basal circular layer appears as two rows. The units of the outer row are more developed. The posterior and anterior sphincters are absent. 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. It consists of inner longitudinal and outer circular layers.

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 Fig 1 F,G are oval and situated one behind the other. The ductus ejaculatorius unites with the metatherm before getting into the genital papilla. The pars prostatica is cylindrical. It is connected to the pars musculosa, which makes a few loops before joining the vasa differentia. The vasa differentia 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* Nasmark, 1937). The genital papilla is short. There is no sphincter papilla. The ventral sphincter is small and poorly developed. The ventral atrium is shallow. The eggs are operculate and light-green, with small granules scattered in the yolk.

4. DISCUSSION

Systematic studies on paramphistome infections in sheep and goats have shown that sheep are better host than goats [11, 18, and 24]. Our study showed the same trend of sheep being better hosts than goats. The drinking points for sheep and goats are the same such that they are equally exposed to water borne parasites. Goats develop better immunity than sheep which also accounts for the differences in the parasite loads between them as shown by our studies [11, 12].

In general more sheep were infected with paramphistomes than goat from the same area. The presence of smaller dams and ponds as sources of drinking water can lead to severe infections during periods when the water level are getting low. This is because the number of cercaria per unit volume of water becomes more and thus cattle take in high doses of parasites [17].

The genital atrium was the most reliable for identifying the parasites to species level as previously documented [4]. Although no disease symptoms were observed the lesions in the affected areas could lead to opportunistic bacterial infections. While the number of parasites per herd was relatively low, it should be noted that the percentage take of paramphistomes is less than 20% [12, 17]. Thus these seemingly low figures of less than 1000 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 sub mucosa away from the harsh conditions in the gut lumen. The fluctuations of numbers among individual animals could arise as a result of some flocks drinking from drying water bodies where the levels of cercaria always tend to increase per unit volume of water [17]. For effective diagnosis and control of parasitic diseases, it is essential that parasite isolates be accurately identified by method that is simple and reproducible [15]. In our study we have positively identified three species.

C. microbothrium was identified based on the histology of the structures of the acetabulum pharynx and the genital atrium, which are in agreement with earlier descriptions [4, 12, 21, and 26]. In identifying paramphistome at species level several workers used the parameters as we have presented in Table 1 and showed that minor variations in measurements in fixed specimens do occur [4, 5, 10, 12, 13, 20, and 22]. It seems that *C. microbothrium* is the

most frequently reported ruminant paramphistome in most parts of Africa in view of the fact that it has been recovered from all places where studies on ruminant paramphistomes have been made [4,6,10,12,16,19,21,22,23,25]. The present study shows it is the most frequently encountered paramphistome in goats and sheep from Gwanda district and this should be a cause for concern because it has resulted in outbreaks of paramphistomiasis in the same animals elsewhere [11, 12, 18, and 24].

Chronic infections with paramphistomes can result in loss of, weight, milk production and plasma proteins [12]. The results of our study point to the fact paramphistome infections should not be ignored as untold loses due to them could be occurring which have not yet been evaluated.

C. clavula, which resembles *C. microbothrium* in many respects, was identified based on the histology of the genital atrium, which are peculiar to this species, which is in agreement with previous descriptions [6,16,26] *Ceylonocotyle dicranocoelium*, which is being reported in Zimbabwe for the first time, was confirmed by its characteristic pharynx and acetabulum, which correspond to earlier descriptions [6,16,26]. While no report of outbreaks among goats and sheep have not been received to date within the district, it is suggested the watering of live stock be organized in a way that would reduce incidence of paramphistomes.

There is also the probability that various species could be infecting the same snails *in* which case they could affect development in the snail synergistically or antagonistically as was demonstrated for *Paramphistomum daubneyi* and *Fasciola hepatica* [1].

Acknowledgements:
NUST Research Board funded this work. R. Dube and M. Dube, both technical staff, assisted in the collection of samples.

5. REFERENCES

[1] Abrous, M., Ronde, D. and Dreyfuss, G. (1996). *Parmphistomum daubneyi* and *Fasciola hepatica* the effect of dual infection on prevalence and cercarial shedding in preadult *Lymnaea glabra*. *The Journal of Parasitology* **82** (6): pp1026-1029

[2] Boray, J. C. (1969) studies on intestinal Paramphistomosis in sheep due to *Paramphistomum ichikawai* Fukui, 1922. *Veterinary Medical Review*. **4**: 290 – 308

[3] Buttler, R. W. and Yeoman, G.H. (1962). Acute intestinal paramphistomiasis in Zebu cattle in Tanganyika. *Vet. Rec*. **74**. 227 - 231.

[4] Dinnik, J.A. (1964). Paramphistomum *sukumum* sp. nov. and other stomach flukes from cattle in the Sukumaland areas of the Lake Region, Tanganyika. *Parasit*. **54**: 201 - 209.

[5] Durie, P.H. (1951). The Paramphistome (Trematoda) of Australian ruminants 1. *The systematics. Proc. Linn. Soc. N. S. W. Australia* **76**: 41 - 48.

[6] Eduardo, S. L. (1983). The taxonomy of the family Paramphistomidae Fischioeder, 1901 with special reference to the morphology of species occurring in ruminants. III. Revision of the genus *Calicophoron* Nasmark.1937. *Systematic Parasitology*. **5***: 25-79.*

[7] Eduardo, S. L. (1982a). The taxonomy of the family Paramphistomidae Fischoeder, 1901 morphology of
species occurring in ruminants. II.Revision of the genus *Paraniphistomum* Fischoeder, 1901. Systematic *Parasitology* .**4**:189-238.

[8] Eduardo, S. L. (1982b). The taxonomy of the family Paramphistomidae Fischoeder, 1901 morphology of species occurring in ruminants. I General considerations. Fischoeder, 1901. Systematic *Parasitology* .**4**:189-238.

[9] Frandsen, F and McCullough, F. (1980) Practical *guide to the identification of African fresh water snails*. pp. 12. Danish Bilharziasis Laboratory. Denmark.

[10] Gretillat, S. (1960). Amphistomes (Trematodes) des ruminants demestiques de la Republique du Tchad description d'un Gastrothylacidas nouveau *Carmyerius graberi* n. sp. *Ann. Parasit. Hum*. Com. **35** (4): 509 – 527.

[11] Horak, 1. G. (1967). Host parasite relationships of Paramphistomum *microbothrium* in experimentally infested ruminants with particular reference to sheep. *Ondestepoort Journal of Veterinary Research* **34**: 451-540.

[12] Horak, I. G. (1971). Paramphistomiasis of domestic ruminants. *Advances in Parasitology*. **9**: 33 - 70. Academic Press London, England. New York U. S. A.

[13] Lee, S. K. and Lowe, C. Y. (1971). Comparative histological and anatomical studies on amphistomes (Trematoda) from Malayan - Thai buffaloes and Malayan cattle. *Zool. Anz*. **187**(0.5): 25 - 61.

[14] Mahoney, R. (1973). *Laboratory Techniques in Zoology*. Second Edition London Butterworths.

[15] McManus, D.P. and Bowels, J. (1996). Molecular genetic approaches to parasite identification: their value diagnostic parasitology and systematics. *International Journal for Parasitology.* **26** (7): pp 687-704

[16] Nasmark, K.E. (1937). A revision of the tramatode family Paramphistomidae. *Zool. Bidr. Uppsala*. **16**: 301 - 565.

[17] Rolfe, P.F., Boray, J.C., Nichols, P. and Collins, G.H. (1991). Epidemiology of Paramphistomosis in cattle. *International Journal for Parasitology*. **21** (7): 813 - 819

[18] Rolfe, P.F. Boray, J. C. and Collins, G.H. (1994). Pathology of infection with Paramphistomum ichikawai in sheep. *International journal for Parasitology* 24(7): 995-1004.

[19] Round, M.C. (1968). *Checklist of the Helminthes Parasites of African mammals*. Commonw. Agric. Bereaux. Pp 8 - 19.

[20] Sey, O. (1975). Histological examination on the muscular organs of some amphistomes. (Trematoda: Paramphistomata) *Parasit. Hung*. **8**: 55 - 59.

[21] Sey, O. (1976). Studies on the stomach Flukes of Buffalo in Egypt (Trematoda: Paramphistomata). *Follia Parasit*. **23**: 237 - 242.

[22] Sey, O. (1977). Examination of Amphistomes (Trematoda: Paramphistomata) Parasitizing Egyptian ruminants. *Parasit. Hung*. **10**: 27 - 50.

[23] Sey, O. (1980). Re-examination of an amphistome (Trematoda) collection deposited in the Geneva Museum with a description of *Orthocoelium saccocoelium* sp. n. *Revue Suisse Zool*. **87**: 431 - 437.

[24] Singh, R. P., Sahai, B. N. & Jha, G. 1. (1984). Histopathology of the duodenum and rumen during experimental infections with *Paraniphistomum cervi: Veterinary Parasitology*. **15***:* 39-46.

[25] Swart, P.J. (1954). The identity of so-called *Paramphistomum cervi* and *P. exp1anatum* two common species of ruminant trematodes in South Africa**.** *Ondestepoort Journal of Veterinary Research***. 26**: 463 **-** 473.

[26] Yamaguti, S. (1971) Synopsis of digenetic trematodes of vertebrates. **Vols. I & II**. Tokyo: Keigaku Publishing Co. **1**: 285-293, **2**:695-714.