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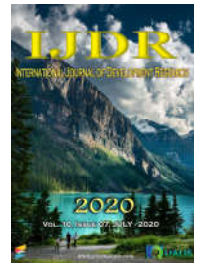
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RESEARCH ARTICLE

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MORPHOMETRIC ANALYSIS OF PARAMPHISTOMES COLLECTED FROM THE RUMEN WALL OF INFECTED GOATS IN KOLKATA, WEST BENGAL

Sutapa Datta^{1*} and Sabana Yeasmin²

¹Assistant Professor, Parasitology and Immunology laboratory, PG Department of Zoology, Bethune College, Kolkata, 700 006, West Bengal, India; ²M.Sc, Parasitology and Immunology laboratory, PG Department of Zoology, Bethune College, Kolkata, 700 006, India

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*Corresponding author: Sutapa Datta

ABSTRACT

The digenetic trematodes platyhelminth (flatworm) parasites (platyhelminth: Trematoda-Digenea) are pear-shaped, thick, fleshy worms, especially prevalent in the reticulum and rumen, while immature parasites in the small intestine mainly in the duodenum. Paramphistomes are responsible for "Paramphistomosis" which is a gastrointestinal disease characterized by low milk production associated with gastroenteritis with high morbidity and mortality rates in domesticated and wild ruminants, which causes prime economic losses to the Livestock industry to the tune of several thousand crores of rupees annually. The accurate identification of Paramphistomes is much needed for defining pathogenicity, treatment and disease control strategies. Not all paramphistomes species are responsible for disease in Livestock. It is 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. Although various paramphistomes have been incriminated as etiological agents of paramphistomosis, here only *Paramphistomum cervi* and *Cotylophoron cotylophorum* were extensively studied in this regards.

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INTRODUCTION

The digenetic trematodes platyhelminth (flatworm) parasites (platyhelminth: Trematoda -Digenea) are pear-shaped, thick, fleshy worms, characterized by two distinct suckers, one of which is posterior sucker, (acetabulum) for attachment and the other is aboral sucker consists of muscular pharynx. Their genital pore pre-equatorial and the ovary usually post testicular, and has di-heteroxenous life cycle, which require an intermediate host for completion. Several species are found in fish, amphibians, reptiles, birds and mammals. Here in our work, we have considered the Genus-*Paramphistomum*, which belongs to the family paramphistomidae (Nasmark, 1937). *Paramphistomum* is considered to be one of the most important species of paramphistomes and are parasitic in the alimentary canal of many ruminants around the world (Dinnik, 1962). Mature parasites are especially prevalent in the reticulum and rumen (Fig.1), while immature parasites in the small intestine mainly in the duodenum (Eduardo, 1982a). Immature migrating parasites 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 which results in anorexia, polydypsia, profuse foetid diarrhoea, drop in plasma protein concentration and anaemia, which weaken the host.

Mature paramphistomes are also responsible for rumenitis, irregular rumination, unthriftiness, lower nutrition conversion and loss of body condition. (Urquhart *et al.*, 1996). Paramphistomes are responsible for "Paramphistomosis" i.e. gastrointestinal parasitic disease which causes prime economic losses to the Livestock industry to the tune of several thousand crores of rupees annually. It has been a neglected trematode infectious disease in ruminants; but has recently come out as a significant cause of productivity loss in wool, meat and milk production. Distribution of paramphistomosis is worldwide, but the highest prevalence have been accounted in tropical and subtropical regions, particularly in Africa, Asia, Australia and Eastern Europe. (Nasmark, 1937). The present study is conducted to identify two different species of paramphistomes by using the technique include morphometric analysis of whole mount flattened specimens.

MATERIALS AND METHODS

Adult flukes were collected from local abattoir and kept in freshly prepared Phosphate buffer saline (PBS, pH – 7.6; Fig 2). For fixation and stretching of live trematodes Alcohol, Formaldehyde, Acetic acid mixture was used. Different alcohol grades were used for dehydration followed by staining with Semichon'scarmin (1%). After mounting the stained stretched trematode were studied under microscope with the help of Stage and Ocular Micrometer.

RESULTS

The number of parasites per infected animal ranged between 100 to 1000, were found in the folds of rumen and between papillae in the reticulum, where they adhere to knobbed parts of the mucosa. Two distinct species from the family paramphistomidae, Fischoder, 1901 were recovered in the rumen of infected goats, which were identified as *Paramphistomum cervi* (Swart, 1954; Jha *et al.*, 1984) and *Cotylophoron cotylophorum*. The morphometric analysis of the recovered species were done by using an eye piece micrometer and all measurements are taken in millimeter (mm).

Description

- Body is conical and measures 5.04 mm long and 1.89 mm maximum breadth, concave ventrally and convex dorsally. Surface tegument is marked by well-defined transverse wrinkles, sometimes anteriorly provided with small dome-shaped papillae, more numerous in circumoral region.
- Acetabulum is sub-terminal, muscular and measures about 0.81mm in diameter. The ratio of acetabulum to body length is 1:6.22.
- Pharynx (oral sucker) is of the Liorchis type, with or without papillae and measures 0.45mm in length. The ratio of pharynx to body length is 1:11.2 (Fig 3).
- Oesophagus is small about 0.57mm long and bifurcates into two caeca that run along both sides of the body.
- Testis slightly lobed and measure about 0.54mm in length and 0.69 mm in wide, situated in the mid-third of the body (Fig.4).
- Genital atrium is not clearly visible due to presence of diverticulum, arise from the caecal bifurcation. Ovary is spheroid to ovoid, post testicular, i.e. situated between the testis and the margin of acetabulum and measure about 0.37mm in diameter.

Comparing the data with the available reference data as tabulated in table 1 the specimen seems to be *Paramphistomum cervi*. The identification was done according to Zeder, 1790.

Description

- The body is conical, bending ventrally and measures 6.3 mm in length and 2.04 mm in breadth.
- Acetabulum is sub-terminal and absence of dorsal and ventral exterior circular muscle and measures about 1.32 mm in diameter. The ratio of acetabulum to body length is 1:4.77.
- Pharynx is calicophoron type, lacks diverticulum and measures about 0.57 mm in diameter which leads to oesophagus. The ratio of pharynx to body length is 1:11.05.
- The oesophagus is shorter than pharynx and made two layers, an outer circular layer measures 0.51 mm in diameter and an inner longitudinal layers, and leads into two caeca that run along both sides of the body.
- The testes are situated diagonally in the mid-third of the body. They are deeply lobed. Anterior testis measures 0.78 mm in length and 0.96 mm in width, while posterior 0.72 mm in length and 0.93 mm in width.
- Genital atrium is of cotylophoron type surrounded by muscularized genital sucker and measured about 0.12 mm in diameter, opens at the end of first-third of the body.
- Ovary and Mehlis gland lie adjacent to each other between the posterior testis and the acetabulum towards the dorsal side, spheroid to ovoid and measured about 0.48 mm in diameter.

Comparing the data with the available reference data as tabulated in table 2 the specimen seems to be *Cotylophoron cotylophorum*. The identification was done according to Romero *et al.*, 2009.

Table-1. Measurements in millimeters (mm) for diagnostic morphological characters in the flattened whole mount of paramphistomes from rumen wall of goat

Description	Reference Slide	Slide-A1	Slide-B1
Body length	5-12	9.42	6.09
Body breadth	2-3	2.58	2.13
Acetabulum diameter	2	2.04	1.2
Ratio of acetabulum diameter to body length	1:4.4-1:6	1:4.61	1:5.07
Pharynx length	0.84	0.87	0.51
Ratio of pharynx length to body length	1:10.5	1:10.82	1:11.94
Oesophagus length	0.777	0.69	0.48
Anterior testis length	1.2	1.14	1.14
Anterior testis breadth	2	1.26	1.32
Posterior testis length	0.98	0.96	0.96
Posterior testis breadth	-	1.23	1.05
Ovary diameter	-	0.72	0.65
	<i>Paramphistomum cervi</i> , According to Zeder, 1790	Hence the specimen seems to be <i>P. cervi</i>	

Table 2. Measurements in millimeters (mm) for diagnostic morphological characters in the flattened whole mount of paramphistomes of goat from rumen wall

Description	Reference Slide	Slide-A2	Slide-B2
Body length	6.02-8.36	6.3	7.17
Body breadth	2.02-2.61	2.04	2.34
Acetabulum diameter	1.50-1.70	1.32	1.32
Ratio of acetabulum diameter to body length	1:4.1-1:4.9	1:4.77	1:5.43
Pharynx length	0.64-0.84	0.57	0.6
Ratio of pharynx length to body length	1:9.7-1:11.2	0.51	1:11.95
Oesophagus length	0.57-0.81	0.51	0.57
Anterior testis length	0.98-1.20	0.78	1.26
Anterior testis breadth	1.00-1.36	0.96	1.44
Posterior testis length	0.80-0.91	0.72	1.17
Posterior testis breadth	1.50-1.75	0.93	1.29
Ovary diameter	0.45-0.61	0.48	0.45
	<i>Cotylophoron cotylophorum</i> , According to Romero <i>et al.</i> 2009	Hence the specimen seems to be <i>C. cotylothorum</i>	

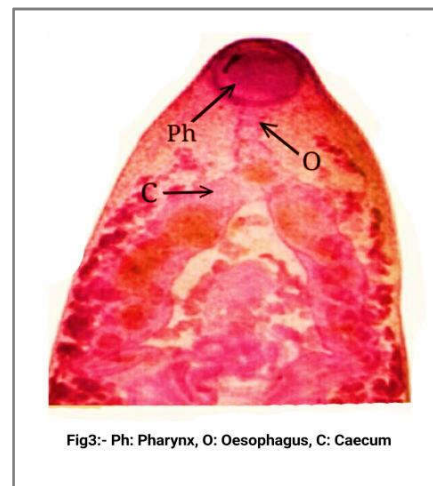


Fig3:- Ph: Pharynx, O: Oesophagus, C: Caecum

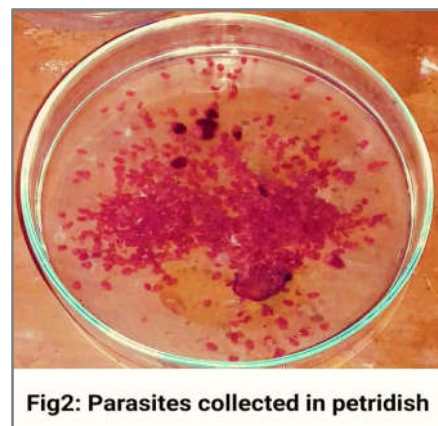


Fig2: Parasites collected in petridish

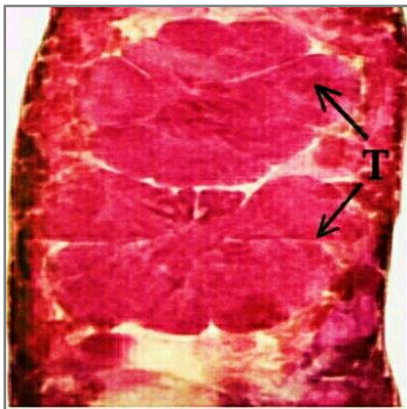


Fig4:- T: Testis

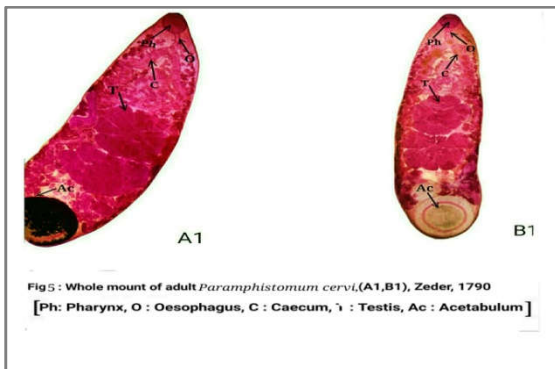


Fig5: Whole mount of adult *Paramphistomum cervi*(A1,B1), Zeder, 1790
[Ph: Pharynx, O : Oesophagus, C : Caecum, T : Testis, Ac : Acetabulum]

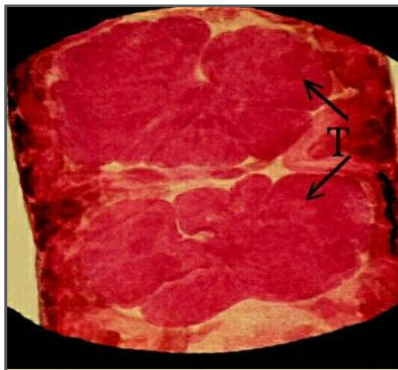


Fig6:- T: Testis

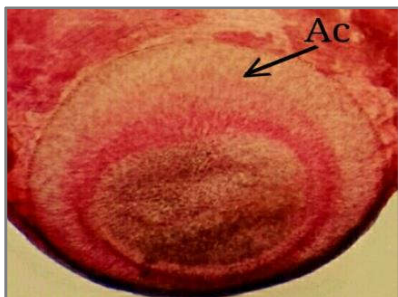


Fig7:- Ac: Acetabulum

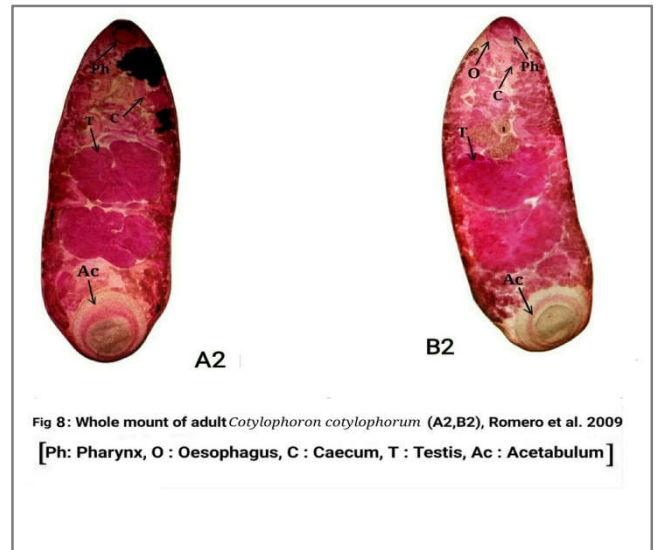


Fig 8: Whole mount of adult *Cotylophoron cotylophorum* (A2,B2), Romero et al. 2009
[Ph: Pharynx, O : Oesophagus, C : Caecum, T : Testis, Ac : Acetabulum]

taxonomic characteristics (pharynx, acetabulum, testis, ovary, genital atrium) in different paramphistome species (Sey O, Eslami, 1981-82; Rounud, 1968). Paramphistomes are responsible for paramphistomosis i.e gastrointestinal parasitic disease in domesticated animals, which cause heavy economic losses to the livestock industry to the tune of several thousand crores of rupees annually. According to Eduardo (1982), the occurrence, distribution and shape of the tegumental papillae have an important taxonomic value and can be employed to support other characteristics in the specifics identification of some mammalian amphistomes. The pharynx of *C. cotylophorum* as a highly muscularised organ following the oral opening, with no mention of an oral sucker, referred to as musculature pharynx as a oral sucker surrounding the mouth opening. Several authors agreed that the genital atrium which was redescribed later as terminal genitalium, is the most valid characteristics used in differentiating species belonging to paramphistomidae (Dube et al, 2005). Nasmark (1937), Yamaguti (1971) agree with the description for the genus *Paramphistomum* on the basis of body shape, the ratio of the acetabulum diameter to body length, the position and shape of the acetabulum, ovary, testes and genital pore. For effective diagnosis and control of parasitic disease, parasite isolation and identification is very essential. In this study, two species of paramphistomes were identified on the basis of shape, structure and relative position of organs with valid taxonomic characteristics.

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DISCUSSION

The study was undertaken for morphometric comparison of adult paramphistomes, to be the common cause of paramphistomosis in infected goats. This study has helped to understand the shape, structure and relative position of different organs with valid

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