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Studies on Larval Digenetic Trematodes of Godavari River, Gangapur Project : Xiphidiocercariae

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ABSTRACT

The earlier work on the snails and cercariae of this region was carried out by Karyakarte and Yadav(1974 to 1979 under PL-480 Project on "Control of Molluscan Agents of Helminth Parasites of Agricultural and Veterinary Importance" (Project No. A 7-ADP-39). The account of their finding was published in the year 1981. They examined 8 species of snails viz , Viviparous bengalensis, Melanoids tuberculatus, Melanus scabra, Lymnea acumulata , Lymnea luteola, Lymnea auricularia, Indoplanorbis exustus, Anisus (Gyrulus) convexiusculus. Out of these 8 species, they reported cercarial infection in 6 species of snails and 2 species, M. scabra and V. bengalensis were free from larval infection. Their work included description of 11 species of fresh water cercariae belonging to Monostome, Amphistome and Distome groups. The work on larval trematodes was further continued in this region in the Trematology Laboratory. Present paper deals with two species of freshwater cercariae which belong to Xiphidio cercariae. The group xiphidio cercariae having a piercing spine or stylet in the oral region is represented here with two different types of cercariae. The cercariae are abundant during the period between December and May in the hepatopancreas and gonads of the snails occurring in Nashik region. The collection is mainly from the snails Lymnea Luteola and Melania tuberculata The snails were collected at Gangapur project (Water reservoir) Godavari river, Darna river, Waldevi river, Girnare, ponds and ditches around Godavari river. The cellulosa group is represented hereby two cercariae a known Cercariae indicae LVII sewell, 1922 and a new Cercaria disciforma n.sp.

Keywords: Monostome, Distome, Amphistome, Trematology, Cellulosa group.

I. INTRODUCTION

Luhe (1990) made the first attempt to classify the cercariae in a comprehensive manner. He classifies various cercariae into five different groups. The groups were Monostome cercariae, Distome cercariae, Amphistome cercariae. Lophocercous cercariae, Gastrostome cercariae. Labour (1911) made a survey of British marine cercariae and divided into two main groups Gastrostomata and Prosostomata. Cort (1914) made a survey of larval trematodes from Norh American freshwatrer snails Faust (1919b,1921,1924,1926) studies larval trematodes from South Africa and China. Miller (1936) made a comparative account of Furcocercus cercariae and in 1936 he studied the North American cercariae. Sewell (1922) studied the freshwater cercariae from India and he modified the Luhe's classification and divided major groups into number of smaller groups. Porter (1938) studied the freshwater Larval trematodes found in certain South American Mollusca. While classifying the cercariae he followed the ideas of Luhe (1909) and Sewell (1922). Soparkar (1921) gave a note on some Furcocercuos cercariae from Bombay. Chandler (1953) gave a key to the Furcocercuos cercariae. Khan (1960 to 1961) studied larval trematodes infecting

freshwater snails in London and some adjoining area. Nasir (1964) gave a key to the cercariae from British freshwater Molluscs. In 1972 he gave some aspect of Xiphidocercarial classification and in 1973 he reported twenty new species of Venezuelan cercariae, Ito et al(1977) study on the freshwater cercarial in Leyte Island, Philippines. After Sewell, few workers have described some cercariae from India (Singh 1952, P, Srivastava 1958, Malaki and Singh 1962, Gupta and Taneja 1970, and 1970a, Mohandas 1977 and 1979, Karyakarte and Yadav 1981, A Farahank 2006, 2007, Nkwengulila 1998, Gulam M.A. 2011, Eric 2005, Shimura 1980, Oleg Ditrich 1997, Sami Bdir 2011, Sey 2003, Todd 2004, Thapana 2011, Uthpala 2010,),Karkaykarte & Yadav
1981) Present paper deals with two species of freshwater cercariae which belong to Xiphidiocercariae. The classification followed in the paper is of Luhe (1909), Sewell (1922) and Porter (1938).

II. METHODS AND MATERIAL

(1) Collection and maintenance of snails:

Studies on cercariae commenced with collection of first intermediate host (snails). They were collected either hand picked or dragging a net through water and were transported to the laboratory. The snails were then transferred to glass water bowls and well aerated acquaria already provided with a rich water plants such as Vallisneria, Hydrilla, Chara, Spiirgyra and fimbria etc. After a short period of acclimatization the snails were transferred to individual test tubes kept on wooden rocks in order to detect the cercariae. In the laboratory most preferably the same pond water was used for the snails from which they were collected as the purified tap water supplied to the laboratory proved unsuitable perhaps due to chemical purification

(2) Observations:

The snails collected were kept under observation for some time. The snails which are fully grown showed larval infection while the young ones were normally free from larval infection. Due to the infection, it was observed that the snails grow in size and show a phenomenon of gigantism. Many a time the shell grow enormously and ballooning was observed.

For the study of cercariae heavily infected snails were selected. Two methods were followed for the morphological observations.

1) Natural emerging method
2) Crushing method.

1) In natural emerging method the snails (2to3 at a time) were kept in separate test tubes. This was a constant source of living cercariae naturally emerging from the snails. The sunlight and artificial light play an important positive role in stimulating th emergence of cercariae . It was observed that some cercaraie emerge only in darkness.

2) Crushing method

This method of investigation of cercariae found suitable for morphological observation on various developmental stages such as sporocysts and rediae. This quick method was useful for studying the seasonal percentage of infection of cercariae.

The cercariae collected were subjected to various artificial methods for the study of various internal structures.

3) Movement relaxation :

Sometimes cercariae were found to be so active that observation under power was impossible without some method interfering with or controlling their

movement. Hence dilute solutions of gum, starch, gelatin were used to slow down their movements.

4) Vital stains :

For the study of structural details in live condition vital stains were used such as Neutral red, Methyl green, Nile blue, Azur II and Nile blue sulphate.
For the study of flame cells Indian ink and Amphibian ringer solution were found to be suitable.
For the preparation of permanent mounts the cercariae were fixed in 1% hot formalin, stained in Delafieid's haematoxylin, cleared in clove oil and mounted in D.P.X.

(5) Measurements :

Most of the specimens were measured in live state. In the preset work the measurements given for two species of cercariae and their parthenitae represent averages of twenty specimens of each species. The diagrams have been made with the aid of a camera lucida. Sketches were drawn at different magnification using oil immersion objective if necessary. This method gave the most uniform results.

All the measurements are in millimeters.

The most suitable time making the diagrams for morphological study of living cercariae was immediately after they emerged from the snails without vital staining otherwise became opaque after remaining in water for half an hour.

Responses :
The responses of cercariae to various stimuli were studied in the laboratory conditions at temperature 28®C
(A) For the study of phototaxis a glass apparatus was fabricated and used. The cercariae allowed to move into four limbs of the apparatus. Three limbs were subjected to various light intensities and fourth the dark one.
(B) For geotaxis U tube was used.
(C) Emergence of cercariae was concluded after series of such observations.

I) XI PHIDIOCERCARIAE

CER CAR IAE MICROCOTYLAE CELLULOSA CROUP
I) CERCAR IAE INDICAE LVII SEWELL , 1922

In the present work xiphidiocercariae are represented by two species. These cercariae were collected from the snail host Lymnea luteola and Melania scabra. The snails collected at Gangapur Project, Godavari river, Darna River, Waldevi river and nearby places. As indicated in collection data of snails and cercariae it is evident that the larval forms of Cercariae indicae LVII Sewell, 1922 are abundant during the month from December to May for both years 2015 and 2016. Two cercariae belonging to Cercariae Microcotyle group of Cellulosa in Xiphidiocercariae are described here. The Cellulosa group is represented here by two cercariae, a known Cercariae indicae LVII Sewell, 1922 and a new Cercaria disciforma n.sp.

COLLECTION DATA

1) Cercariae indicae LVII Sewell, 1922

Percentage of infection during the years 2014 and 2015

Percentage of infection (Mean) = 4.16

The cercaria is greenish in colour with spinose cuticle. The eye spots are absent. It is an active swimmer and has a tendency to swim at the bottom. The main body of cercaria is oval. The tail is two times longer th. The cercaria measures 0.34 (0.30 to 0.38) in total length (main body 0.11 X (0.09 to 0.13) and Tall

is 0.23 (0.20 to 0.26). The breadth of main body Is 0.08 (0.05 to 0.11) and that of tail is 0.03 (0.02 to 0.04). The oral sucker Is bigger than acetabulum, subterminally located and measures 0.03 (0.03 to 0.04) in length and 0.04 (0.03 to 0.05) in breadth. The stylet is pointed and measures 0.03 (0.02 to 0.04) in length. The ventral sucker is located in the posterior half of the body. Its anterior margin just touches the equator. It is 0.02 (0.01 to 0.03) long and 0.03 (0.02 to 0.04) wide. The mouth leads into a very short prepharynx which is turn opens into a pharynx which is muscular and measures 0.010 (0.007 to 0.013) in length. Oesophagus and intestinal caeca are totally wanting. The cercaria is typical in haying two pairs of salivary glands. The glands are dimorphic and differ in their location. One pair (smaller) is located in between oral and ventral suckers. The other pair (larger) is spindle shaped end extends in the postacetabular region in between ventral sucker and excretory pore. Both the ducts open slightly anterior to mouth.

The excretory bladder is spindle shaped and the excretory tubules branch marginally in the acetabular region into anterior and posterior sub-branches. The flame cells could not be traced. The caudal excretory duct opens at the tip of the tail.

Sr. NO.	Month	Locality	No.of snails examined	No,of snails infected	% of infec-tion
1	January 2015	Gangapur Project Girnare Godavari River Waldevi River	513	45	8.96
2	February 2015	-do-	215	21	9.76
3	March 2015	-do-	232	27	11.63
4	April 2015	-do-	292	38	13.01
5	May 2015	-do-	345	51	14.78
6	June 2015	-do-	-	-	-
7	July 2015	-do-	-	-	-
8	August 2015	-do-	-	-	-
9	September 2015	-do-	-	-	-
10	October 2015	-do-	-	-	-

SPOROCYST :

The sporocyst is oval, containing 3.0 - 11 mature cercariae at a time. It is greenish in colour and measures 0.38 (o.31 to 0.45) in length and 0.17 (0.14 to 0.20) in width. The wall of the sporocyst is dotted over with a number of irregularly distributed orange granules.

RESPONSES :

(1) Phototaxis Negative
(2) Geotaxis Positive

11	November 2015	-do-	-	-	-
12	December 2015	-do-	12	6	2.83
13	January 2016	-do-	425	17	4.00
14	February 2016	-do-	293	15	5.11
15	March 2016	-do-	410	29	7.07
16	April 2016	-do-	340	31	9.11
17	May 2016	-do-	312	34	10.89
18	June 2016	-do-	-	-	-
	July 2016	-do-	-	-	-
20	August 2016	-do-	-	-	-
21	September 2016	-do-	-	-	-
22	October 2016	-do-	-	-	-
23	November 2016	-do-	-	-	-
24	December 2016	-do-	255	7	2.74
	Annual Percentage of infection 2016	Total	2035	133	3.24

III. DISCUSSION

As the oral sucker Is provided with a stylet, the present form belongs to Xiphldlocercaria In Leptoceroous group.

In possessing undivided tail, main body less than 0.20 acetabulum smaller than the oral sucker and located in the posterior region of the body, salivary glands two pairs confined in the pre-acetabular region the present form belongs to Microcotyle group - Luhe 1909 of Xlphldiocercariae. Amongst Mlcrocotyle group it falls in with the members of sub- group Cellulosa as It possess two pairs of salivary glands.

In Cellulose group the present form resembles Cercaraie Indicae LVII Sewell, 1922. As the present form Is reported from Lymnea luteola a new host record and also from a different local It y# it was thought advisable to report it here.

Host : Lymnea luteola
Habit : Hepatopencreas and gonads
Locality : Gangapur Project , Girnare Godavari River, Waldevi River, Nashik District, Maharashtra State India.

2) Cerearia disciforma n.sp

This cercaria, belonging to XiphIdlocercaria group

Sr No.	Months	Locality	No.of snails examined	No, of snails infected	% of infec-tion
1	January 2015	Gangapur Project Girnare Godavari River Waldevi River	400	48	12.00
2	February 2015	-do-	325	26	8.00
3	March 2015	-do-	370	25	6.75
4	April 2015	-do-	380	22	5.78
5	May 2015	-do-	420	21	5.00
6	June 2015	-do-	420	16	3.80
7	July 2015	-do-	325	-	-
8	August 2015	-do-	312	-	-
9	September 2015	-do-	285	-	-

10	October 2015	-do-	415	-	-
11	November 2015	-do-	315	-	-
12	December 2015	-do-	295	-	-
	Annual Percentage of infection 2015	Total	4259	201	4.67
13	January 2016	-do-	292	33	11.30
14	February 2016	-do-	515	29	6.98
15	March 2016	-do-	400	24	6.00
16	April 2016	-do-	440	22	5.00
17	May 2016	-do-	310	12	3.87
18	June 2016	-do-	390	11	2.82
19	July 2016	-do-	200		
20	August 2016	-do-	472		-
21	September 2016	-do-	390	-	-
22	October 2016	-do-	360	-	-
23	November 2016	-do-	320	-	-
24	December 2016	-do-	272	38	13.97

was collected from the snail Melanla tuberculata The collection was observed in the hepatopancreas and also in gonads. The snails collected at Gangapur Project (Water Reservoir) Godavari river, Darna river, Waldevi river and near by places. The snails occur in Nashik region 2012 and 2013 but the infection was found from Decemeber to June during both the years and 2012 and 2013.

3) Collection data
4) Percentage of infection during the years 2015 &

in having a length of 0.05 (0.04 to 0.06) and width of 0.011 (0.010 to 0.012) with a pointed anterior end. It shows small thickening about one third the length from the anterior end. The ventral sucker is in the posterior part of the body, smaller than the oral and measures 0.03 (0.02 to 0.04) in diameter. The ratio between width of ventral to oral sucker is 1: 1.6. The mouth is ventral and leads into a pharynx which measures 0.02 (0.01 to 0.03) in length and 0.03 (0.02 to 0.04) in width. There is no trace of oesophagus and intestinal caeca. There are two pairs of salivary glands. The outer pair is larger than the inner one. The glands are confined in the preacetabular region and open near the mouth with the help of narrow ducts.

The excretory bladder is Y-shaped. The protonephridial tubules are coiled and at the tip of them fairly prominent flame cells are observed. The caudal excretory canal is present in the tail but flame cells are not observed.

SPOROCYST

The sporocyst is yellowsih in colour and oval in shape with limited number of mature cercarie (Four at a time). It measures 0.41 (o.39 to 0.43) in length and 0.25 (0.23 tfc 0.27) in width. The wall of the sporocyst is dotted over with a number of small round retractile granules

DISCUSSION

The present Xiphidiooercaria reported from Melania tuberculata belongs to Cercariae microcotylae group, the characters being long and undivided tail, body length less than 0.20 and acetabulum smaller than the oral sucker lying behind the middle of the body.

As there are only two pairs of salivary glands, it is included in the cellulose group From India only two species are reported in Cellulosa group namely Cercariae indicae

LVII Sewell, 1922 and Cercaria naukuchlensis Malaki and Singh, 1962.

Amongst these two forms the present form shows affinities with C. indicae; LVII Sewell, 1922.

C. indicae; LVII and the present form have same type of stylet, oral sucker larger than the acetabulum and same type of excretory bladder. Close examination, however, shows that the present form is quite different from the known one.

The body is oval in C. indicae LVII and rounded in the present form.

The acetabulum is located very near to the equator in the known form and away from it by a considerable distance in the new form. Both the pairs of salivary glands are preacetabular in the form under discussion whereas one pair is preacetabular and other paracetabular in the form described by Sewell.

The excretory system differs into two. In the known form there are eight flame cells whereas, in the present form the excretory tubule terminates into single flame cell. Thus there are only two flame cells in the present form.

The known form is reported from Melanoides tuberculatus from a tank, Indian Museum, Calcutta whereas, the new form from MeIania tuberculata at Gangapur Project, Godavari river, Nashik. Maharashtra State, India.

A new species is established and named as Cerceria disciforman.sp.

Host : Melania tuberculata

Habitat : Hepatopancreas and gonads

Locality : Gangapur Project (Water Reservoir) Girnare,Godavari River Waldevi River

Dharna river , Girnare District Nashik Maharashtra State, India.

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