

BRC 2001078/14410

## Excystment of the metacercaria of the trematode *Clinostomum marginatum*

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(Received May 9, 2001)

**ABSTRACT:** The *metacercaria* of *Clinostomum marginatum*, a trematode parasite of heron and other fish eating birds excysted in chicken and toad bile and human urine. Excystment occurs at 32°C in Chicken bile, 31°C in toad bile and at 33°C and 35°C in human urine. Temperature less than 30°C do not cause excystment in all the media. Excystment in *C. marginatum* involves activation of the metacercaria by the media and its emergence through a hole created at one end of its spherical or ovoid wall. The rest of the cyst wall remains intact after the emergence of the metacercaria.

**Key words:** Metacercaria; Bile; Human urine; Heron; Excystment; *Clinostomum marginatum*.

### Introduction

*Clinostomum marginatum* is a common digenetic trematode having a cosmopolitan distribution with definitive hosts being several species of herons and other fish eating birds. Hazen and Esch (1978) through studies carried out on the ecology of *clinostomum marginatum* in the large mouth of Bass fish, noted that the percentage infection by metacercaria could not be related to the body condition or length of the fish.

According to Soulsby (1978) and Adekunle (1989), the body of the metacercaria worm particularly that of *Clinostomum marginatum* is dorso – ventrally flattened with no segmentation.

Krull (1934), and Hunter and Hunter (1934; 1935), observed that the *cercaria* of *Clinostomum marginatum*, is forked-tailed and penetrates the fish which is the immediate host where it develops into large metacercaria popularly known as ‘Yellow grub’. When infected fish are eaten by certain species of water bird, the yellow grubs are freed and they migrate to the oral cavity of the host where they attain sexual maturity in a few days.

Excystment of the metacercaria is considered to be an active process. Immediate activity of the *metacercariae* is commenced when any substance capable of bringing about excystment is added. The metacercaria rotates vigorously within the cyst wall and probes in all directions until a bulge appears on the wall. The anterior part of the metacercaria bursts through the bulge and escapes, leaving the cyst wall behind.

## Materials and Methods

Tilapia species consisting of *Sarotherodon melanotheron*, *Sarotherodon galilaeus*, *Oreochromis niloticus*, *Tilapia Zilli* and *Hemichromis fasciatus* were collected using dragnets from Eleyele Dam in Ibadan between January and April, 1998. The specimen were kept in a bucket of water to keep the fish alive. Some of the fish died while many remained alive. The fish specimen were autopsied and all organs, tissues and body musculature were scrutinized for the presence of *metacercariae* parasites. The *metacercariae* found all encysted were brought out using forceps and transferred into normal saline. Some *trematodes* cysts were released from their cyst wall and were immediately observed under light and dissecting microscope for observation of the nature of their movement and longevity. The *metacercariae* worms were stretched out on the slide by pressing gently with another transparent slides placed on the parasite to make the structures of the immature worm as distinct as possible.

Excystment of *metacercariae* of the *trematode* was observed in various media: Chicken bile, *tode* bile and human urine. Bile was extracted from gallbladders of freshly killed chickens and toads using a hypodermic syringe. Reactions of the meta-*cercaria* to various media were observed under the microscope for some minutes. Two to four metacercaria were put into the media inside the petri dishes at a time. All the media with the exception of the human urine were at room temperature.

## Results

All the media induced excystment by puncturingcyst wall while the metacercaria emerge. Water on the other hand act as control and did not bring about excystment. It has influence on the survival of both the encysted *metacercariae* and the excysted adult. Chicken bile brings about excystment of *clinostomum marginatum* at the minimum temperature of 32°C and at the maximum temperature of 33°C between 97 seconds and 124 seconds (Table 1).

Table 1: Excystment of Metacercariae of *clinostomum marginatum*

Temperature (°C)	Duration of Excystment (Second)
30.0	No Excystment
31.0	No Excystment
32.0	97
32.5	116
33.0	124

Toad bile brings about excystment at the minimum temperature of 31.9°C and at the maximum temperature of 32°C. The excystment occurred between 342 and 218 seconds. Excystment failed to occur at temperature below 31°C. Excysted parasite in toad bile in all cases, soon died in medium shortly after emergence (Table 2).

Table 2: Excystment of metacercaria of *C. marginatum* in toad bile.

Temperature (°C)	Duration of Excystment (Second)
30.0	No Excystment
31.9	342
32.0	218

Human urine brings about excystment at the minimum temperature of 33.5°C and at the maximum temperature of 52°C (Table 3). Human urine cooled to 28°C and 33°C did not cause excystment.

Table 3: Excystment of Metacercariae of *C. marginatum* in human urine.

Temperature (°C)	Duration of Excystment (Second)
28.0	No Excystment
32.0	No Excystment
33.5	72
35.0	69
36.0	77
52.0	87

With increasing temperature, the cyst took longer time to excyst in chicken bile and human urine while in the toad bile, excystment time became significantly shorter. In chicken bile and human urine, the higher the temperature, the higher the time of excystment of the *metacercariae*. However, in toad bile, the higher the temperature, the shorter the time of the emergence of the *metacercariae*. The details of *metacercariae* of *C. marginatum* removed from its cyst is shown in Fig. 1.

## Discussion

*Clinostomum marginatum* is a parasite of herons and other fish eating birds (Krull, 1934); Hunter and Hunter (1934 and 1935a). Therefore, finding encysted *metacercariae* on the body of fish, was not very surprising as this only aids its lifecycle. Excystment of the *metacercariae* in the definitive host must have to occur before parasites can attain maturity. The procedure for excystment was described by Fashuyi (1986), which involves “activation of the metacercari and its emergence through a hole created at one end of its spherical or ovoid wall”. This imply that excystment cannot occur without activation and activation should be triggered by the right medium.

The role of bile salts in the excystment of metacercaria conformed with the highlights of Oshima et al (1958). Excystment occurring at 32°C, 32.5°C and 33°C in 97, 116 and 124 seconds respectively (Table1) in chicken bile while it occurred at 31.9°C and 32°C in 342 and 218 seconds respectively.(Table 2) in the toad bile. However, excysted parasite in toad bile in all cases, soon died in the medium shortly after emergence. Human urine on the other hand, has been reported to contain substances like bile pigments, the enzyme diastase and ions which can act in conjunction at a temperature of 36°C to effected excystment of



Plate 1: Metacercaria of *Clinostomum marginatum*.

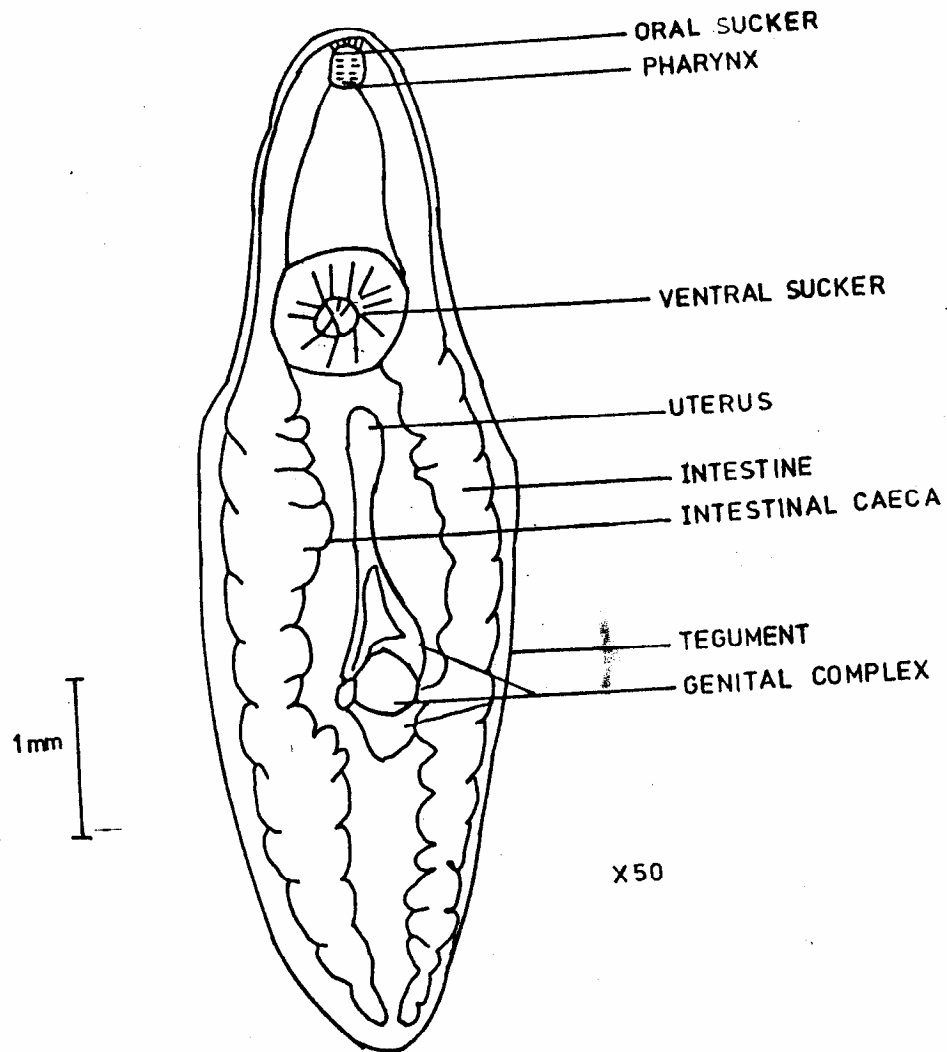


Fig. 1: Details of metacercaria of *Clinostomum marginatum* from its cyst.

*trematode metacercariae* (Fashuyi, 1986). Although excystment occurred at 33.5°C and 35°C in 72 and 69 seconds respectively, it also occurred at 36°C in 77 seconds. However, excystment did not occur in human urine cooled to 28 and 32°C (Table 3).

The role played by these bile salts was described by Dixon (1966) namely:

1. Increasing the permeability of enclosing membrane
2. Stimulating muscular movement and
3. Increasing the effects of enzymes secreted either by the parasite or host.

These work to create a hole in the cyst wall, from which the juvenile escape. Findings from this research showed that *Clinostomum marginatum* more or less uses cichlids as second intermediate hosts, as five different species were shown to harbour metacercariae. From this observation, it was shown that *S. galilaeus* and *T. zilli* harboured more cysts. From the observed findings, *C. marginatum* excysted in avian bile. This suggests that probable zone of excystment in the heron would be in the duodenum, from where it migrates to the oral cavity, where it becomes sexually matured. Although not a parasite of man, Chandler and Read (1961) reported that the *metacercariae* may cause an acute irritation of the throat from temporary attachment of worms eaten with raw food. This disease is also referred to as parasitic, *Laryngo-pharyngitis*.

Excystment in human urine incriminates it as a suitable medium. However, its chances of entering man is obsolete as eating raw fish is not a common feature in man. Investigation on the excystment of *clinostomum marginatum metacercariae* in human bile would definitely be rewarding but Herculean, hence may not be accomplished. In toad bile, excystment occurred but emergent worms soon died. This makes it convenient to suggest that *C. marginatum* in herons have been suggested. This would still call for further research to actually validate the proposed migratory route from the duodenum to the oral cavity as suggested from this research.

**ACKNOWLEDGEMENT:** The authors acknowledged Mr. M.O. Oniya of the Federal University of Technology, Akure, for his interest and love during this research.

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