

**OBSERVATION ON THE MORPHOLOGICAL CHARACTERS ON  
METACERCARIAE AND ADULTS OF *POSTHODIPLOSTOMUM  
CUTICULA* (TREMATODA: DIPLOSTOMATIDAE) IN CULTURED  
*OREOCHROMIS NILOTICUS***

By

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**ABSTRACT**

This study was conducted on 400 *Oreochromis niloticus* fish that randomly collected from the earthen ponds of El-Abbassa area. These fish were subjected to clinical and post-mortem, parasitological and histopathological examinations. Besides, an attempt to control the infection by metacercariae was performed in this study. The clinical signs and postmortem lesions of diseased fish were manifested as restlessness, excessive mucous secretion, roughness of the skin and small blackspots measured about 1 millimeter in diameter scattered on the skin. Experimental infection was established in puppies, farm reared chickens, ducklings and laboratory reared Albino rats by feeding on encysted metacercariae. Dissections of experimental hosts were made between 48 and 72 hours post infection in order to obtain adults. *Posthodiplostomum (Neascus) cuticula* was only obtained from rats. The freezing at -20 C for 24 hours killed the encysted metacercariae in fish skin. Praziquantel at a concentration of 10 mg/L for 1 hour and Metriphonate at 0.15 mg/L were succeeded to control blackspot disease among diseased tilapia. Histopathologically, the encysted metacercariae were embedded in the tissue between the fin rays and numerous melanomacrophages were scattered in the tissue. The morphological characters and zoonotic importance of the encysted metacercariae were discussed.

**INTRODUCTION**

Tilapia were touted as the aquatic chicken by more than 20 years ago. They are likely to be the most important of all aquacultured fish in the 21<sup>st</sup> century (Fitzsimmons, 2000). Fish diseases constitute one of the most important challenges confronting tilapia farming.

Black spot trematodes have been reported from fishes in different countries and caused by many species of metacercariae (**Wittrock et al., 1991**) who reported metacercarial cysts of black spot trematodes of *Uvulifer ambloplitis* from green sunfish, *Lepomis cyanellus* and *Neascus pyriformis* from redshiners, *Notropis lutrensis* .

**Alexandrino et al., (1996)** found that the metacercariae of *Posthodiplostomum* sp. cause blackspot on the skin and fins of hybrid freshwater fish. **Muzzal., (1995)** collected metacercariae of *Neascus* sp. and *Posthodiplostomum* sp. from the skin of *Perca flavescens* fingerlings. **Lasee, (1995)** and **Kurochkin and Biserova, (1996)** stated that blackspot disease of fishes is caused by infection with *Posthodiplostomum cuticula* metacercariae in fresh and salt water fishes .

Also, **Badran et al., (1996)** recorded that encysted metacercariae of *Posthodiplostomum cuticula* as a skin parasitic disease of hybrid tilapia in Egypt. These parasites do not usually cause mortality, but can be very unsightly.

The purpose of this study is to identify the metacercariae causing the blackspot disease in *Oreochromis niloticus* through light microscopical examination, histopathological examination and experimental infection to obtained the adult worms. Also, evaluate the effect of some treatments on the viability of encysted metacercariae.

## MATERIAL AND METHODS

### Fish:

Four hundreds of *Oreochromis niloticus* fish of different body weights and lengths in this study were randomly collected from fish ponds of El-Abbassa, Sharkia Governorate, Egypt.

### Clinical and Postmortem Examinations:

The collected fish species were subjected to clinical and postmortem examinations as described by **Lucky, (1977)** and **Woo, (1995)**.

### Parasitological Examination:

Fish specimens were transported alive to the laboratory, the specimens under investigation were grossly examined for detection of encysted metacercariae in skin and fins and were dissected with needles from fish, placed in phosphate buffer solution and then transferred to liquid fixative for light microscopy (**Wittrock et al., 1991**).

When the cysts were needed for excystation, the infected tissue (skin) containing the parasite cyst removed and incubated in digested solution at 37 °C for 15 – 20 minutes. The digest solution consists of pepsin 0.5 gm, 0.1 N HCl (10 ml) and Ringer solution (90 ml), according to **Williams, (1967)**.

### **Experimental Infection:**

Experimental infection was established in 3 puppies, 5 farm reared chickens, 5 ducklings and 5 laboratory reared Albino rats by feeding viable encysted metacercariae. Dissections of experimental hosts were made between 48 and 72 hours post infection in order to obtain adults. In addition, two of the previously mentioned experimental animals were kept as a control. All of the experimental animals were free from infection and in a good health state.

### **Effect of Freezing and Chemotherapeutic agents on Viability of *Posthodiplostomum cuticula* metacercariae :**

**Freezing:** 20 *O. niloticus* fish naturally infected with encysted metacercariae were preserved by freezing at -20 C for a period of 24 hours and then fish were examined for the viability .

**Chemotherapy:** 60 live *O. niloticus* naturally infected with encysted metacercariae on the external surface were divided into three equal groups in glass aquaria supplied with continuous aeration and dechlorinated tap water at 23 C. Group (1) was treated with Praziquantel (Bayer AG, Germany) at concentration of 10 mg/L for 1 hr as described by **Woo (1995)**. Group (2) was subjected to Metriphonate (ADWIA, Egypt) at a dose of 0.15 ppm according to **Woo, (1995) and Paperna, (1996)**. Group (3) was left as a control group. Then after 24 hr all treated fish were examined for the viability of encysted metacercariae.

### **Histopathological Examination:**

Skin with cysts for light microscopy were fixed in Bouins fixative, dehydrated in ethanol, cleared in benzene, embedded in paraffin, sectioned at 5µ and stained with hematoxylin and eosin (**Roberts, 2001**).

## **RESULTS**

The clinical signs and postmortem lesions of diseased fish were represented as restlessness, excessive mucous secretion and roughness of the skin. Fish had small blackspots measured about one millimeter in diameter. Blackspot larvae look like grains of peppers scattered on the surface of the skin. The disease may be associated with rapid breathing in the cases of gill infections. Young fish and fry may be retarded in growth where badly

infected and became more susceptible to predation or adverse conditions. The presence of small number of encysted metacercariae in the skin of fish caused little harm. Fry may be dead when exposed to heavy infection (plate, 1).

The cysts were transparent and oval in shape. It measured 0.95 – 1.32 mm in length and 0.683 – 0.976 mm in width. The cyst wall was composed of an outer thick layer of host origin and inner layer formed by the parasite and the space between the host capsule and parasite cyst was filled with a viscous material (plate, 1&2).

The Neascus *Posthodiplostomum cuticula* was opaque and white. The two regions of the body were easily distinguishable, the fore body was about twice as long as the hind body. The fore body measured 0.53 – 1.01 mm in length and 0.28 – 0.58 mm in breadth, but the hind body measured 0.18 – 0.39 mm in length and 0.17 – 0.33 mm in breadth. The oral sucker was small and terminal and there was a short prepharynx, a short esophagus and the intestinal caeca pass into the hind body (plate, 2).

The adult parasite has been obtained experimentally from the lower part of intestine of experimentally infected albino rats. The body is small 1.3 – 1.5 mm long, fore body 0.54 – 0.73 mm long and 0.56 – 0.84 mm width, the hind body 0.54 – 0.73 mm long and 0.32 – 0.41 mm width. The oral sucker is small and terminal and measuring 0.04 – 0.06 mm long and 0.05 – 0.06 wide. The oral sucker leads into a short prepharynx and esophagus. The ventral sucker is slightly larger than the oral one, measuring 0.06 – 0.73 mm in length and .06 - .07 mm in width. Immediately behind the ventral sucker lies the adhesive organ measuring 0.056 – 0.07 mm in length and 0.10 – 0.14 mm in width. A very large gland lies above the adhesive organ. The ovary is oval in shape 0.04 – 0.1 mm in length and 0.10 – 0.16 mm in width. The anterior testes measuring .14 .19 mm in length and 0.2 – 0.23 mm in width. The posterior testis is larger, measuring 0.18 - .22 mm in length and 0.25 – 0.32 mm in width. The vitellaria are present as small follicle in the area between ovary and gland of adhesive organ (plate, 2).

Freezing of the infested fish at –20 C for 24 hours had lethal effect on encysted metacercariae in the diseased fish.

Regarding with the effect of some drugs on the viability of encysted metacercariae, the present study recorded that praziquantel at a concentration of 10 mg/L for 1 hour or Metriphonate at a dose of 0.15 ppm had lethal effect as recorded by light microscopic examination after 24 hr post-treatment. The signs indicating blackspot disease were gradually disappeared. Also, no signs of toxicity among treated fish were recorded during and after treatment.

Histopathologically, the encysted metacercariae were embedded in the tissue between the fin rays. Numerous melanomacrophages were scattered in the tissue between the fin rays with few small ones of them around the

encysted metacercariae. The outer layer of such metacercariae was almost free from melanin. Other areas showed numerous encysted metacercariae with almost complete absence of melanomacrophages. However, the external layer of such encysted metacercariae was diffusely embedded with melanin pigment. The encysted metacercariae heavily colonized the terminal portion of the fins. Some deeply embedded encysted metacercariae partly destroyed the fin rays (plate, 3).

## DISCUSSION

Blackspot disease is widespread and can be found in many different fish species. Also, referred to as black ich, black grubs or Neascus infection.

Blackspot disease causes an economic problem because the aesthetic value of the fish is compromised. The presence of encysted metacercariae in the skin of fish in a small number usually causes little serious harm. Heavy infected tilapias showed excessive mucous secretion, retarded growth, signs of respiratory distress in case of heavy infection of gills with pin-headed black spots. These clinical alteration were in harmony with those founded by **Wittrock et al., (1991); Lasee, (1995); Woo, (1995); Badran et al., (1996); Paperna, (1996); Kurochkin and Piserova, (1996); Alexandrino et al., (1996); Ferrara and Cook, (1998); Sepulveda et al., (1999) and Roberts, (2001)** who mentioned that the melanin pigment is deposited around the encysted metacercariae of *Posthodiplostomum cuticula* by the host giving rise to the condition known as blackspot.

Adult specimens of *Posthodiplostomum cuticula* were collected from laboratory reared rats by feeding on metacercariae from the skin of the Nile tilapia (*O. niloticus*), fish species endemic to Abbassa fish culture infected with metacercariae of *P. cuticula*, in all cases, dissecting of experimental hosts were made between 48 and 72 hours post-infection in order to obtain adults and this in agreement with the work of **Gerardo (1995)**. The morphological differences between *P. cuticula* in the present study was the gland of adhesive organ is anterior to it and not behind as in *P. manum*.

**Niewiadomska, (1973); Palmeri, (1976, 1977<sub>a</sub>, 1977<sub>b</sub>) and Shoop, (1989)** suggested that the need for a new approach to classify Strigeoid digeneans, in which the host identity played a subordinate role to be replaced by information from all developmental stages.

**Sepulveda et al., (1999)** stated that great egret (*Ardea albus*) play an important role in the dissemination of the infection of *P. cuticula* to fish.

**Badran et al., (1996)** reported that the encysted metacercariae of *P. cuticula* is one of the skin parasitic diseases of hybrid tilapia. **Ferrara and Cook, (1998)** stated that the black spot disease caused by metacercarial cysts

were recorded from infected fish between September and January and this nearly agree with the present study .

**Malhorta and chatterjeel, (1996)** recorded the zoonotic and transmission of black spot disease from snow trout and catfish in India. **Alexandrino et al., (1996)** reported that black spot disease on the skin and fins of hybrid freshwater fish caused by encysted metacercariae of *Posthodiplostomum* species and infection rate was 100 % in fish examined.

**Kurochkin and Biserova, (1996)** found that the blackspot disease of fishes is caused by infection with *P. cuticula* metacercariae and the same author stated that 8 species of parasites causing black spot were recorded including *Apophallus muehlingi*, *Cryptocotyle concavum*, *C. jeguna*, *C. lingua*, *Metagonimus yokogawai*, *P. cuticula*, *Prohemistomum vivax* and *Rossicotrema donicum* and identification of the parasite by experimental infection is required.

Concerning testing of some measures which may kill metacercariae in fish, our results showed that freezing at -20 C for 24 hours was sufficient for killing them. However, **El-Leathy, (1997)** and **El-Ashram, (2003)** indicated that freezing temperature for a period more than two days was sufficient for destroying metacercariae in frozen fish. Longer period (7 days) was recommended by **Paperna (1995)**. This may be attributed to the presence of encysted metacercariae on the external surface of fish. In spite of the fish be unappetizing, fish that is properly cleaned and cooked can not transmit any parasites to consumers.

Our results suggested that praziquantel as a bath treatment at concentration of 10 mg/L for 1 hr and Metriphonate at 0.15 mg/L were effective treatment in cultured tilapia which is in accordance with **Woo, (1995); Paperna, (1996) and Noga, (2000)**. **Singhal et al., (1986)** recorded that for the fish infected with black spot , immersion in 0.03 mg/l picric acid solution for 1.5 hr reduced the infection. **Badran et al. (1996)** reported that formalin at concentration of 1:4000 (250 mg/l) for 1 hr succeeded to control the encysted metacercariae of *P. cuticula* among cultured hybrid tilapia. **El-Ashram, (2003)** mentioned that praziquantel may be the drug of choice for application in fish farm by incubating the parasitized fish with *Clinostomum tilapiae* in aerated water bath containing 10 mg/L water. Indirect control has been attempted by controlling the snails that act as intermediate hosts to the parasite. It is possible to reduce the incidence of infection by removing all snails from the ponds to cut the cycle of infection.

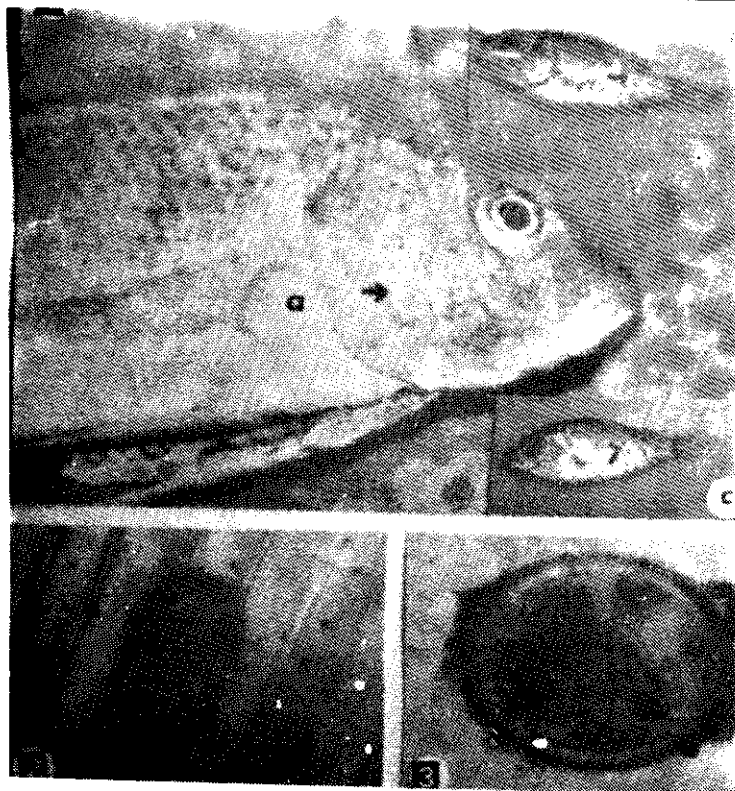
Similar pictures of histopathological observations were recorded by **Wittrock et al., (1991); Woo, (1995); Badran et al., (1996); Alexandrino et al., (1996) and Roberts, (2001)** who noticed that the body defense was presented by presence of melanomacrophages.

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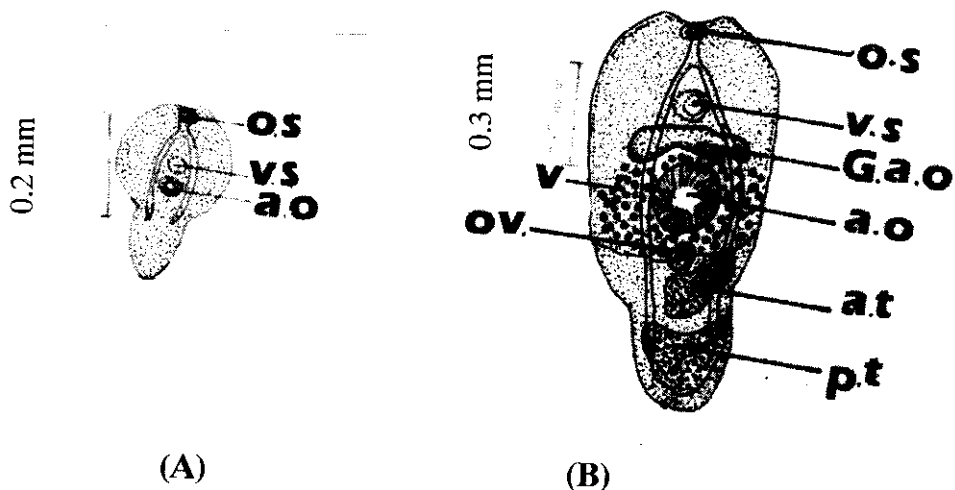
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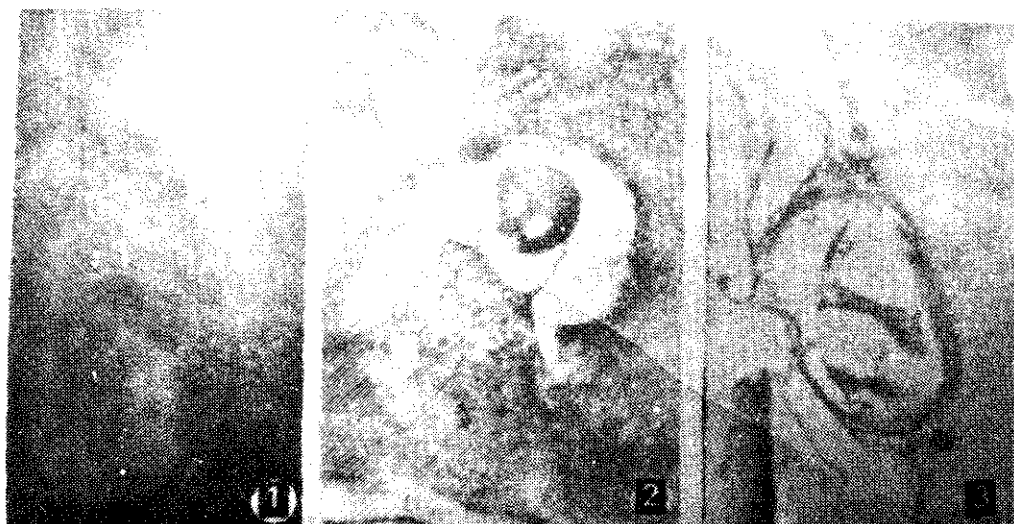




**Plate (1):** Fig. (1): a, b, c, black encysted metacercariae of *P. cuticula* scattered on the external surface of fingerlings and adult *O. niloticus* . Fig. (2): An encysted metacercaria on the fin x 40 . Fig. (3): An encysted metacercaria of *P. cuticula* x 60 .



**Plate (2):** (A) excysted metacercaria (B) adult of *P. cuticula*. oral sucker (o.s.), ventral sucker (v.s.), adhesive organ (a.o.), gland of adhesive organ (G.a.o.), vitellaria (v.), ovary (ov.), anterior testes (a.t.) and posterior testes (p.t.).



**Plate (3):** Fig. (1, 2 & 3) showed the histopathological alterations due to *P. cuticula* metacercariae infections. H.& E., x 100.

## الملخص العربي

ملاحظات على الخواص المورفولوجية لكلا من السركاريا المتحوصلة والديدان

البياضة (بوثودبلوستومم كيونتيكولا) في أسماك البلطي النيلي المستزرعة

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تم إجراء هذه الدراسة على عدد ٤٠٠ سمكة من البلطي النيلي والتي تم الحصول عليها من الأحواض الترابية بمنطقة العباسة - محافظة الشرقية - مصر.

تمثلت العلامات المرضية والصفة التشريحية في ضعف النمو، خشونة في الجلد، زيادة في إفراز المخاط ووجود علامات صعوبة التنفس في حالة إصابة الخياشيم ووجود بقع سوداء في حجم رأس الدبوس (١مليمتر عرض) متناثرة على السطح الخارجي للسمكة.

بالنسبة لتأثير التجميد على الإصابة بيرقات بوثودبلوستومم فقد أوضحت النتائج أنه بتجميد الأسماك عند -٢٠م لمدة ٢٤ ساعة كانت كافية تماما لقتل هذه الطفيليات.

كما أثبتت الدراسة أنه يمكن علاج هذه الإصابة باستخدام عقار البرازيكونتيل كحمام مائي عند تركيز قدره ١٠مجم/لتر لمدة ساعة أو المتروفونات (١٥, ٠مجم/لتر) وعند فحص الأسماك بعد ٢٤ ساعة من العلاج تبين نفوق السركاريا المتحوصلة.

تم إجراء العدوى التجريبية للجراء، الفئران البيضاء، البط والفراخ ولكن لم يتم الحصول على الديدان البالغة إلا من الفئران فقط.

تبين من الفحص الهستوباثولوجي ترسيب صبغة الميلانين في اليرقات المتحوصلة والتي تتسبب في لونها الأسود.

كما تم عمل وصف تفصيلي لليرقات والديدان البالغة بالإضافة إلى مناقشة الأهمية الصحية لهذه اليرقات.