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ELECTROPHORETIC ANALYSIS TO CONFIRM THE IDENTIFICATION OF SOME KINDS OF ENCYSTED METACERCARIAE FROM *OREOCHROMIS NILOTICUS*

(With 2 Tables and 3 Plates)

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التحليل الكهربي للتعرف على بعض أنواع الميتاسركاريا المتحوصلة في أسماك البلطي النيلي

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تم تجميع أربعة أنواع مختلفة من الميتاسركاريا المتحوصلة في أسماك البلطي. وقد استخدمت هذه الأنواع منفصلة في عدوى فئران معمليا وقد نتج عن ذلك تكون أربعة أنواع مختلفة من الديدان المفلطحة البالغة في أمعاء الفئران تم تجميعهم و التعرف عليهم وتصويرهم. والأنواع الأربعة هي: بروهيمستومم فيفاكس و بيجيديوبسيس جيناتا وبروسيروفم فاريم وهابلوركس بوميليو. وكذلك تم تحضير مولدات الضد من الميتاسركاريا المتحوصلة للأنواع المختلفة منفصلة وذلك لتحليل كل نوع كهربيا باستخدام الفصل الكهربي بطريقة الجل وصبغة بالكوماسي الزرقاء. وقد تم تحديد البروتينات المختلفة في كل نوع. وقد أظهرت النتائج أن المولد المضاد المحضر من بروهيمستومم فيفاكس و بيجيديوبسيس جيناتا وبروسيروفم فاريم و هابلوركس بوميليو أعطى ٨ ، ٧ ، ٣ ، ٥ من البروتينات المختلفة وكان الوزن الجزيئي لهم يتراوح بين ١٣ – ١١٢ كيلو دالتون. وقد أعطت النتائج صورة واضحة لإمكانية استخدام هذه الطريقة للتعرف على الأنواع المختلفة للميتاسركاريا المتحوصلة في الأسماك.

SUMMARY

The present study was designed to collect four kinds of encysted metacercariae (EMC) from *Oreochromis niloticus*. The EMC were used separately to infect rats experimentally. The adult flukes were recovered

from the experimentally infected rats 5-7 days after infection. The collected adult flukes were identified and photographed. The four adult flukes were *Prohemistomum vivax, Pygidiopsis genata, Proverovum varium* and *Haplorchis pumilio*. The *P. vivax, P. genata, P. varium* and *H. pumilio* EMC antigens were fractionated on SDS-PAGE and stained with Coomassie blue stain. The molecular weights of each fractionated antigen were determined. The *P. vivax, P. genata, P. varium* and *H. pumilio* EMC antigens gave 8, 7, 3 & 5 bands with different molecular weights ranging from 13 – 112 KDa. The distribution of the yeild bands in each lane gave the characteristic feature of each antigen.

Key words: *Electrophoretic analysis, encysted metacercariae, oreochromis niloticus.*

INTRODUCTION

The largest endemic focus is located in the Nile Delta, where favorable conditions exist for propagation of the heterophyiasis (Acha & Szyfres, 1991). Moreover, in the Nile Delta lakes, *Oreochromis niloticus* is important host of heterophyids and cyathocotylids including zoonotic pathogen (Taraschewski, 1984 and Paperna, 1996). The major genera infecting man in Korea were *Pygidiopsis, Haplorchis* and *Procerovum* (Ito, 1964 and Seo, 1979). While, the major species enlisted infecting man in Egypt were *Prohemistomum vivax* (Nasr, 1941); *Heterophyes heterophyes* (Khalil, 1933 and Rifaat et al., 1980); *Pygidiopsis genata* (Boulos, 1979 and El-Mokaddem, 1982) and *Haplorchis pumilio* (Khalifa et al., 1977 and Tadros & El-Mokaddem, 1983). Furthermore, *Haplorchis* and *Procerovum* were reported to be able to cause fatal erratic parasitism in heart, brain and spinal cord with their eggs (Africa et al., 1940).

The known methods for the identification of microscopic metacercariae are using suitable hosts for experimental infection, as metacercariae have no mature reproductive organs. It is usually impossible to identify metacercariae for different species (Noga, 1996). Recently, several authors are using the SDS-PAGE for identification and characterization of different micro-organisms. Jean & John (1991) and Nieme et al. (1993). The latter authors isolated different strains of *Caulobacters* and *Streptococci*, respectively on the basis of protein band profiles on SDS-PAGE.

So, the aim of the present study was planned to fractionate some identified encysted metacercariae on SDS-PAGE to determine the number of specific bands for each metacercaria. This method might be used as a recent role to confirm the identification of different metacercariae instead of the old methods which were based on experimental infections of different final hosts to confirm the identification.

MATERIALS and METHODS

Seventy *Oreochromis niloticus* (5-15 gm b.w.) were collected from the endemic Heterophyiasis area at Dakahlia, Egypt during the period from October, 1998 to March, 1999.

Collection of encysted metacercariae (EMC):

Fish were dissected as immediately as possible and examined for the presence of EMC according to Paperna (1980). The fish were minced and artificially digested with 0.25% pepsin in 0.85% Na cl for one hour at 37°C in shaking water bath (Srisawangwong et al., 1997). The digested tissues were strained through sieves and sedimented in 0.85% Nacl. The investigated EMC were separated using the binocular microscope into the 4 kinds depending on their morphological characters according to Paperna (1980) and Mahdy (1991). Each kind of collected metacercariae were divided into two parts, the first part was used to infect rats while, the second part was used for preparation of specific antigen.

Experimental infections:

Fifteen white rats of three weeks old were divided into 5 groups, each group contains three rats. They were reared on dry bread and clean water. Fecal samples of all rats were examined daily for 7 days before infection to be insure that they are free from trematode infection. Each group were inoculated with 300 EMC (each rat was given 100 EMC) from each separated kind of EMC, while, the last group was kept as control.

Dissections of experimentally infected rats:

The definitive hosts were killed between 5-7 days p.i. The rats were narcotized by a blow on the head or by chloroform. The intestines were removed immediately; placed in a dish with warm saline and scraped. The small intestines of each group of rats (single infection)

were divided into 3equal parts. Each part was transferred separately into a dish with warm saline and the mucosa was scraped.

Collection and identification of flukes:

The flukes were collected from the dishes under a binocular microscope and stored separately. Genera were differentiated using the key given by Velasquez (1973) and Mc Donald (1981). Flukes determination and morphological variation were studied. The obtained EMC and flukes were prepared and mounted according to Pritchard & Kruse (1982). They were identified and photographed.

Preparation of different soluble EMC antigens:

Four types of identified EMC were collected separately from *Oreochromis niloticus*. The four types of EMC were *Prohemistomum vivax, Pygidiopsis genata, Procerovum varium* and *Haplorchis pumilio.* EMC of each worm were individually homogenized for 15 minutes on ice using a tefflon glass homogenizer followed by sonication for 5 minutes to disrupt the remaining intact EMC. The homogenates were centrifuged at 20,000 rpm for 45 minutes at 4°C. The protein contents of each of the supernatants containing EMC antigenic material were determined using Lowry`s method (Lowry et al., 1951). The various preparations were aliquoted and saved at –70°C until used.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis(SDS-PAGE):

Ten µg of *Prohemistomum vivax, Pygidiopsis genata, Procerovum varium* and *Haplorchis pumilio* EMC antigens were electrophoresed using 12 % SDS-PAGE under non-reducing conditions (Laemmli, 1970). The fractionated antigens were visualized by Coomassie blue stain. The gel was soaked overnight in the Coomassie blue R (0.25% Coomassie blue powder dissolved in de-staining solution.). The gel was then de-stained by de-staining solution (45% methanol, 5% glacial acetic acid and 50% distilled water) with several changes, till the bands became clear. The gel was dried by Gel drier, then photographed. The different antigenic bands of each EMC antigens were determined by using low molecular weights, pre-stained standard (Bio-Rad).

RESULTS

The total number of metacercariae collected from 70 *Oreochromis niloticus* were about 8400 EMC. Therefore, the average metacercarial density/one fish was 120. The metacercariae collected

from the viscera and muscles of head, trunk and tail are displayed in (Table 1 & Plate 1). It was difficult to confirm the identification of 4 kinds of metacercariae when they were encysted in fish or even after isolation. For this reason the metacercariae from various parts of the fish were pooled and used for experimental infection to rats. 5-7 days p.i., 279 adult flukes were recovered from the experimentally infected rats. The flukes were fully grown and mature, containing many eggs in the uteri. Careful observations on the worms after staining led us to consider that they are 4 morphologically different types, which were proposed by Velasquez (1973) and Mc-Donald (1981). The four types were *Prohemistomum vivax, Pygidiopsis genata, Procerovum varium* and *Haplorchis pumilio*. (Table 2 & Plate 2).

Table 1: Distribution of metacercariae in the different parts of 70 *Oreochromis niloticus.*

Body portion	Number of metacercariae	Percentage (%)	Mean number/ one fish
Muscles of head	1100	13.0	16
Muscles of trunk	4403	52.4	62
Muscles of tail	2797	33.3	40
Viscera	100	01.2	2
Total	**8400**	--	**120**

Table (2): The types and numbers of collected flukes from experimentally infected rats.

Types of collected flukes	No. of infected rats	No. of EMC used for infection	No. of collected flukes	%
Prohemistomum vivax	3	300	121	40.00
Pygidiopsis genata	3	300	77	25.66
Procerovum varium	3	300	34	11.33
Haplorchis pumilio	3	300	47	15.66
Control	3	--	--	--
Total	**15**	**1200**	**279**	**24**

Coomassie staining of fractionated antigens derived from the different EMC of 4 parasites (*Prohemistomum vivax, Pygidiopsis genata, Procerovum varium* and *Haplorchis pumilio*) revealed several bands of molecular weights ranging from 13-112 KDa. *Prohemistomum vivax* EMC antigen gave 8 bands of molecular weight in the range from 13–102 KDa. There were 5 common bands with molecular weights of 20,22,32,37.5 and 102 KDa and 3 faint bands with molecular weight of 13,34 and 51 KDa. However, 7 and 3 bands from the range of 13–102 and 21–112 KDa were stained from *Pygidiopsis genata* and *Procerovum varium* EMC antigens, respectively. The fractionation of *Pygidiopsis genata* EMC antigen had 4 prominent bands (13,20.5,39 &77 KDa) and 3 faint bands (27.5,85 & 102), while *Procerovum varium* EMC antigen had two prominent bands (41 & 112 KDa) and one faint band (21 KDa). Staining of *Haplorchis pumilio* EMC antigen revealed 5 bands from the range of 14–112 KDa. There were 4 prominent bands (14,20.5,39 & 112 KDa) and one faint band of 102 KDa. (Plate 3).

DISCUSSION

The present data showed that four genera of flukes were obtained from experimentally infected rats with four kinds of EMC collected from *O. niloticus* from Dakahlia Governorate. These flukes were *P. vivax, P. genata, P. varium and H. pumilio*. These species of flukes were previously recorded in the small intestines of mammals and birds fed on naturally infected fish with different kinds of EMC from Austeralia (Pearson, 1964 and Velasquez, 1973), Korea (Seo, 1979) and Egypt (Shalaby, 1982; Maklouf et al., 1987; Mansour et al., 1987; Mahdy,1991; Mahdy et al.,1995; Abbass,1997 and Tantawy et al.,1998). Furthermore, in the present study all the detected genera of flukes; *P. vivax, P. genata, P. varium and H. pumilio* were transmissible to human (Nasr, 1941; Ito, 1964; Khalifa et al., 1977; Boulos, 1979; Seo, 1979; El-Mokaddem, 1982 and Tadros & El-Mokaddem, 1983).

The known methods for identification of microscopic metacercariae are infection to laboratory animals (Noga, 1996). The present study was designed to identify four kinds of EMC collected from *O. niloticus* by SDS-PAGE instead of the experimental infection of the suitable final host. In the present study the *P. vivax, P. genata, P. varium and H. pumilio,* EMC antigens were fractionated by SDS-PAGE and stained with Coomassie blue, The P. vivax, *P. genata, P. varium and H.*

pumilio, EMC antigens gave 8, 7, 3, 5 bands with different molecular weights, some of these bands were prominent and the others were faint gave the specific characteristic feature of each EMC antigen. These data are recorded for the first time concerning the available literature. The application of SDS-PAGE to confirm the identification of different microorganisms were previously applied by several authors, Jean & John (1991) and Nieme et al. (1993) applied the SDS-PAGE to confirm the identification of different strains of *Caulobacters* and *Streptococci*.

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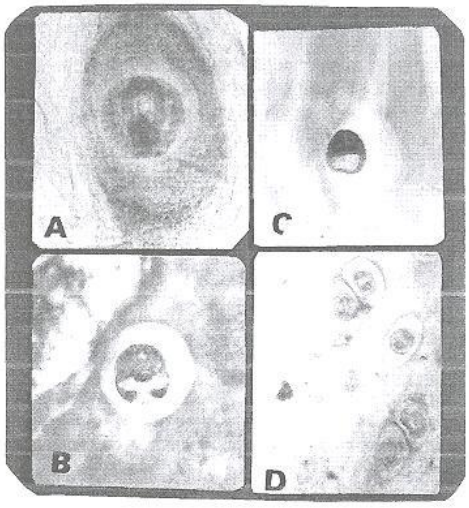

Plate (1): Shows the different kinds of EMC (X 100) collected from *Oreochromis niloticus.*

(A): *Prohemistomum vivax.*

(B): *Pygidiopsis genata.*

(C): *Procerovum varium.*

(D): *Haplorchis pumilio.*

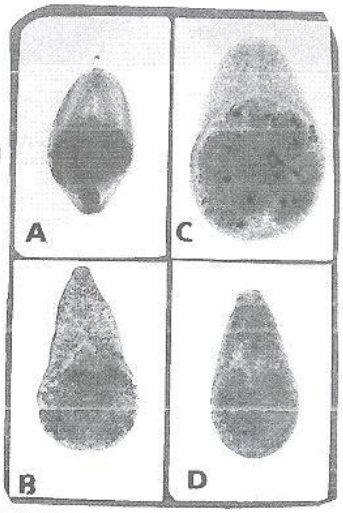

Plate (2): Shows the adult flukes (X 100) collected from experimentally infected rats.

(A): *Prohemistomum vivax.*
(B): *Pygidiopsis genata.*
(C): *Procerovum varium.*
(D): *Haplorchis pumilio.*

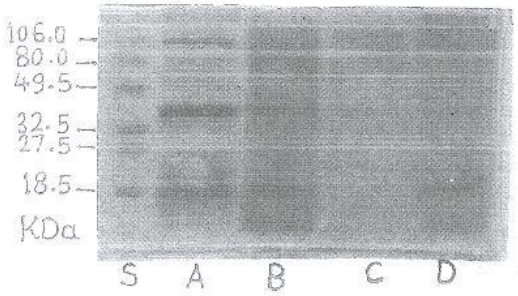

Plate (3): Shows the electrophoretic analysis of 4 kinds of EMC antigens.
Lane (1), Pre-stained standard, low molecular weight (Bio-Rad).
Lane (2), *Prohemistomum vivax* EMC antigen.
Lane (3), *Pygidiopsis genata* EMC antigen.
Lane (4), *Procerovum varium* EMC antigen.
Lane (5), *Haplorchis pumilio* EMC antigen.