SOME STUDIES ON INFECTION OF CHRYSICHTHYS AURATUS WITH TRYPANOSOMA MANSOURI

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ABSTRACT

Chrysichthys auratus was collected from River Nile at Giza province during the period from the being of June 2003 till the end of May 2004 .Prevalence of Trypanosoma mansouri was determined using a haematocrit centrifuge technique (70%) and percentage of haematocrite was recorded. Parasitaemia was estimated by counting trypanosomes in 30 fields (10 ocular x 10 objectives) of wet preparation. Individual fish carrying heavy and medium infection were most common in summer and spring respectively. Seasonal changes showed high prevalence in summer (100%), spring (92%) and autumn (60%) and comparatively low in winter (28%). In vitro, Cultivate the Trypanosoma mansouri have been attempt in uniphasic sheep blood agar medium. Minimum Essential medium (MEM) contains no serum and in MEM supplemented with 10% foetal bovine serum. During 7days from initial inoculum growth of trypanosome was followed up in culture media. Medium containing no serum failed to support the growth of parasites and growth on uniphasic blood agar not continued after 5 days.

INTRODUCTION

Haemoflagellates of the genus Trypanosoma is prevalent in both freshwater and saltwater fishes and is transmitted by leeches of various genera and species as vectors **(Post, 1987)**. Natural fish trypanosomiasis is widespread, apparently nonpathogenic and generally regarded as a well-balanced host-parasite relationship **(Baker, 1960)**. However, adverse conditions are thought to upset this balance **(Lom, 1979)**. On the other hand, the potential of fish trypanosomes depends on the intensity of infection which may results in a series of changes in the fish host as well as transient or irreparable histological changes and eventually mortality **(Overath, et al., 1999)**. Trypanosoma infection may lead to economic losses, lowering production and destruction of haemopioetic organs and immune system **(Khan, 1991, and Lom and Dykova, 1992)**.

Trypanosoma infection in fish is usually governed by some epidemiological aspects related directly to the environments, parasite and host. Studies on the morphology, physiology, and biochemistry and drug responses of many pathogenic mammalian Trypanosomes have been aided by their vitro cultivation **(Davies, et al 1995)**.

Few detailed studies have been carried out on vague profiles of blood parasites in vitro cultivation from Egyptian fishes **(Elssa and Mohamed, 2001)**. The aims of this study were to investigate natural trypanosoma infection in C. auratus including seasonal prevalence, percentage of haematocrite and parasitaemia. In addition, to determine whether T. mansouri could be cultivated in uniphase sheep blood agar medium and in medium supplemented with foetal bovine serum and to compare in vitro growth in serum free medium and follow up the nature of such growth in its different stages through an in vitro culturing system which is the focus of this study.

MATERIALS AND METHODS

Collection and examination of fish:

One hundred C.auratus fish, average body weight were 50±5 gram were randomly collected during the period from the being of June 2003 till the end of May 2004 from different localities of River Nile at Giza province. Fish were transferred alive to laboratory, kept in glass aquaria supplied with dechlorinated aerated water and maintained on a commercial diet. Any clinical signs and internal gross lesions were recorded according to **Schaperclaus, (1992)**.

Fish were anaesthetized with MS222 (tricaine methane sulphate) (Sandoz). Blood samples obtained from caudal blood vessels were examined according to haematocrite centrifuge technique (HCT) **(Woo, 1969)**, percentage of haematocrite was recorded and parasitaemia was estimated by counting trypanosomes in 30 fields (10 oeular x 10 objective) of wet preparation. Percentage of low, medium and high parasitaemia were recorded based on average number of trypanosomes per field in wet preparation .Fish were subdivided into low infection (up to 2), medium infection (2 to<4) heavy infection (4 to<12) according to **A Zintl et al (1997)**. Seasonal fluctuations were estimated for prevalence of trypanosome infection.

Cultivation in vitro:

Trypanosomes were cultured in selected three medium 10% sheep blood agar, Minimum Essential medium (MEM) and MEM supplemented with 10% foetal bovine serum (FBS). Standard aseptic technique was undertaken within a laminar flow hood throughout in vitro culture.

Blood samples (0.5ml) corresponding to the positive blood films were taken from fish and

aseptically cultured on sheep blood agar plates **(Diamond and Hernan, 1954, Eissa and Mohamed, 2001)** and in two screw —topped bottles, one contain 4.5ml MEM and other contain 4.5ml MEM with 10% (FBS) **Bienek and Belosevic (1997)**. Inoculated plates and bottles were incubated at 22 ±10 0C and examined every 6 hours through out 7 days (experimental time) for morphological characteristics of the cultured parasite. Type of haemolysis in blood agar plates and microscopic follow up of different developmental stages of parasitic division were recorded. Films taken from culture were air dried, fixed in methyl alcohol for 3 minutes and allowed to dry. The smears were stained by Giemsa (one drop Giemsa, 1ml of phosphate buffered saline[(PBS, 2.4g Na2H1 po4.2H2o , 0.54g NaH2 po4.2H2o and 0.34g Nacl) , pH 7.2] and examined with oil immersion **(Van Meirvenne ,1988)**.

Statistical analysis:

The data were statistically analyzed according to **Petrie and Watson (1999)**.

RESULTS

The clinical sings observed in natural infected C. auratu with T. mansouri were emaciation and lethargic appearance with some fish suffer from ascitis and discoloration of skin. Post mortem examination was showing splenomegaly and/or hepatomegaly.

The prevalence of trypanosome infections were 70% of fish examined during the entire year. The Seasonal prevalence were 28%, 60%, 92%, and 100% during winter autumn, spring and summer seasons respectively (Table (1).

According to the level of infection the Fish were subdivided into categories of low, medium, and heavy infection .Fish carrying heavy and medium infection were most common in summer and autumn seasons respectively (Table (1). Changes of haematocrit values throughout the year were tested for all infected and uninfected fish table (2) and showed that increase in parasitaemia were associated with a significant decrease of percentage haematocrit.

Growth of T.mansouri looked promising within the first few hours in blood agar plates and in MEM contain 10% FBS medium, Medium containing no serum failed to support the growth of parasites .Growth was very rapid over 5 days in MEM but declined rapidly and no growth was observed in blood agar media after 7days of inoculation and wide hemolytic zones surrounding each of the inoculum s areas. Trypanosome morphology was noticed during culture in two medium (sheep blood agar media and MEM & 10%FBS) since this medium showed similar growth patterns. Fig: (1).

In the culture medium, blood stream stages transform into a mixture of trypomastigotes, and

epimastigotes. At 8 hours post inoculation, tryponastigotes transformed into swollen amorphous flagellates, the free flagellum coiled at one end of the organism. At 56 hours rounded forms with 2 nuclei and a kinetoplast were observed. At 96 hours, observed that these forms divided by unequal binary fission to elongated forms (n=10, mean 12.3±0.6µm) with production of new flagellum, kinetoplast, nucleus and cytoplasm with large numbers of granules.

At 110 hours the fusion of 2 unequal form of T.mansouri was traced in detail. At 114 hours individuals increased in size (n=20, mean 50.45 ±6.4 m). In fifth day post inoculation, trypanosome cell wall in agar plate was destroyed (lyses) and stops the follow up of trypanosome growth.

DISCUSSION

Trypanosomes are always transmitted by blood sucking leeches and most species are not known to cause mortality **(Woo, 1995)**. Trypanosomes were common in fish of all African waters and recorded from the Nile, east African lakes, Congo, Niger and southern African **(Paperna, 1996)**. In the present study, the prevalence of trypanosome infection in C.auratus were high (70%) and morphologically identified as T. mansouri and similar to the description given by **Negm El Din, (1997)**. The high prevalence of trypanosome infection which was recorded for Nile C.auratus agrees well with previous reports of extremely common and widespread flagellate infections in nature fish populations **(Negm El Din, 1997, Paperna, 1996, Mohamed, 1997 and Eissa and Mohamed 2001)**.

The obtained clinical sings of natural infected C.auratus with T.mansouri coincided with that previously noticed by **Mohamed, (1997) and (Ahmed), 2001** who found that fish naturally infected with trypanosomiasis showed emaciation, sunken abdomen pale coloration of gills and swelling in spleen and liver.

Prevalence of trypanosome infections were decreased during autumn and remained low during the winter, which may have been due to direct effects of water temperature on the rate of parasite multiplication. These results agreed with those reported by **Lom, 1979, Woo et al., 1983, A Zintl et al., 1997, Mohamed, 1997 and Ahmed, 2001**. The high prevalence of trypanosome infections were recorded in summer and spring. Rising water temperature may have enhanced trypanosome proliferation, rather than the feeding behavior of the vector (leeches) **(A Zintl et al., 1997 and Ahmed, 2001)** The increase in parasitaemia were associated with a significant decrease of percentage haematocrit as recorded by **Awad, 1997, A Zintl et al., 1997 and Ahmed, 2001** who found that Haemoflagellate infection cause anemia manifested by reduced haematocrit and hemoglobin and a fall in the number of erythrocytes. Haemolysis, caused by lytic or immune-complex forming components of parasite antigens were suggested as possible

causes by **Laidley et al, (1988)**.

The development of T.mansouri in sheep blood agar media and MEM contain 10% FBS were succeeded and MEM media containing no serum failed to support the growth of parasites, and the morphology were observed allover 5days.These findings are consistent with **Islam and Woo (1992)** who showed that T. danilewskyi was cultured and subculture as trypomastigotes in MEM with 10% FBS without fish cell and did not multiply in diphasic blood agar media with sheep blood and various vitamins. Also, with **Bienek and Belosevic (1997)** showed that medium contain no serum or mammalian serum failed to support the growth of trypanosomes and added that in vitro cultivation ,growth in 10% GFS (golden fish serum) was faster and greater than that in 5% FBS and 5% GFS. In the present study, successful results were obtained from in vitro cultivation of T.mansouri in uniphasic sheep blood agar medium and growth not continued after 7days. This results agrees with that described by **Eissa and Mohamed (2001)** who recorded that uniphasic sheep blood agar medium were less expensive than biphasic medium and highly succeeded in vitro cultivation of T.mansouri but they follow the trypanosome growth for 10 days.

Table (1): Seasonal changes in parasitaemia and Prevalence of *T.mansouri* infected *C.auratus.*

Items	Total examined	light infection	Medium infection	Heavy infection	Total No. of infected	%
winter	25	5	2	-	7	28
autumn	25	5	7	3	15	60
summer	25	-	6	19	25	100
spring	25	3	15	5	23	92
Total	100	13	30	27	70	70

Low infection: up to 2 mean number of *T.mansouri* per field x 100 magnification

Medium infection: 2 to >4.

Heavy infection: 4 to >12.

Table (2): Haematocrit percentage of *C.auratus* infected with *T.mansouri*

Items	Light Infection	Medium infection	Heavy infection	Free of infection
Haematocrite%	38.16±8.2	33.77±6.2*	30.3±9.5**	41.24±6.7

Significance *P<0.5 **P<0.1

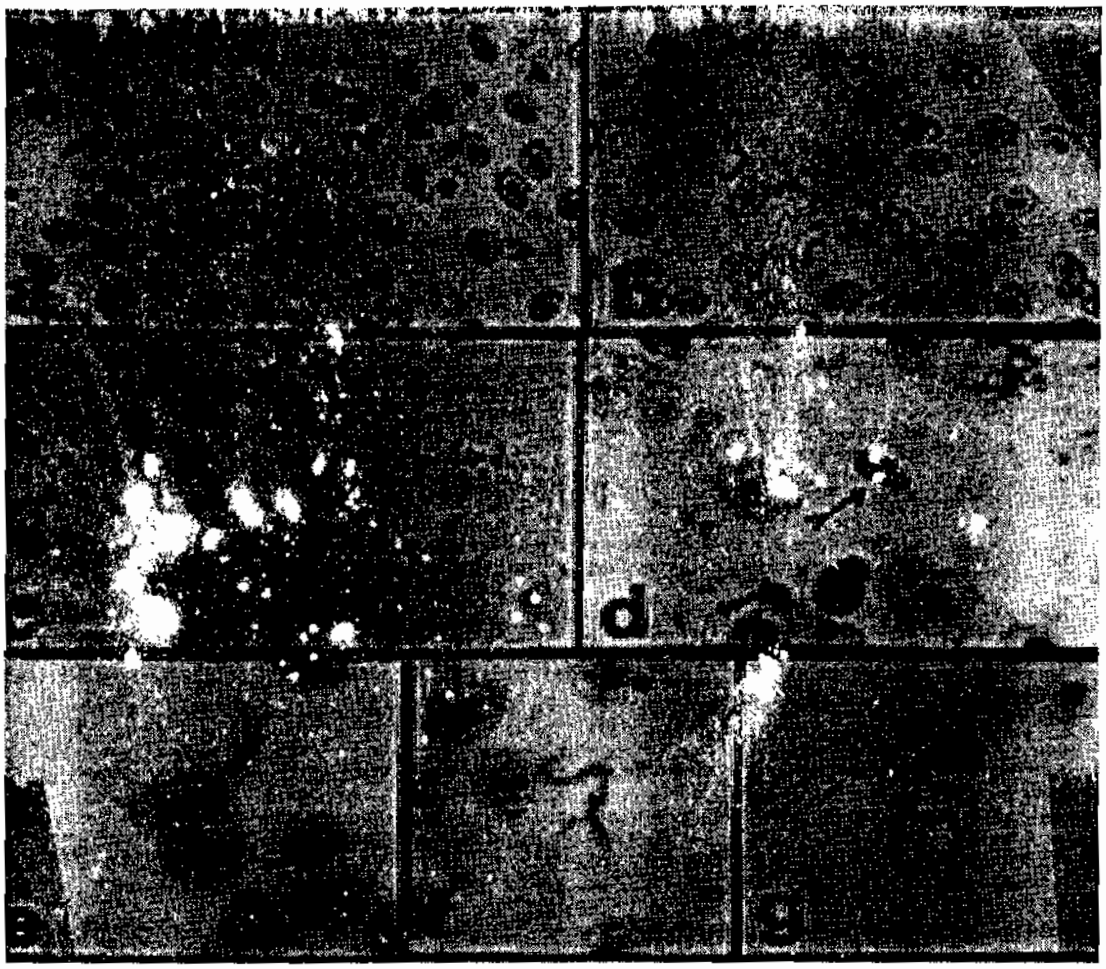

Fig (1) : T.mansouri: culture forms on Giemsa – stained smears

a- Blood stream form _ 1000

b- Transformation into epimastigote _ 1000

c- Young developed stage _ 1000

d- Spheromastigote form _ 2000

e- Elongated metatrypanosome form _ 2000

f- Unequal division, elongated metatrypanosome _2000

g- Trypanosoma destroyed cell after 5 days in blood agar plate_2000

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الملخص العربى

بعض الدراسات على إصابة أسماك الكراكير بطفيل التريبانوسوما منصورى

المشتركون فى البحث

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تمت هذه الدراسة على أسماك الكراكير المجمعة من نهر النيل محافظة الجيزة على مدار سنة من يونيو ٢٠٠٣ حتى مايو ٢٠٠٤، وتم تحديد نسبة إصابة الأسماك بطفيل التريبانوسوما منصورى باستخدام طريقة التركيز الخلوى الدموى مع دراسة التغيرات فى نسبة التركيز الخلوى الدموى بالنسبة إلى شدة الإصابة، وقد تم حساب شدة الإصابة على أساس عدد طفيل التريبانوسوما فى ٣٠ حقل ميكروسكوبى (١٠ عينة × ١٠ شيئية) لمسحة من دم الأسماك، وكانت معظم الأسماك التى أصابتها بالطفيل شديدة فى فصل الصيف والأسماك متوسطة الإصابة فى فصل الربيع، وسجلت إصابة أسماك الكراكير بطفيل التريبانوسوما منصورى فى هذه المنطقة نسب عالية ١٠٠٪، ٩٢٪ ، ٦٠٪ و٢٨٪ فى فصل الصيف والربيع والخريف والشتاء على التوالى.

تم عمل محاولة لزراعة طفيل التريبانوسوما منصورى على ميديا وحيدة الطبقة من اجار دم الأغنام ١٠٪ وعلى ميديا الميم دون إضافات وكذلك على الميم مضاف إليه ١٠٪ مصل بقرى، تم تتبع نمو الطفيل فى الميديا لمدة ٧ أيام من الزرع وقد تم بنجاح نمو الطفيل فى ميديا الميم المضاف إليه ١٠٪ مصل بقرى وكذلك ميديا اجار دم الأغنام حتى ٥ أيام ولم يستمر النمو وميديا الميم الخالية من المصل لم ينمو فيها الطفيل.