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A STUDY ON TRYPANOSOMIASIS IN SOME FRESHWATER FISHES AT ASSIUT GOVERNORATE

(With 8 Tables and 4 Figures)

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دراسة عن الإصابة بالتريبانوسوما في بعض أسماك المياه العذبة في محافظة أسيوط

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استهدفت هذه الدراسة قياس معدل انتشار مرض التريبانوسوما في ستة أنواع من أسماك المياه العذبة و قد أوضحت النتائج الآتي: وجود أعلى نسبة إصابة في أسماك أبو ريالة (٤٢,٣ %) والقراميط (٨%) بينما نسبة الإصابة في أسماك البوز كانت (٣,٥%) وفي أسماك البياض (٢,٥%). أما عن أسماك البلطي واللبيس وجد أنهما خاليان تماما من العدوى بمرض التريبانوسوما. وأوضحت الدراسة أن معدل انتشار مرض التريبانوسوما في أنواع الأسماك المصابة في الشتاء أعلى منها في الصيف. و بدراسة معدل انتشار المرض في الأعمار المختلفة اتضح أن نسبة الإصابة في الأسماك ذات الأوزان الصغيرة أعلى من الأسماك ذات الأوزان الكبيرة في أسماك أبوريالة. أما بالنسبة للصورة الدموية: اتضح أنه يوجد نقص معنوي في عدد خلايا الدم الحمراء و كمية الهيموجلوبين ونسبة الهيماتوكريت في أسماك أبوريالة المصابة بمرض التريبانوسوما. و يزداد معدل النقص كلما زادت درجة الإصابة. وتم تسجيل الأعراض الإكلينيكية للإصابة بمرض التريبانوسوما في الأسماك المصابة وكذلك تم إجراء التحليلات الإحصائية بين الأسماك المصابة و السليمة.

SUMMARY

The study was carried out to investigate the prevalence of Trypanosoma infection in six freshwater fishes. The prevalence of trypanosoma infection was recorded in *Chrysichthys auratus* (42.3%) followed by *Clarias garipienus* (8%). The lowest infection was found in *Morymyrus kanumme* (3.5%) and *Bagrus bajad* (2.5%) while *Oreochromis niloticus* and *Labeo niloticus* were free from the infection. The clinical findings of infected fish species were described. The rate of infection with *Trypanosoma spp.* in naturally examined fish was higher in winter than

in summer. The young ages of infected *Chrysichthys auratus* were more susceptible than old ones. The total hemoglobin (g/L), hematocrit percent and red blood cells count were decreased significantly with the increase of the degree of infection in *Chrysichthys auratus*. Statistical analysis of the obtained data between healthy and diseased fish was performed.

Key words: *Trypanosomiasis, Freshwater fishes.*

INTRODUCTION

Trypanosomiasis is one of the most important haemoflagellate parasitic diseases of freshwater and marinewater fishes causing considerable levels of economic losses, lowering their production and acting as a devastating agent to haemopiotic and immune system (Dykova and Lom, 1979; Khan, 1990; Lom and Dykova, 1992 and Woo, 1995).

A large number of trypanosoma species have been described as parasites of freshwater fishes (Schäperclaus et al., 1992).

Mohamed (1978) recorded Trypanosoma mansouri and Trypanosoma cynophilum in Egyptian Nile fish, Chrysichthys spp. While Trypanosoma alhussaini described in Clarias lazera. Trypanosoma spp. was transmitted to fish by leech vectors (Duijan, 1973; Post, 1983; Kabata, 1985; Brown, 1993 and Woo, 1995).

Infected fish were anemic, anorexic, lethargic with emaciation and splenomegaly, moreover, the mortality rate varied according to the degree of infection and the susceptibilities of fish species (Khan, 1977, 1985; Kabata, 1985; Mehlhorn et al., 1986; Islam and Woo, 1991.b,c and Zintl et al., 1997).

Significant hematological variations in different fish species infected with trypanosoma were recorded by Tandon and Joshi (1973); Khan et al. (1980); Gupta and Gupta (1985); Boon et al. (1990) and Zintl et al. (1997).

The current study aims to determine the prevalence of trypanosomiasis among some freshwater fishes with emphasis on the clinical pictures and influence of seasonal variation. Besides hematological changes in the infected *Chrysichthys auratus*.

MATERIALS and METHODS

I-Materials:

Fish:

A total number of 1300 freshwater fishes were collected from various Nile tributaries at Assiut governorate during winter (average water temperature, 15± 3°C) and summer (average water temperature, 20± 3°C) as shown in the following table:

Fish species	Total number of samples	Number of samples in winter	Number of samples in summer
Chrysichthys auratus	300	150	150
Clarias gariepinus	200	100	100
Mormyrus kannume	200	100	100
Bagrus bajad	200	100	100
Oreochromis niloticus	200	100	100
Labeo niloticus	200	100	100

Fish were transported in tanks aerated with oxygen to the wet lab. At the Dept. of Animal Medicine. Faculty of Vet. Med. Assiut University

II-Methods:

1- Determination of condition factor (K value):

The total length (TL)of fish and the body weight (W) were measured to estimate the condition factor (K value) according to Oni et al. (1983).

This factor is given by the following formula:

$$K \text{ value} = \frac{W \times 100}{(TL)^3}$$

2- Clinical and postmortem examinations:

All the fish were subjected to clinical and postmortem examinations, The external and internal gross lesions were recorded according to Inglis et al. (1993).

3- Collection of the blood:

The blood was collected from each individual fish by section of the caudal peduncle in small plastic vials containing 1 mg Di-potassium

salt of Ethylene Diamine Tetra-acetic acid (EDTA) as anti-coagulant for each 1 mg blood.

4-Examination of the blood smears:

Blood smears were prepared from all examined fishes on clean glass slides and stained with Giemsa-stain. The stained blood smears were examined microscopically for the presence of *Trypanosoma* species according to Lucky (1977).

5-Parasitemias estimation:

The total number of *Trypanosoma* /ml blood were estimated by means of a haemocytometer according to Khan et al. (1980).

6-Haematological parameters:

Erythrocytic counts (RBC_s) hemoglobin content (Hb, g/L), and hematocrit values of the fish species, *C. auratus*, in summer were evaluated according to the methods of Stoskopf (1993).

7-Statistical analysis:

The data were statistically analyzed by the ANOVA.

RESULTS

The most common clinical and postmortem findings observed in fish naturally infected with trypanosomiasis were emaciation , sunken abdomen, pale coloration of gills and swelling in spleen and kidney.

Only 8.7% of examined fish revealed ascitis and 3.3% have yellow discoloration of musculature and skin. These signs were more pronounced in *C. auratus*, while in other fish species no obvious clinical signs were observed.

Table (1) shows that the highest infection rate was found in *C. auratus* (42.3%) followed by *C. garipienus* (8%). While it was 3% , 2.5% for *M. kannume* and *B. bjiad*. There was no infection recorded in *O. niloticus* and *L. niloticus*.

In summer, the percentages of the infection rates were 40%, 6%, 3% and 2% in *C. auratus*, *C. garipienus*, *M. kannume* and *B. bajad* respectively (Table 2). While in winter the percentages of infection with trypanosomiasis were 44.6%, 10%, 4% and 3% in *C. auratus*, *C. garipienus*, *M. kannume* and *B. bajad* respectively, Table (3).

Table (4) revealed that the condition factor of examined *C. auratus* was found to be size free (insignificant correlation with length). The statistical analysis of such factors showed that in summer the

different types of infection did not affect the well being of the fish, whereas, in winter the condition factor reflects such impact of trypanosoma infection ($P<0.0001$).

The degrees of infection in terms of the total number of trypanosoma in 1 ml blood of *C. auratus* were recorded in Table (5).

Except for *O. niloticus* and *L. niloticus*, the examination of the stained blood films taken from all collected fishes revealed the presence of Trypanosoma species (Figures 1, 2, 3 and 4).

Tables (6 & 7) show the degree of infection in different classes of body weight and seasons. From such table, the younger ages of *C. auratus* were more susceptible to Trypanosoma infection.

At ($p<0.05$) level of significance the total hemoglobin, hematocrit and red blood cells were decreased significantly in the medium and heavy infection of *C auratus* . Also, the results revealed a non-significant decrease in total hemoglobin, hematocrit and red blood cells in the light infection (Table 8).

Table 1: Prevalence of trypanosomiasis in some species of freshwater fishes

Fish species	Total number of samples	Number of infected fish	% of infection
Chrysichthys auratus	300	127	42.3
Clarias garipeinus	200	16	8
Morymyrus kannume	200	7	3.5
Bagrus bajiad	200	5	2.5
Oreochromis niloticus	200	0	0
Labeo niloticus	200	0	0

Table 2: Prevalence of Trypanosomiasis in some species of freshwater fish in summer

Fish species	Number of samples	Number of infected fish	Number of non infected fish	% of infection
Chryichthys auratus	150	60	90	40
Clarias garipienus	100	6	94	6
Mormyrus kannume	100	3	97	3
Bagrus bajiad	100	2	98	2
Oreochromis niloticus	100	0	100	0
Labeo niloticus	100	0	100	0

Table 3: Prevalence of Trypanosomiasis in some species of freshwater fish in winter

Fish species	Number of samples	Number of infected fish	Number of non infected fish	% of infection
Chryichthys auratus	150	67	83	44.6
Clarias garipienus	100	10	90	10
Mormyrus kannume	100	4	96	4
Bagrus bajiad	100	3	97	3
Oreochromis niloticus	100	0	100	0
Labeo niloticus	100	0	100	0

Table 4: Basic statistics of the condition factors (K. value) of *C. auratus*

Degree of infection	Summer		Winter	
	Number	Means±S.D	Number	Means±S.D
Heavy infection	24	1.387±0.25	24	1.09±0.24
Medium infection	23	1.442±0.173	34	1.18±0.32
Light infection	13	1.361±0.206	9	1.24±0.36
Free from infection	90	1.396±0.266	83	1.544±0.23

Table 5: Degree of infection according to the number of trypanosoma/ 1 ml blood in *C. auratus*

Degree of infection	Number of Trypanosoma in 1 ml blood	Number of infected samples in summer	Number of infected sample in winter
Heavy infection	More than 30.000	24	24
Moderate	More than 500	23	34
Light	Less than 500	13	9

Table 6: Percentages of occurrence of the degree of infection according to the weight classes of *C. auratus* in winter

Weight classes (g)	Heavy infection		Moderate infection		light infection		Free from infection	
	No.	%	No.	%	No.	%	No.	%
12 --- 23	15	31.9	19	40.4	3	6.3	10	21.2
24 --- 33	7	26.9	10	38.4	1	3.8	8	30.8
34 --- 43	1	3.4	3	10.3	2	6.9	23	79.3
44 --- 53	0	0	2	8	1	4	22	88
54 --- 63	1	7.1	0	0	0	0	13	92.8
64 --- 73	0	0	0	0	2	28.6	5	71.4
74 --- 83	0	0	0	0	0	0	0	0
84 --- 93	0	0	0	0	0	0	1	100
94 --- 103	0	0	0	0	0	0	1	100

Table 7: Percentages of occurrence of the degree of infection according to the weight classes of *C. auratus* in summer

Weight classes (g)	Heavy infection		Moderate infection		light infection		Free from infection	
	No.	%	No.	%	No.	%	No.	%
12 --- 23	3	11.5	3	11.5	1	3.8	19	73
24 --- 33	4	13.3	3	10	4	13.3	19	63.3
34 --- 43	9	30	9	30	4	13.3	8	26.7
44 --- 53	2	6.3	4	12.5	2	6.3	24	75
54 --- 63	2	14.3	1	7.1	0	0	11	78.6
64 --- 73	1	12.5	2	25	1	12.5	4	50
74 --- 83	0	0	0	0	0	0	2	100
84 --- 93	1	20	1	20	1	20	2	40
94 --- 103	2	100	0	0	0	0	0	0
104 --- 113	0	0	0	0	0	0	1	100

Table 8: Means of hemoglobin, hematocrit and red blood cells in naturally *C. auratus* with trypanosomiasis

Blood parameters	Degree of infection	Means	S.D	Range
Hemoglobin (g/L)	Heavy	3.41	0.57	2.4-4.2
	Medium	5.77	0.69	4-7
	Light	7.87	0.57	6.9-8.9
	Free from infection	8.14	0.48	7.3-9.0
Hematocrit (%)	Heavy	10.67	1.24	13-18
	Medium	15.4	1.34	18-23
	Light	23.67	1.05	22-25
	Free from infection	24.00	1.00	22-25
Red blood cells count	Heavy	719.333	91.68	600.000-950.000
	Medium	907.333	92.46	810.000-1.200.000
	Light	1.453.333	235.63	1.100.000-1.800.000
	Free from infection	1.546.667	378.42	1.200.000-1.900.000

DISCUSSION

The most important obtained clinical signs of natural infected fishes with trypanosoma species were emaciation, sunked abdomen, pale coloration of gills, anorexia, swelling in spleen and kidney, ascitis and yellow discoloration of musculature and skin. These clinical findings coincided with those previously described by Tandon and Joshi (1973), Mehlhorn et al. (1986) and Awad (1997) in different species of fishes infected with *Trypanosoma spp*. It was noticed that those clinical signs became more obvious with the increased degree of infection especially in *C. auratus*, While in other species of fishes and during light infection, clinical findings could not be recorded.

Becker (1977) mentioned that alteration of the host physiologically may result in increased numbers of the trypanosoma in blood. While Kabata (1985) recorded that fish with only a few trypanosoma in their blood show no outward signs of infection. while those heavily infected display the usual signs associated with anemia.

The highest prevalence of trypanosoma infection was found in *C. auratus* (42.3%) followed by *C. garipienus* (8%). The lowest infection was recorded in *M. kannume* (3.5%) and *B. bajiad* (2.5%). The infection was prevalent in the aforementioned fish species may be due to the absence or weakness of scales and contact with vector. In addition to that, these fishes are bottom feeders and less active. While the variation in prevalence of infection among these fish species may be due to

susceptibility of the fish to infection. Awad (1997) supported these findings. Also the obtained results were confirmed with Khan (1982) and Khan (1984) who recorded that the prevalence of trypanosoma infection was observed in benthic fish. One of the underling causes of these variations might be the distribution and prevalence of leeches that serve as vectors.

The present study revealed that *O. niloticus* and *L. niloticus* were free from infections., this is may attributed to the presence of hard scales the and activity of those fishes may retard the contact of vectors with fish for transmission of the infection with trypanosoma. Also, susceptibility of those fish species may play a role in retardation of the infection. Meanwhile, Laya (1994) recorded that the prevalence of *Trypanosoma tilapiae* in wild *O. niloticus* was 2.33%.

The results indicated that the rates of infection with trypanosoma species in examined fish species in winter were higher than those in summer. This may be attributed to depression of the immune status of the fish, decrease of food intake in winter and abundance of leeches, where the examined fish are known to be warmwater fishes. Lom (1973) showed that as the temperature slightly above 28°C or below 18°C, the infections developed more slowly and the final parasitaemias were low. Cottrell (1977) recorded that the highest infection levels (20%) of trypanosoma in plaice coinciding with the lowest sea temperatures conversely, the lowest infection levels in July corresponded to some of the highest sea temperatures. Also, the same author added that lower temperatures not only prolonged the latent periods for generation of specific immunity, but also increased susceptibility of fish to pathogenic and opportunistic parasites. Moreover, Cottrell (1977) added also that, the lowest incidence of trypanosoma in plaice in summer may have resulted from the increased immunological competence, conversely, the highest incidence of trypanosoma occurred at the lowest environmental temperatures when the immune response would be more depressed. Gupta and Gupta (1988) revealed that, the highest intensity of infection with *Trypanosoma trichogasteri* in Indian fishes tended to be at the peak in winter.

According to the weight classes of fish, the infection rates with trypanosoma species decreased by increasing the weight of fish (*C auratus*) Tables (6 & 7). This shows that smaller fishes were more susceptible to be infected with trypanosoma than large ones. Tondon and Joshi (1973) found that small *Clrias butrachus* and *Mystus vittatus* were more susceptible to infection with *Trypanosoma majuri* than large ones.

The condition factor findings of the present work revealed that, the susceptibility of the fish to infection increased in winter, which were manifested by emaciation of fish. Whereas, in summer, the fish seemed to be in a well condition. Khan (1984) stated that, the condition factor decreased in the infected fish without referring to seasons or temperatures.

The total hemoglobin (g/L), hematocrit (%) and total red blood cells count were decreased significantly ($P<0.01$), ($P<0.05$) and ($P<0.01$) respectively with the increased degree of infection in *C. auratus*. Such decrease in hemoglobin , hematocrit and red blood cells count may be caused by hemolysins secreted by trypanosoma which destroy and lyse red blood cells and also leads to hemodilution which were manifested by appearance of pale gills, anemia and yellow discoloration on fish. Tandon and Joshi (1973) recorded that both R.B.Cs. and Hb values were higher in a healthy *Clarias butrachus* and *Mystus vittatus* if compared with an infected one. Awad (1997) found that, the case of heavy infection with trypanosoma in fish leads to a significant decrease in R.B.Cs. count, Hb content and PCV%.

The present study led to the following conclusions:

1- The susceptibility of freshwater fishes to trypanosoma infection varies with season, fish species, temperature and size.
2- The results of the monitoring survey of the infected fish must be followed by bioassay to manage the different factors controlling the process of infection.

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LEGENDS

Fig. 1: A blood film showing *Trypanosoma species* from *C. auratus* (X10)

Fig. 2: A blood film showing *Trypanosoma species* from *C. garipienus* (X40)

Fig. 3: A blood film showing *Trypanosoma species* from *M. kannume* (X40)

Fig. 4: A blood film showing *Trypanosoma species* from *B. bajad* (X40)

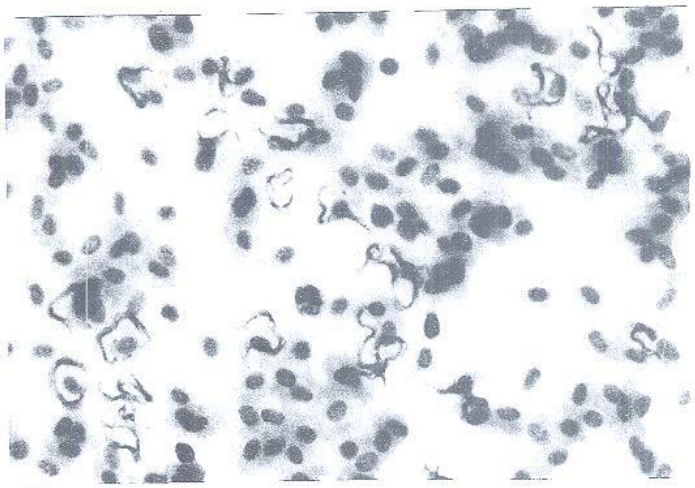

Fig. (1)

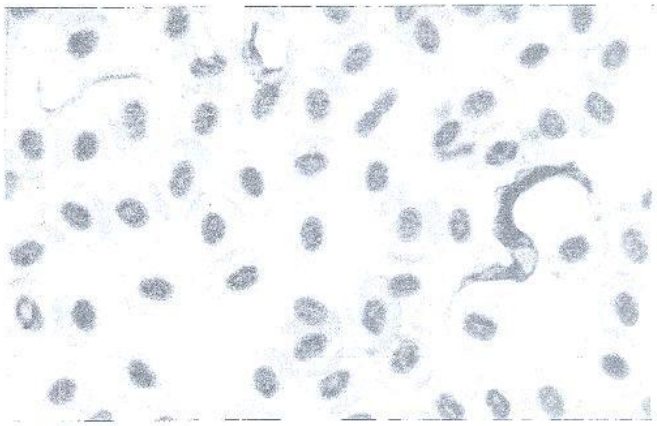

Fig. (2)

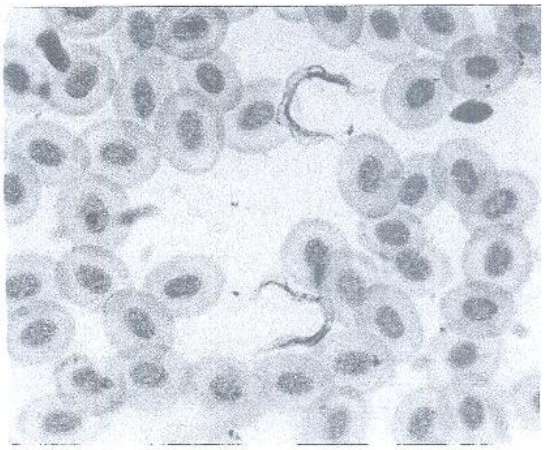

Fig. (3)

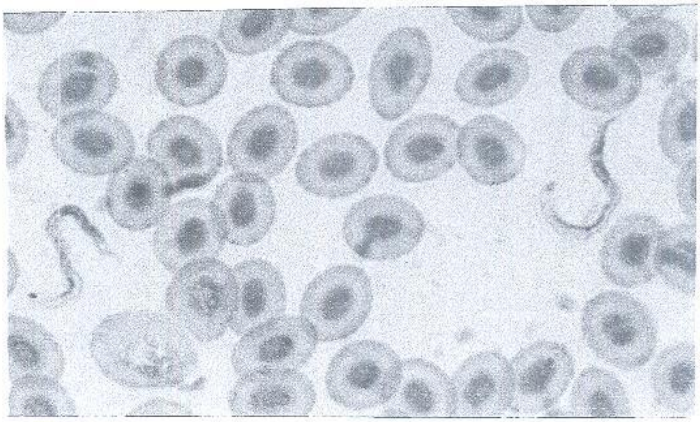

Fig. (4)