Vet.Med.J.,Giza. Vol.46, No.4. A (1998):543 -553.

BLOOD PROTOZOA INFECTING CLARIAS LAZERA IN LAKE MANZALA WITH ELECTROPHORETIC, HAEMATOLOGICAL AND BIOCHEMICAL STUDIES ON TRYPANOSOMA MUKASAI (HOARE, 1932).

By

EZZ EL-DIEN, N. M.* and MOUSA, W. M.*

* Dept. Parasitol., Fac. Vet. Med., Cairo Univ.

SUMMARY

Examination of 180 live catfish, *Clarias lazera* from Lake Manzala was carried out for blood protozoan parasites from January to December 1997. 112 samples (62.2%) were found infected with blood protozoan parasites, *Trypanosoma mukasai* (57.2%), *Babesiosoma aegyptiacus* (9.4%) and *Haemogregarina aegyptiacus* (11.7%). The collected blood parasites were re-described and microphotographed.

Electrophoretic analysis of sera from infected *Clarias lazera* with *Trypanosoma mukasai* showed significant increase in β–globulin with significant decrease in α–globulin and albumin, while the γ–globulin has no significant value. Also, significant decrease in Hb %; PCV and RBcs count with leucocytosis. The biochemical analysis revealed significant decrease in albumin and albumin globulin ratio as well as in iron, however no significant value in globulin.

INTRODUCTION

Blood protozoa infection among the Egyptian fish population attracted minor attention with regard to their pronounced pathological affections which might cause significant economic losses among cultured and wild fish (Hoffman, 1970). *Clarias lazera* is one of the popular economic cat fish species and known as a highly susceptible species for many protozoa infection (El Naffar, 1970 and Fahmy et al., 1971). The present study aimed firstly to investigate the blood protozoa infecting *Clarias lazera* in one of the most important fish sources, Lake Manzala, including incidence and identification.

The other important aim of the present work was to evaluate the humoral immune response against Trypanosoma infection. Moreover, determination of the haematological and biochemical changes resulted from experimental infection of *Clarias lazera* with *Trypanosoma mukasai* were adopted.

MATERIALS AND METHODS

Parasitological examination:

From lake Manzala 180 live catfish; *Clarias lazera* were examined for blood protozoan parasites from January to December 1997. For the rapid diagnosis of haemoflagellates, fresh blood

smears were examined. Thin blood films from heart, gills and caudal artery were obtained as well as impression smear from cut surfaces of viscera after clotting the excess blood (Carleton et al, 1967) were air dried, fixed and stained with Giemsa stain. The detected parasites were measured and illustrated with microphotographs.

Experimental Trypanosomes transmission:

Inoculum preparation:

From *Clarias lazera* fish which proved to be heavily infected with *Trypanosoma mukasai* by examination and appeared to be free from other blood parasites, the blood was drown from the caudal artery into a sterile syringe containing citrated saline with dilution of 1:10 (Negm El Dien, 1991). The number ($2x10^6$) trypanosomes/cm3 of blood was counted using the haemocytometer according to Hoffman, 1977.

Fish inoculation:

Ten alive *Clarias lazera* fish proved to be free from trypanosomes according to Letch, (1979) were kept in aerated glass aquarium with chlorine free tap water, each was intra-peritoneally inoculated with 2ml of the diluted inoculum (Lom, 1973). Every other day blood smears from each inoculated fish were examined for trypanosome detection up to 60 days.

Electrophoretic analysis of serum protein in fish experimentally infected with T. mukasai:

Electrophoretic analysis of sera collected from Trypanosoma free fish before experimental infection (control) as well as sera collected from the same fish after experimental infection with *T. mukasai* was done by polyacrylamide gel columns according to Davis (1964) and Ornstein (1964). 10 ul of the examined sera were placed separately on the surface of the partially polymerized large bar in a cylindrical glass tube containing columns of polyacrylaminde gel, the tube was then fixed vertically into the electrophoresis apparatus.

Electrophoresis was carried out by applying a current of 1.5 ml. amp. / tube for 90 minutes. When electrophoresis was completed, the gel was immediately removed, fixed and stained for 2 hours in 0.25% commassie blue. Clearing of the gel was carried out by washing them several times with de-staining solution (45% methanol, 5% glacial acetic acid and 50%-distilled water). Reading of the gel was carried out by scanning technique described by Harb et al. (1973). The relative mobility (R.m.) values of each zone were determined basically as described by Glick (1968) which was modified by Schellner (1970). That was achieved by dividing its migration distance from the origin and confirmed by the determination of albumin in the same gel, which usually appeared as a heavily stained and prominent band.

Haematological study:

Fresh blood samples were collected from caudal vein of both control and experimentally infected fish 3 weeks post-inoculation by hypodermic syringe coated with 1% EDTA solution. Samples were examined to determine haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count and total leucocytic count according to Kaneov (1985).

Serum samples were obtained from both control and infected fish by centrifugation of blood samples at 3000 rpm. for 5 minutes. Serum iron, total protein and albumin were determined using commercial diagnostic kits from BioMerieux Laboratory Reagents and Products, France. Globulin and albumin/globulin (A/G) ratio were also calculated.

RESULTS

Incidence of the detected blood protozoa:

In 112 (62.2%) out of 180 examined *Clarias lazera* fish were found to harbor protozoa in their blood, including, *Trypanosoma mukasai, Babesiosoma aegyptiacus*, and *Haemogregarina aegyptiacus*. As displayed in table (1).

Table (1): Incidence of the detected blood protozoa:

Total examined	Total		Trypanosoma spp.		Babesiosoma spp.		Haemogregarina spp.	
	Infested	%	Infested	%	Infested	%	Infested	%
180	112	62.2	103	57.2	17	9.4	21	11.7

Morphological description

1. Trypanosoma mukasai (Hoare, 1932), Plate (1)

The parasite was detected in the fresh smear by its active motility. In the stained films, the trypanosomes length ranged from 38 to 65 u with tapered body ends, and were considered to exist in two forms of *T. mukasai*, the small form (Plate 1-A) up to 45u, in which the trypanosomes had a large rounded or oval kinetoplast lying at the bluntly pointed posterior end. The flagellum arises from the kinetoplast and passed forward along a fairly developed undulating membrane reaching the tapered body end and the free flagellum was found. The nucleus was oval in shape measuring 5.1-6.2 (5.6 mean) u by 3.0-4.1 (3.5) u. with its long dimension parallel to the long axis of trypanosome, it lays centrally or slightly forward to the mid point of the body. In the large (Plate 1-B) forms (from 45-65 u in length), the kinetoplast was large, mostly rounded and near the bluntly pointed posterior end. The undulating membrane was well developed and clearly visible for most of its length. The free flagellum was present. The nucleus was oval to round lying somewhat anterior to the mid point of the body and measured 3.0-5.5 (4.3)u by 2.6-5.0 (3.8) u.

2.Babesiosoma aegyptiacus (Negm El Din, 1991), Plate (2):

The parasites were seen intra-erythrocytic and

underwent schizogony resulting in the production of four merozoites arranged in the form of rosette cruciform stage characteristic for the genus. The merozoites were in the form of small slightly curved rods filled with cytoplasm. The chromatin granules were indistinct, and restricted to the body periphery. It measured 4.2-6.4 (5.3) u in length and 1.3-2.6 (1.9) u in width (Plate 2-A). The trophozoites (Plate 2-B&C) were elliptical to oval in shape measuring 6.1-8.2 (7.2) u in length and 4.1-6.2 (5.2) u in width. The cytoplasm appeared as faint light blue strands throughout the body but more densely near the periphery where the chromatin granules were condensed. The process of schizogony was noticed in stages started by early schizont, the binucleated schizont (Plate 2-D) was 6.8-7.9 (7.4) u by 3.0-4.1 (3.5) u. and the tetranucleated schizont (Plat2-E, F &G) was 6.9-8.0 (7.5)u by 4.0-5.3 (4.6)u. The later became constricted at the middle with a cleft at each pole and as a result of cytoplasmic cleavage. The typical rosette cruciform stage was formed (Plate 2-H) and lastly, 4 merozoites each was capped with chromatin material were detected (Plate 2-I&J). Forms interpreted as gametocyte were large and lacked the central vacuole, those were differentiated as macrogametocyte (Plate 2-L) were more rounded and the nucleus was huge, deeply red stained, while the microgametocytes (Plate 2–K) were club or crescent in shape with huge faint red nucleus.

3. Haemogregarina aegyptiacus (Negm El Din, 1991), Plate (3)

The parasite was represented by the intra-erythrocytic asexual and sexual developmental stages. The trophozoite (Plate 3-A) was sausage in shape with rounded ends, measuring 8.5-9.6 (9.05) u in length and 3.2-4.3 (3.75) u in width. An elongated narrow chromatin mass was found near one of the body poles, representing the nucleus. The cytoplasm was deep blue in color, coarsely granulated containing 1-3 vacuoles. The repeated nuclear division of the trophozoite resulted in the formation of elongated early binucleated schizont (Plate 3-B) and the tetranucleated schizont (Plate –C,D&E), was 9.2-9.6 (9.4) u in length and 6.0-6.5 (6.3) u in width, it appeared in the form of amoeboid mass containing four faint red irregularly distributed chromatin masses and a single vacuole. The fully developed schizont (Plate 3-F) contained 4 nuclei in the form of four short beaded shaped chromatin mass chains with the cytoplasm longitudinally arranged around them. The process of longitudinal fission resulted in the production of daughter merozoites (Plate 3-G). Macrogametocyte (Plate 3-H) was pear shaped with one end more attenuated than the other measuring 11.5-12.5 (12.05) u in length and 4.8-5.5 (5.2) u in width. The nucleus was large, deep red in color and filled about 2/3 of the body size. The microgametocyte (Plate 3-I) was differentiated by its larger size 12.1-12.6 (12.3) u in length and 5.1-5.6 (5.4) u in width, the more rounded body ends, and the faint red stained nucleus which filled about 1/2 the body size. It was important to mention that impression smears of viscera of all the examined fishes revealed negative results for all the detected parasites.

Experimental studies:

The experimental infection of *T. mukasai* revealed the detection of both small and large form of *T. mukasai* up to 60 days post-infection but it was noticed that with the duration of the infection, the trypanosome body length increased and the range of length observed in the experimental infection was narrower than that in natural infection, also the number of large forms of the trypanosomes was gradually predominate the small form.

Electrophoretic analysis of sera collected from fish experimentally infected with *T. mukasai* were displayed in Plate (4) and Fig (1). It was cleared that the percentage of β-globulin was significantly increased. On the contrary there were significant decreased in α-globulin and albumin, while there were no significance in the mean values of γ-globulin.

Table (2): Blood pattern of experimentally infected fish with T. mukasai.

	Hb	PCV	RBcs	WBcs
Mean of infected group	7.3±1.41	21.9±1.41	3650000±2.35	83000±1.65
Mean of control group	13.55±2.51	40.65±2.51	6775000±2.86	60000±1.35

Table (3): Biochemical parameters in serum of experimentally infected fish with T. mukasai.

	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	Iron (ug/dl)
Mean of infected sera	3.43±1.22	1.43±0.36	2.00±0.63	0.72±0.13	246.10±1.87
Mean of control sera	3.87±1.34	1.93±0.49	1.94±0.52	0.99±0.15	409.65±2.63

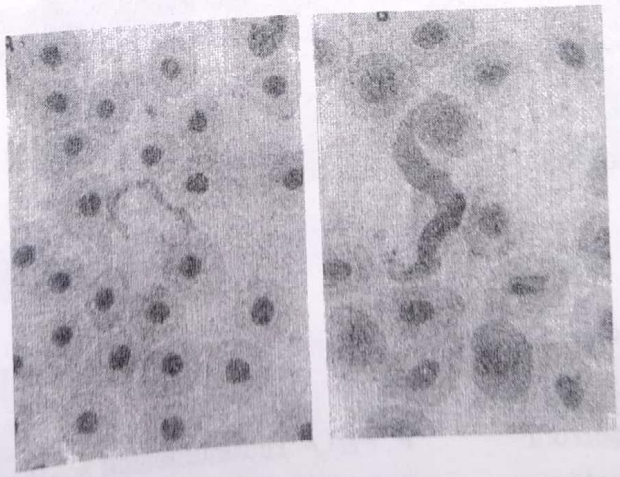

Plate (1): *Trypanosoma mukasai* from naturally infected *Clarias lazera*. (X1000).
A- Small form. B- Large form.

Haematological examination revealed results of the effect of *T. mukasai* on the haemogram of infected fish were recorded in table (2). It can be seen from the obtained result that the values of PCV, Hb and RBcs count in experimentally infected fish were significantly decreased. The pattern of the total white blood cells in infected fish showed significant increase. Results of serum biochemical constituents in control and infected fish were shown in table (3). They revealed significant decrease in the values of total protein, albumin and albumin/globulin (A/G) ratio. Insignificant changes were recorded in the values of globulin. Concerning the mean values of iron, it was revealed that they were significantly decreased.

DISCUSSION

The present study dealt with the blood protozoa infecting *Clarias lazera* fish from lake Manzala in Egypt revealing three protozoan species. *Trypanosoma* species under investigation was morphologically identified as *T. mukasai* (Hoare, 1932), according to Haiba (1963), Imam et al. (1985), Abo El Wafa (1988) and Negm El Din (1991). Small and large forms were detected indicating the dimorphic trypanosome as described by Baker (1960). The detected *Babesiosoma aegyptiacus and Haemogregarina aegyptiacus* were morphologically similar to the original description given by Negm El Din (1991) from Nile *Clarias lazera* and *Chrysichthys*

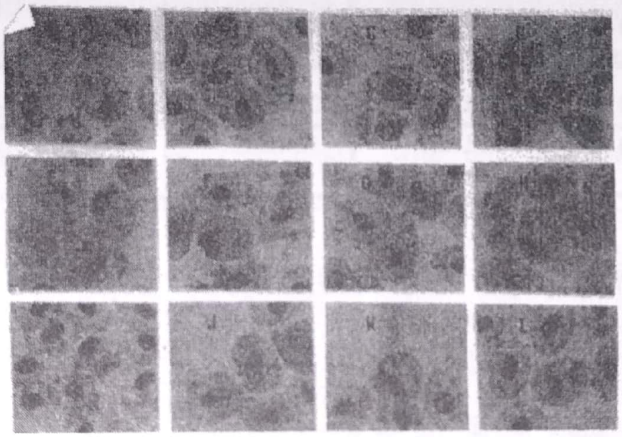

Plate (2): *Babesiosoma aegyptiacus* from naturally infected *Clarias lazera.* (X 1000).

A- Merozoite. B&C- Trophozoite.
D- Binucleated schizont. E, F&G- Tetranucleated schizont.
H- Rosette stage schizont. I&J- Daughter merozoite.
K- Microgametocyte. L- Macrogametocyte.

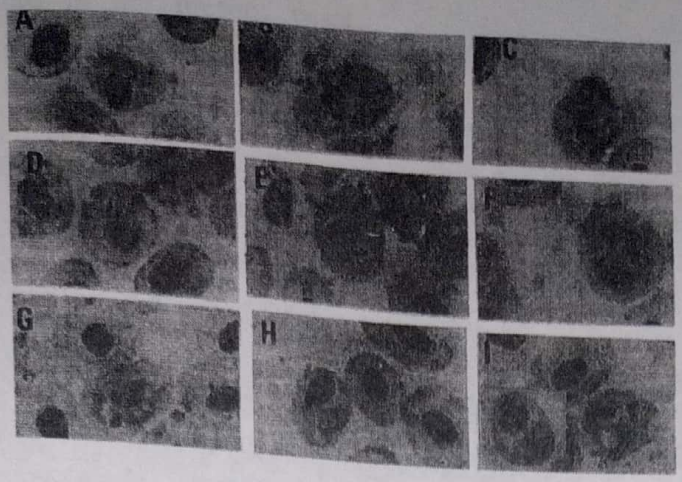

Plate (3): *Haemogregarina aegyptiacus* from naturally infected *Clarias lazera*. (X 1000).

A- Trophozoite.
B- Binucleated schizont.
C, D&E- Tetranucleated schizont.
F- Schizont.
G- Daughter merozoite.
H- Macrogametocyte.
I- Microgametocyte.

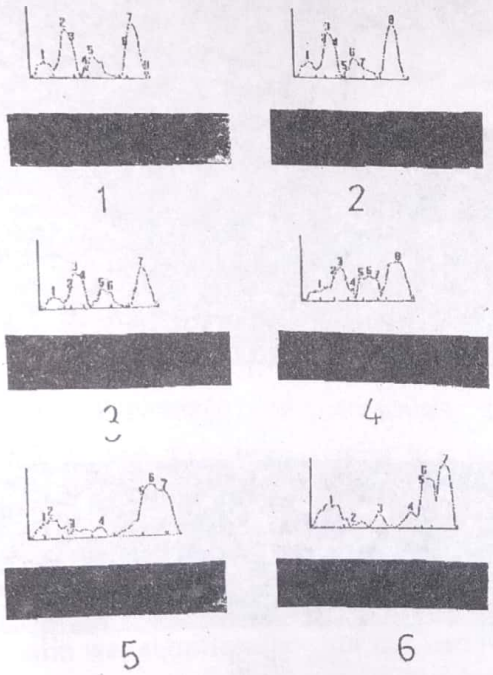

Plate (4): (1, 2, 3, 4) Electrophoretic analysis of sera from fish experimentally infected with *Trypanosoma mukasai*.

(5, 6) Electrophoretic analysis of sera from non-infected fish.

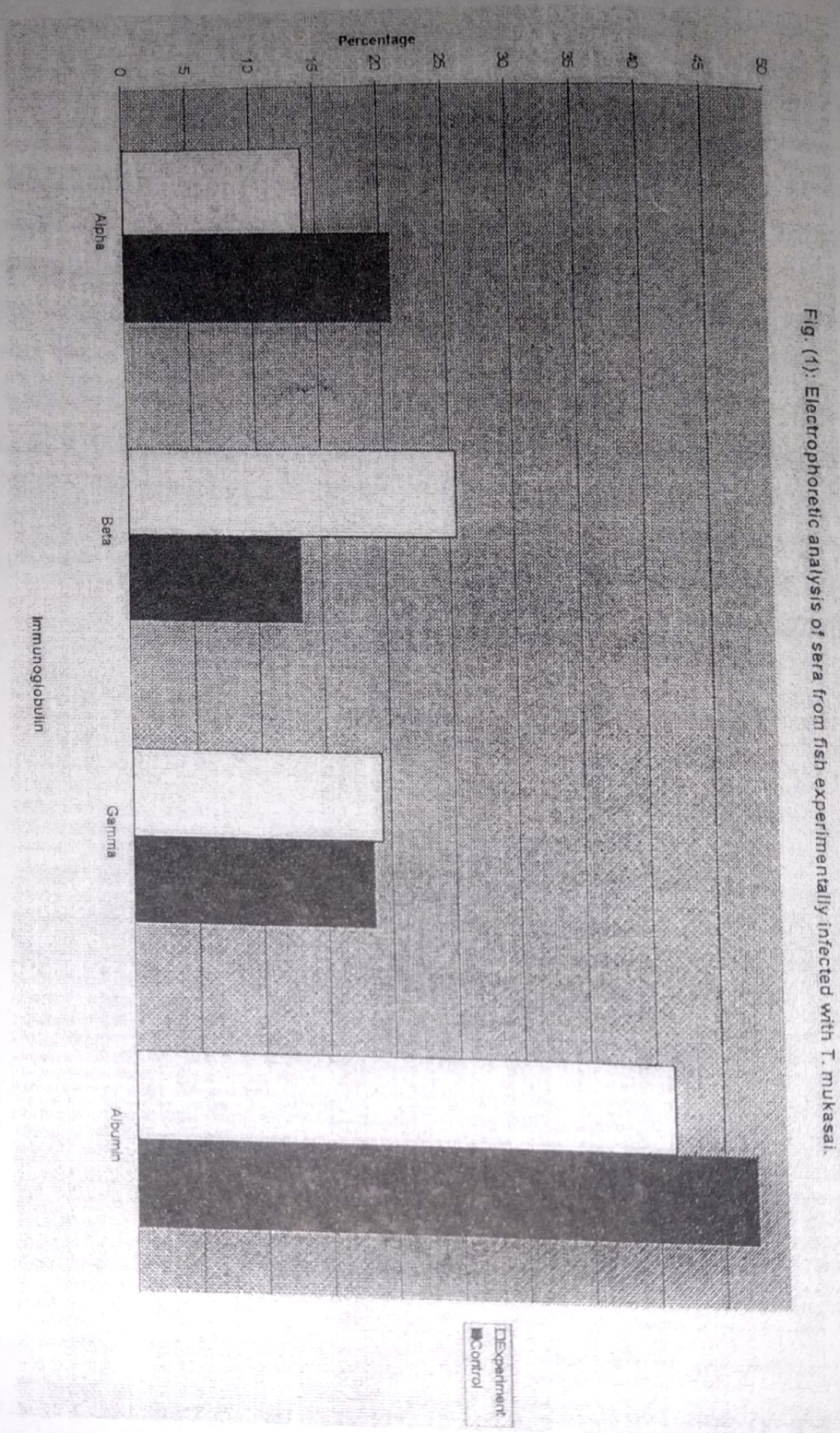

Fig. (1): Electrophoretic analysis of sera from fish experimentally infected with T. mukasai.

auratus respectively, so in the present work, *Clarias lazera* is considered as a new host for *H. aegyptiacus* in a new locality record (Lake Manzala). The incidence of blood protozoa was 62.2% among the examined *Clarias lazera* in which *T. mukasai* was particularly with high incidence 57.2%. *T. mukasai* was reported from the same fish species but with relatively lower incidence by Haiba, 1963 (14%), Abo El Wafa, 1988 (9%), Alyain, 1990 (11.4%), Negm El Din, 1991 (50%) and Alyain et al., 1994 (21%). *B. aegyptiacus* was reported also in higher incidence (9.4%) than that given by Negm El Din, 1991 (5.47%) from the same host at Sharkya and Khalubia governorates.

Experimental transmission of *T. mukasai* between *Clarias lazera* was succeeded and could considered as a second report but among new host, although Negm El Din (1991) transmitted other *Trypanosoma* species in Egypt between *Chrysichthys auratus* fish.

In the present study, electrophoretic analysis of sera collected from fish experimentally infected with *T. mukasai* as well as sera from non-infected fish were investigated. It was cleared that no significant changes in the percentage of γ-globulin. These results were in agreement with Barrow (1954& 1955) who reported the serum antibodies in fish against trypanosomal infection were very low. Also the present study revealed that the percentage of β-globulin was significantly increased, these result were in agreement with Cottrell (1976&1977) who reported the β-globulin more prominent in the serum of fish infected with *T. platesae* which might be associated with antibody production. So the present work revealed that the humoral immunity has no important role for protection of fish against infection with *T. mukasai*. These data are carried out for the first time in Egyptian media.

The lack of standard methods and the survey approach to fish haematology has resulted in little baseline information of value for clinicians interested in the diagnosis of disease (Stoskopf, 1993). Ferguson (1989) recorded that anaemia in fish resulted from either exposure to bacterial haemolysins or also occurred with heavy infection of trypanosomes. Anaemia caused in infected fish might be attributed to functional iron deficiency (Jain, 1986). The obtained anaemia might cause defect in iron containing myeloperoxidase enzyme, which constitutes one of the important oxidative mechanism (Davis and Drutzmills, 1982). Also these results are in agreement with these described by Gupta and Gupta (1985) who reported significant decrease in Hb% and RBcs count among *Clarias batrachus* infected with *T. batrachi*. The observed leucocytosis in infected fish with *T. mukasai* might be caused by a secondary bacterial infection occurred during migration of the parasites (Coles, 1986). Regarding the serum biochemical constituents, our data revealed marked decrease in the values of total protein, albumin and A/G ratio in serum of infected fish. It is worth to mention that (Jones and Junt, 1983) had reported that the presence of trypanosomes in the blood and possible

involvement of the liver and other organs with trypanosome antigens resulted in lowering of total protein and albumin. Both authors added that, hepatic dysfunction during the course of trypanosomiasis have been described.

ACKNOWLEDGMENT

The authors would like to express their thanks to Dr. Bashandy, M. staff member of clinical pathology division, Fac. Vet. Med., Cairo Univ. for his help and different facilities to complete this study.

REFERENCES

Abu El Wafa, S. A. D. (1988): Protozoa parasites of some fresh water fishes in Behera governorate. M. V. Sc. Thesis, Fac. Vet. Med. Alex. Univ. Egypt.

Alyain, S. A. (1990): Studies on certain internal protozoa infections of Egyptian fresh water fish. Ph. D. Thesis, Alex. Univ. Egypt.

Alyain, S. A.; Soheir, M. A. El Menyawe and Mahmoud, N. A. M. (1994): A revision study on some protozoa infections in Nile cat fish (Clarias lazera) in Upper Egypt after 20 years. Vet. Med. J. Giza. 42(2): 21-26.

Baker, J. R. (1960): Trypanosomes and dactylosomes from the blood of fresh water fish in East Africa. Parasitol. 50: 515-526.

Barrow, J. H. (1954): Observations of some host specificity and immunological reactions of trypanosome infections in some fresh water fish of Europe. Anat. Rec. 120: 750-751.

Barrow, J. H. (1955): Social behavior in fresh water fish and its effect on resistance to trypanosomes. Proc. Nat. Acad. Sci. 41: 676-679.

Carleton, M. A.; Druvy, R. A.; Wallinton, F. A. and Cameron, H. (1967): Carleton's histological technique 4th ed. Oxford Univ. Press New York, Toronto.

Coles, E. M. (1986): "Vet. Clinical pathology". 4th ed., W. B. Saunders Comp., Philadelphia, London, Toronto.

Cottrell, B. J. (1976): The immune response of plaice, Pleuronectes platessa to tissue parasites. Parasitol. 24: 74.

Cottrell, B. J. (1977): A trypanosome from the plaice Pleuronectes platessa L. J. Fish Biol. 11: 35-47.

Davis, J. and DrutzMills, J. (1982): "Immunity and infection". In Stites DP. Stobo J D, Fudenberg HH, Wells JV (eds) "Basic and Clinical Immunology" 4th ed. Lod Altos, CA: Lange Medical Public. P. 209.

Davis, P. J. (1964): Disc electrophoresis. Method and application to human serum proteins. J. Ann. NY. Acad. Sci. 121: 409.

El Naffar, M. K. (1970): Studies on parasites of Nile fishes (some parasites in Assiut province). Ph. D. Thesis, Fac. Sci., Assiut Univ. Egypt.

Fahmy, M. A.; Mandour, A. M. and El Naffar, M. K. (1971): A survey of Myxosporidia of fresh water fish collected from River Nile at Assiut province. J. Egypt Soc. Parasitol. 4&5: 93-102.

Ferguson, H. W. (1989): "Systemic pathology of fish". Iowa state Univ. Press. Ames, Iowa, USA.

Glick, B. (1968): Serum protein electrophoresis patterns in acrylamide gels patterns from normal and bursa-less birds. Poult. Sci. 47: 807.

Gupta, N. and Gupta, D. K. (1985): Haematological changes due to Trypanosoma batrachi and T. aligaricus infection in two fresh water teleosts. Angewandte Parasitologie. 26: 193-196.

Haiba, M. H. (1963): On the Nile fish parasites in Egypt part II. A new host record with detailed study of Trypanosoma mukasai, Hoare, 1932 in the Egyptian Nile fish Clarias lazera. J. Arab. Vet. Med. As. 23: 27-34.

Harb, N.; Hafeez, M. A. and Voaden, D. J. (1973): A regulated temperature disc electrophoresis apparatus. Chem. And Indust., P. 643.

Hoare, C. A. (1932): On protozoa parasites collected in Uganda. Parasitol. 24: 210.

Hoffman, G. L. (1970): Parasites of North American fresh water fishes. Univ. Calif. Press., Berkley and Los Angeles 21.

Hoffman, G. L. (1977): Methods for the diagnosis of fish diseases. Trans. From Zdenek Lucky, Franklin Book Programs Inc. Cairo.

Imam, E. A.; Marzouk, M. S. M.; Hassan, A. A.; Derhally, F. S. and Itman, R. H. (1985): Studies on blood parasites infection in Nile fishes. J. Egypt Vet. Med. Ass. 2: 97-108.

Jain, N. C. (1986): "Shalm's Veterinary Hematology." 4th Ed. Lea and Febiger, Philadelphia, USA.

Jones, C. T. and Hunt, R. H. (1983): "Vet. Pathology". 5th. Ed., Lea and Febiger, Philadelphia.

Kaneov, A. E. (1985): "Vet. Hygiene in fish farming". Moscow: 140-149.

Lom, J. (1973): Experimental infections of fresh water fishes with blood flagellates. J. Protozool., 20(4): 537.

Negm El Din, M. (1991): Morphological and biological studies on some blood parasites of fresh water fishes of Egypt. Ph. D. Thesis, Parasitol. Fac. Vet. Med. Zag. Univ. Benha, Egypt.

Ornstein, L. (1964): Disc electrophoresis, background and theory. J. Ann. NY. Acad. Sci. 121: 321.

Schellner, H. P. (1970): Gesanteiweissbestimung und elektrophoretische aufirennung in polyacrylamid gel puten seren. Archiv fur Geflugelkunde, 2: 72.

Stoskopf, M. K. (1993): "Fish medicine". W. B. Saunders Company, Harcourt Brace Jovanovich, Inc. Philadelphia, London, Toronto.