Diagnosis of some internal parasitic diseases in some freshwater Fishes

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ABSTRACT

The present study was carried out on three freshwater fishes *Oreochromis niloticus* (wild and cultured), *Clarias gariepinus* and *Lates niloticus* which were collected from different localities from February to November (2013). The infested fishes showed no pathognomonic clinical signs except slight abdominal distention. Fish species subjected to parasitological revealed presence of encysted metacercaeiae which including (*Clinostomum spp, Euclinostomum spp, Haplorchid, Cyanodiplostimatid* and *Prohemistomatide*). Nematodes are including *(Procamallanas laeviconchus, Paracamallanas cyathopharynx* and *contracaecum spps),* Cestodes including *Polyonchobothrium clarias and* Acanthocephalan including *Acanthocentis tilapiae and Neoechinorhychus rutilli*. The highest seasonal prevalence of internal parasites was found in spring followed by summer then autumn and winter also the highest prevalence of the isolated parasites was observed in *Lates niloticus* followed by *Clarias gariepinus* then wild *Oreochromis niloticus* and cultured ones. The PCR amplification of Internal Transcribed Spacers (ITS-1) region of DNA obtained from *Contracaecum species* of *Lates niloticus* and *Oreochromis niloticus* showed different PCR products related to *Contracaecum spps*.

Keywords: Freshwater fishes, internal parasitic diseases, molecular diagnosis, prevalence

1. INTRODUCTION

Internal parasitic diseases reduce fish production by affecting their normal physiology and had detrimental effects on the function of affected organs in addition they induce health hazard of man and other invertebrates that consume such infested fish **(Noga 2010 and Lima dos Santos and Howgate, 2011**). The clinical picture of infested fish with internal parasitic diseases revealed no pathognomonic abnormalities on the external body surface such fish were shown emaciation and postmortem showed that the internal organs were appeared anemic with enlargement and congestion (**Ibtsam, 2004**). Heavily infested fish with Tapeworm (cestodes) and digenetic trematodes have a distended abdomen that led to pathological changes in infested organs, while pathogenic effects of acanthocephalans are due to attachment of the adult parasite in the digestive tract by the proboscis, (**Amany Abbass, *et al.* 2006**).

The nematode superfamily Ascaridoidea contains approximately 52 genera, and many species of these parasites are of medical or economic significance (**Nadler *et al.* 2000**). *Contracaecum* larvae are difficult to differentiate into species except when using molecular analysis or alternatively infecting experimental hosts to obtain adult worms, (**Aloo, 2001 and Barson and Avenant, 2006).** The objective of this investigation was to study the total prevalence, seasonal dynamics and diagnosis of internal parasitic diseases in some cultured and wild freshwater fishes.

2. MATERIALS AND METHODES

2.1. Examined Fishes:

A total number of 643 fishes; including 348 (228 wild *Oreochromis. niloticus* (*O. niloticus*) and 120 cultured ones ,(219) *Clarias gariepinus (C. gariepinus*) and (76) *Lates niloticus (L. niloticus*) which were collected from different localities of El – Riah El- Tawfiki and its tributaries and some fish farms in Kafr Elshikh Governorate from February to November (2013). . Alive and freshly dead fishes were transferred to the laboratory of Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Moshtohor, Benha University as quickly as possible where clinical and parasitological examinations were carried out.

2.2. Clinical and postmortem examination:

The collected fishes were subjected to clinical and postmortem examinations, according to the method described by (**Noga, 2010).**

2.3. Parasitological examination:

Encysted metacercariae were carefully examined according to **Lucky, (1977).** Excystation of recovered encysted metacercariae and examination of gastrointestinal tract for nematodes and cestodes according to **kruse and Pritchard, (1982)**. The collected cestodes were left in refrigerator at 4 °C till complete relaxation. Then, they were fixed in 5% formalin for permanent preparation and stained with Acetic acid alum carmine stain then cleared and mounted in Canada balsam. The collected nematodes were kept in glycerin alcohol then washed in 70% ethyl alcohol, cleared in lacto phenol and mounted in glycerol gelatin, **(Meyer and Olsen, 1992)**. Larvae of contracaecum were preserved according to **Cai *et al*, (2010)** for molecular diagnosis.

2.4. Molecular diagnosis:-

Extraction of genomic DNA was performed according to **McManus and Bowles (1996)** with slight modification: Briefly, nematodes were washed in physiological saline several times and kept overnight. Genomic DNA was isolated from individual specimen by the following three basic steps; a- cell lysis using a detergent (lysis buffer 2X), b – removal of proteins (proteinase K) and c- ethanol precipitation of nucleic acids at cold temperatures (at -20ºC for 30 min).

PCR reaction: The PCR was used to amplify the Internal Transcribed spacers (ITS-1) region with primer sets for family *Anisakidae* (NC2 r 5'- TTA GTT TCT TTT CCT CCG CGT 3') and NC5 f 5'- TAG GTG AAC CTG CGG AAG GAT CAT 3') according to **Umehara *et al.* (2008).** The PCR (in a volume of 25 µl) was

performed in 5 µl green mix, 2 µl forward primer, 2 µl reverse primer, 5µl Tamplate DNA and 11 µl nuclease free water in a thermocycler under the following conditions: 95°C for 5 min (initial denaturation), followed by 30 cycles of 94°C, 30 sec (denaturation), 55°C, 30 sec (annealing), 72°C, 75 sec (extension), a final extension of 72°C for 10 min. and cooling at 4°C forever. PCR product was confirmed by1.5 % (w/v) agarose gel electrophoresis (containing 0.241g/ml Ethidium bromide) then visualized under ultraviolet light and photographed using a gel documentation system.

3. RESULTS

Clinical picture:

The infested fishes with internal parasitic diseases showed postmortem lesions as swelling and congestion in intestinal wall which distended with yellowish exudates and enlargement and congestion of spleen and liver (Figure 1, A B, C).

Parasitological examination:

Clinostomum spp were isolated from the branchial cavity of wild *O. niloticus* appeared as yellow nodules attached on or embedded in the branchiostegal musculature. The excysted metacercaria was elongated and whitish yellow in color and characterized by two very long intestinal caeca, two large suckers one oral and other ventral, (Figure 2A, B).The total prevalence was 12. 2% with seasonal prevalence of 6.6, 27.7, 14.5 and 0 % in spring, summer, autumn and winter, respectively. (Table 3) *Euclinostomum spp* were found in tissues of the anterior and posterior kidneys of *O. niloticus*. They appear as round to oval grayish black cysts. The excysted metacercariae appeared large leaf like. The ventral sucker was larger than oral sucker, (Figure 3A, B). The total prevalence was 18. 3% with seasonal prevalence of 20.0, 27.3, 19.3 and 6.7 % in spring, summer, autumn and winter, respectively. (Table 3). *Haplorchid metacercariae* were isolated from musculature, gill filaments, liver, spleen and kidneys of *O. niloticus* and *C. gariepinus* characterized by spherical to oval in shape. The cyst wall was double layers and transparent, (Figure 4A, B). The excysted metacercariae were elongated and the oral sucker appeared clearly in some specimens, (Figure 4C). *Cyanodiplostimatid* were obtained from musculature of *C. gariepinus*. The color of cyst varied from whitish opaque to yellowish in color, round and large macroscopic cysts, (Figure 5A, B). The excysted metacercariae appeared elongated oval in shape, (Figure 5C). *Prohemistomatide* were observed in musculature, gill filaments, liver, spleen and kidneys of infested *C. gariepinus*. The encysted metacercariae were spherical or sub spherical in shape with double walled cyst and their color varied from grayish white to yellowish brown, (Figure 6A, B, C). The excysted metacercariae were elliptical to oval in shape and the ventral sucker is smaller than oral sucker, (Figure 6D). The total prevalence of different species of encysted metacercariae was 46.9% with seasonal prevalence of 58.8, 62.9, 48.7 and 17.1 % in spring, summer, autumn and winter, respectively. (Table 3)

Nematodes:

Procamallanus laeviconchus was recovered from stomach of *C. gariepinus*. Only female specimen was detected and described as larvaeparous nematodes with chitenous cup- shaped buccal capsule, devoid of cutting plates. The tail of female was narrowed gradually and terminates with three processes. Uterus occupies most space in its body cavity, filled with motile first- stage larvae and eggs of different stages of development, (Figure 7A, B, C). The total prevalence was 10.3% with seasonal prevalence of 7.1, 17.9, 11.1and 4.9 %in spring, summer, autumn and winter, respectively. (Table 3) *Paracamallanus cyathopharynx* was isolated from the posterior part of intestine of *C. gariepinus*. It was transparent yellowish in color with transferse annulations. The buccal capsule was large with funnel shaped prostome and supported by 8 longitudinal ridges ending anteriorly by teeth. The esophagus was of the cylindrical type and was divided into muscular and glandular parts. The tail was tapering and had two unequal spicules in the male as it was curved ventrally. Females were larger than males. The tail was conical, long and ending with narrow processes, (Figure 8A, B, C).The total prevalence was 6.6% with seasonal prevalence of 7.7, 8.2, 5.6 and 4.8%in spring, summer, autumn and winter, respectively. (Table 3).*Contracaecum spp* was isolated from the abdominal or pericardial cavity of *O. niloticus*, (Figure 9 A, B, C). The total prevalence was 4.6% with seasonal prevalence of 0, 18.2, 0 and 0 %in spring, summer, autumn and winter, respectively. (Table 3) and *L. niloticus* (Figure 10 A, B, C). The total prevalence was 84.8% with seasonal prevalence of 81.5, 90, 86.9 and 80.9%in spring, summer, autumn and winter, respectively. (Table 3).They appeared as third-stage larvae (L3). The body was relatively thick, Cuticle annulated, forming collar at the anterior end. Tail sharply pointed, with spine.

Cestodes:

Polyonchobothrium clarias was the only cestodes recovered from stomach and intestine of *C. gariepinus*. Fresh worms were contracted and appeared whitish in color which flat or cylindrical in shape and its scolex is rectangular with slightly raised apex (rostellum). The apex is divided into 2 semicircles. The hooks located at the end of each semicircle. The immature proglottides of storbila not completely segmented while some mature segments appeared fused. The testes were modularly while uterus anterior to ovary which appeared highly folded and occupying the greater portion of gravid segment. The gravid segments were loaded with spherical eggs of variable sizes, (Figure 11 A, B, C). The total prevalence was 11.6% with seasonal prevalence of 17.6, 11.1, 7.7 and 9.8 %in spring, summer, autumn and winter, respectively. (Table 3)

Acanthocephalans:

Acanthosentis tilapiae were collected from the intestines *O. niloticus* characterized by thorny headed worm with anterior narrow retractile proboscis and abroad bulb-shaped posterior body. The parasite tegument has a series of alternative folds and a large number of pores. The testes were unequal in size and usually slightly overlapping each other, (Figure 12A). The total prevalence was 1.5% with seasonal prevalence of 6.19, 0, 0 and 0 % in spring, summer, autumn and winter, respectively. (Table 3). *Neoechinorhychus rutilli* was collected from the intestines *C. gariepinus*. The body of the worm is elongated and cylindrical with truncate ends.

The body was covered with a thin tegument followed by a much thicker hypodermis, (Figure 12 B, C). The total prevalence was 4.7% with seasonal prevalence of 18.8, 0, 0 and 0 %in spring, summer, autumn and winter, respectively. (Table 3). Total prevalence of internal parasitic diseases was (67.5%) in wild *O. niloticus* while (30%) in cultured ones. In *C. gariepinus*; total prevalence was (74.88%) while in *L. niloticus* was (85.5%). The seasonal prevalence of infested wild *O. niloticus* was 43.3, 78.33, 71.08 and 63.63% in winter, spring, summer and autumn, respectively, while in cultured *O. niloticus* was 33.3, 13.2, 21.8 and 46.42 % in winter, spring, summer and autumn, respectively. In addition; *C. gariepinus* was 58.5, 81.17, 76.92 and 74.55 % in winter, spring, summer and autumn, respectively while in *L. niloticus* was 71.4, 81.5, 95.0 and 86.95% in winter, spring, summer and autumn, respectively. (Table 1). The prevalence of the internal parasitic diseases in relation to the sex of *O. niloticus* and *C. gariepinus;* in wild *O. niloticus;* the prevalence was 74.8 and 57%in female and male, respectively while in cultured *O. niloticus*; the prevalence was 29.7 and 30.1% in female and male, respectively. In addition, the prevalence in *C. gariepinus* was 89.1 and 58% in female and male, respectively. (Table 2).

- Molecular characterizations of *Contracaecum* species:

  The PCR amplification of (ITS-1) region of DNA obtained from *Contracaecum spp* of *L. niloticus* showed band at 100 bp while in *O. niloticus* showed band at 150 bp (Figure 13)

3. DISSCUSION

The two main targets of the present study are the investigation of the prevalence of internal parasitic diseases affecting some freshwater fishes and molecular diagnosis of *Contracaecum species* which were isolated from *O. niloticus* and *L. niloticus*.

Regarding the encysted metecercariae clinical signs and postmortem examination; *Clinostomum spp* appeared as yellowish white to orange pea- like cyst measuring 1.5 to 3.5 mm. They were arranged in groups and appear as grape like structure in the branchiostegal musculature and branchial cavity which led to severe gill damage, **(Amany abbass, 1997, Eissa, 2002 and Abd El- Latif, 2007**). *Euclinostomum spp* appeared as round to oval grayish black cysts and give the area around it faint black color, (**Ehssan *et al.* 2012**).The encysted metacercariae infestation of *C. gariepinus* appeared as nodules of varied sizes and reached to hundreds in number in heavily infested cases. Presence of encysted metacercariae may lead to low weight gain, immarketability of infested fish and high mortality. The damage caused by encysted metacercariae can occur to the fish may due to the cercarial larvae first invade through the skin, (**Abd El- Latif, 2007 and Shehab El-Din, 2008)**. Concerning the clinical signs and postmortem lesions of intestinal helminthes in *O. niloticus* and *C. gariepinus* (*Procamallanus laevichoncus* , *Polyonchobothrium clarias*, *Paracamallanus cyathopharynx* and Acanthocephalans) were swelling, congestion, accumulation of yellowish exudates, emaciation, anemia,

loss of body condition and tissue alteration or destruction may be due to proteolytic enzymes that discharged from the adult parasites, mechanical blockage or decrease nutrient absorption from the gut, (**Eissa, 2002 and Mahmoud *et al.* 2006**). Examination of *O. niloticus* and *L. niloticus* showed that presence of third larval stage of *Contracaecum spp*. The main clinical signs of infested fishes were emaciation and abdominal distention and mass mortalities. Accumulation of parasite in the abdominal cavity may make pressure atrophy in the internal organs that affect on their functions which may explain the mass mortalities, (**Aloo, 2002 and Shamsi and Aghazadeh, 2010).**

Regarding the total prevalence of internal parasitic diseases; the infestation rate was higher in *L. niloticus* (85.5%) followed by *C. gariepinus* (74.88%) then *O. niloticus* (67.5%) while lower rate was recorded in cultured *O. niloticus* (30%). This result could be attributed to their feeding behavior where *L. niloticus* is a carnivorous fish; they feed on what is most available and close to them such as detritus, water invertebrates like arthropods, mollusks, mud and young fish. Among these invertebrates, there may be intermediate hosts of various parasites, **(Shamsi and Aghazadeh, 2010 and Adel *et al.* 2013).** In *C. gariepinus*; the infestation rate was 74.88% could be attributed to its feeding behavior as a carnivorous fish (bottom feeder) that assists in the transmission of more enteric parasites through feeding on aquatic animals that harbor the infective stage of these parasites or even young infested fish. In addition, such fish are scaleless, this may permit the penetration of the infective stages into the external body surface. Nearly similar results were reported by **El Seify *et al.* (1997)** 81.3% and **Eissa *et al.* (2009)** 81%. The lowest rate was detected in cultured *O. niloticus* (30%) could be attributed to its shortage period to reach marketable size and cultured *O. niloticus* depends mainly on artificial food and its requirements from natural food are very low.

The total seasonal prevalence of internal parasitic diseases; in wild *O. niloticus* and *C. gariepinus*, the highest rate was recorded in spring followed by summer then autumn and the lowest infestation rate in winter. This could be related to the availability of intermediate hosts of these parasites at these seasons and increase the feeding activity in warm temperature. These results agree with the findings recorded by **Negm El- Din *et al.* (1988) and Gihan Shager (1999)** who found the peak of infestation in spring followed by summer then autumn and winter. Regarding the prevalence of internal parasitic diseases in relation to sex of *O. niloticus* and *C. gariepinus*. The infestation rate was higher in females than that of males. These results were nearly met with the findings recorded by **Aloo (2002), Diab *et al.* (2004) and Taghreed Ibrahim (2005)**. While it didn't came in accordance with **Aloo (2002)** who recorded that prevalence and intensity of the infestation with internal parasitic diseases that recovered from Tilapia species and *C. gariepinus* where as male fish were more heavily infested as females. No seasonality in infestation level was observed. The total prevalence of *Contracaecum spp* in *Lates niloticus* was high reached 84.8%. This result may be attributed to feeding habit of *L. niloticus*. At the season level, no seasonality in infestation rate was observed (81.5, 90, 86.9 and 80.9%in spring, summer, autumn and winter, respectively). These results may due to reduction the effect of *Contracaecum* species

larvae on intermediate hosts, feeding habit of infested fish (carnivorous) and availability of final host all over the year. On the other hand, the lowest rate of infestation was recorded in winter may due to reduction feeding activity of the fish at low temperatures and reducing the chances of infection via copepods. *Contracaecum* eggs hatch in water with an optimum temperature of 21°C might also account for the low parasite prevalence in winter. These results were nearly met the finding recorded by **Barson (2004) and Adel *et al.* (2013)** who found that the parasitisation caused by *contracaecum* spp. has no significant differences in between both sexes. The prevalence in young hosts was lower than in adults and no seasonality in infestation level was observed. The total prevalence of *Contracaecum spp* in *O. niloticus* was low reached 4.6% in compare with *L. niloticus* and was recorded only in summer season. There was wide difference in the infestation rate between *L. niloticus* and *O. niolticus*. This difference may reflect to the difference in feeding habit where *L. niolticus* is carnivorous fish while *O. niloticus* is omnivorous fish. The PCR was used as a confirmatory tool for the diagnosis of *Contracaecum species* in *L. niloticus* and *O. niloticus*. The results showed different PCR products in relation to *species* of *Contracaecum*. This is the first step toward molecular study of *Anisakids*. In *L. niloticus*, the PCR amplification showed band at 100 bp while in *O. niloticus*, the PCR amplification showed band at 150 bp. From these results; is these results refer to different species or the same species in different fish species? The author suggested that the difference in molecular weight bands may be the same species but in different fish species. Identical results need further investigation by using specific primers.

5. CONCLUSION

From the results of the present study, it can be concluded that morphological characters of parasites may be enough to identify type of parasite and molecular identification of *Contracaecum species* may be a promising result and need further studies.

REFERENCES

Abd El- Latif, A.M. (2007): Gill parasitic diseases of some freshwater fishes. Ph. D. thesis (Fish diseases and management), Fac. Vet. Med., Benha Univ.

Adel, M., Azizi, H. R. Nematolahi, A. (2013): Scomberomorus Commerson, a New Paratenic Host of *Contracaecum* sp. and *Anisakis* sp. (Nematoda: Anisakidae) from Persian Gulf. World Journal of Fish and Marine Sciences 5: 310-314.

Aloo, P.A. (2001): Occurrence of larval *Contracaecum* (Nematoda: Heterocheilidae) in three teleostean species from Lake Naivasha, Kenya. *East African Journal of Science*, 3:1–12.

Aloo, P.A. (2002): A comparative study of helminth parasites from the fish *Tilapia zillii* and *Oreachromis leuc ostictus* in Lake Naivasha and Oloidien Bay, Kenya. Journal of Helminthology 76: 95-102.

Amany Abbass A. A. (1997): Role of fish as an intermediate host of some trematodes in birds. Ph. D. Thesis, Fac. of Vet. Med. (Moshtohor) Zagazig Univ, Egypt.

Amany Abbass A. A; Lashein, G.H.A. and Tantawy, A.A. (2006): the prevailing endoparasitic diseases of catfish "*Clarias gariepinus"* with special reference to the associated pathological changes. Zag. Vet. J. 1, PP.120-139.

Barson, M., (2004): The occurrence of *Contracaecum* sp. Larvae (Nematoda: Anisakidae) in the catfish *Clarias gariepinus* (Burchell) from Lake Chivero, Zimbabwe. *Onderstepoort Journal of Veterinary Research*, 71:35–39

Barson, M., Avenant- Oldewage, A. (2006): Nematode parasites of *Clarias gariepinus* (Burchell,1822) from the Rietvlei Dam, South Africa. *Onderstepoort Journal of Veterinary Research*, 73:87–94

Cai, X.Q, Xu, M.J, Wang,Y.H, Qiu, D.Y, Liu, G.X, Lin, A, Tang, J.D (2010): Sensitive and rapid detection of *Clonorchis sinensis* infection in fish by loop-mediated isothermal amplification (LAMP) Parasitol Res 106:1379–1383

Diab, A. S.; El-Bouhy, Z. M.; Sakr, S. F. and Abdel-Hadi, Y. M. (2004): Prevalence of some parasitic agents affecting the gills of some cultured fishes in Sharkia, Damietta and Fayium governorates. Zag.Vet.J.1, : 1-12.

Ehssan A. Hassan, Maha F.M. Soliman, and Abdel Fattah a. Ghobashy (2012): some factors affecting metacercarial infections in *Tilapia Zilli* from Lake Timsah, Ismailia, Egypt. Egypt. Acad. J. Biolog. Sci., 4: 21-28.

Eissa, I. A. M. (2002): Parasitic fish diseases in Egypt. Dar El-Nahda El-Arabia Publishing, 32 Abd El-Khalek St. Cairo, Egypt

Eissa, I.A. M, Badran, A.F., Sohair, Y., Mohamed, and Heba, I. Abdel- Mola (2009): Entero- parasitic infestation and associated pathological lesions in *Clarias gariepinus* at Ismailia Province. Journal of agricultural and veterinary science, Qassim University. 1, pp. 21-32.

El- Seify, M. A.; N.A. Mahmoud; S.A. Abu El- Wafa and A.M. I. Abd El- Aal (1997): Studies on some enteric helminthes of Nile fishes from Sharkia province, Lower Egypt. Egypt. J. Aquat. Biol. & Fish. 2:43 1-449.

Gihan Shager E.A.E. (1999): Enteric Helminth parasites .M. Sc. Thesis (Parasitology), Fac. Vet. Med, Zagazig Univ.

Ibtsam, E. B.E.D. (2004): Studies on some prevailing parasitic diseases among cultured Tilapia fish. Ph. D. Thesis, Fac. Vet. Med., Suez Canal University.

Kurse, G.O.W. and Pritchard, M.H. (1982): The collection and preservation of animal parasites. Nebraska Univ. Press. USA.

Lima dos Santos C. A. M. and Howgate, P. (2011): Fish borne zoonotic parasites and aquaculture: A review. Aquacult., 318: 253–261

Lucky, Z. (1977): Methods for the diagnosis of fish diseases. Amerind Publishing Co. pvt, Ltd., New Delhi, Bombay, Calcutta and New York.

Mahmoud E. Bayoumy, Osman K. Abd El-Hady, Hussein A.M. (2006): Site adaptations of Acanthogyrus (Acanthosentis) tilapiae: Observations through light and scanning electron microscopyJ. Vet. Sci., 7, 339–342

McManus D. and Bowles J. (1996): Molecular Genetic Approaches to Parasite Identification: Their value in Diagnostic Parasitology and Systematics. Int J Parasitol, 26,687-704.

Meyer, C. M. and W. O. Olsen. (1992): Essentials of parasitology. W. M. C. Brown Co. publishers, USA: P. 288.

Nadler, S.A.; D'Amelio, S.; Fagerholm, H. P.; Berland, B.; and Paggi, L. (2000): "Phylogenetic Relationships among Species of *Contracaecum* Railliet & Henry, 1912 and *Phocascaris* Høst, 1932 (Nematoda: Ascaridoidea) Based on Nuclear rDNA Sequence Data" *Faculty Publications from the Harold W. Manter Laboratory of Parasitology.* 121, pp. 455–463

Negm El- Din, M. M.; Nagwa Eid and Fayed, S.A. (1988): Some studies on helminthes parasites of fresh water in Egypt. Alex. J. Vet. Sci., 4: 357-367.

Noga, E.J. (2010): Fish Diseases, Diagnosis and Treatment. 3rd Edition, Iwa State University, Press, Ames.

Shamsi, S.1 and Aghazadeh-Meshgi, M. (2010). Morphological and genetic characterisation of selected *Contracaecum* (Nematoda: Anisakidae) larvae in Iran. Iranian Journal of Fisheries Sciences 10: 356-361.

Shehab El-Din, M.T.E. (2008): Studies on some nodular parasitic diseases of fish. Ph. D. thesis, Faculty of Veterinary Medicine, Benha University.

Tagreed, B. Ibrahim (2005): Studies on infection with encysted parasite in some fresh water fishes. Thesis Ph.D. Fac. of Vet. Med. Cairo Univ.

Umehara, A., Kawakami, Y, Araki J, Uchida A (2008): Multiplex PCR for the identification of *Anisakis simplex* sensu stricto, *Anisakis pegreffii* and the other anisakid nematodes. *Parasitology International*, Issue 1, Pages 49-53

Table (1) Total prevalence and seasonal prevalence of internal parasitic diseases among examined fish species.

Fish species	Total prevalence	Seasonal prevalence			
		Spring	summer	autumn	winter
Wild *O. niloticus*	67.5	78.33	71.08	63.63	43.3
Cultured *O. niloticus*	30	13.2	21.8	46.42	33.3
C. gariepinus	74.88	81.17	76.92	74.55	58.5
L. niloticus	85.5	61.5	95.0	86.95	85

Table (2) The prevalence of the internal parasitic diseases in relation to the sex of *O. niloticus* and *C. gariepinus:*

Species		Male	Female
Wild *O. niloticus*	Number of examined fish	93	135
	Number of infected fish	53	101
	Prevalence	57	74.8
Cultured *O. niloticus*	Number of examined fish	83	37
	Number of infected fish	25	11
	Prevalence	30.1	29.7
C. gariepinus	Number of examined fish	100	119
	Number of infected fish	58	106
	Prevalence	58	89.1

Table (3) The type of parasite in each fish species, total prevalence, seasonal prevalence and the most susceptible organs:

Fish species	Type of parasite	Total prevalence	Seasonal prevalence				Most Susceptible organs
			Spring	summer	Autumn	winter	
Wild *O. niloticus*	*Clinostomum tilapiae*	12.2	6.67	27.7	14.5	0	Branchial cavity
	Euclinostomum tilapiae	18.3	20.0	27.3	19.3	6.7	Kidneys
	Acanthocephalans spp	1.5	6.19	0	0	0	Intestine
	Contracaecum species	4.6	0	18.2	0	0	Pericardial and abdominal cavity
Cultured *O. niloticus*	Encysted metacercariae	12.1	9.4	12.7	17.9	8.3	Musculature
C. gariepinus	Encysted metacercariae	46.9	58.8	62.9	48.7	17.1	Musculature, liver
	Procamallanus laeviconchus	10.3	7.1	17.9	11.1	4.9	Stomach
	Paracamallanus cyathopharynx	6.6	7.7	8.2	5.6	4.8	Intestine
	Acanthocephalans	4.7	18.8	0	0	0	Intestine
	Cestodes	11.6	17.6	11.1	7.7	9.8	Stomach and intestine
L. niloticus	*Contracaecum spp*	84.8	84.6	90	86.9	80.9	Abdominal cavity

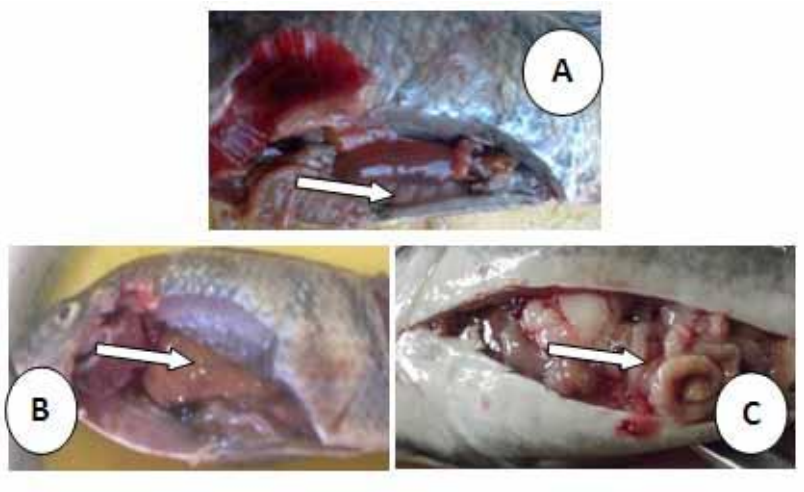

Figure (1) A: *O. niloticus* intestine infested with acanthocephalans spp showing swelling and congestion. B: *O. niloticus* liver infested with encysted metecercariae showing grayish white nodules. C: *C. gariepinus* intestine infested with acanthocephalans spp showing swelling and congestion

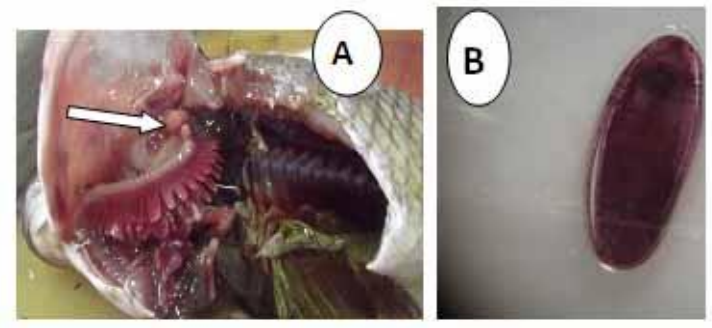

Figure (2) A): showing yellow nodules embedded in branchiostegal musculature of *O. niloticus*. B): Excysted *Clinostomum spp* stained with Acetic acid alum carmine

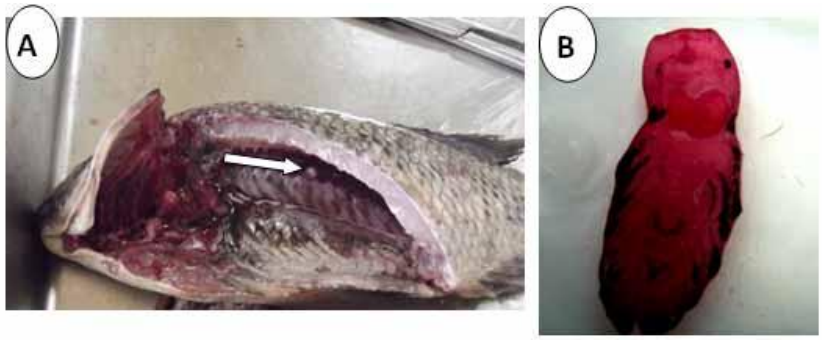

Figure (3): A) kidneys of *O.niloticus* showed grayish white cysts which embedded in kidneys tissue. B) Larvae of *Euclinostomum spp* stained with acetic acid alum carmine

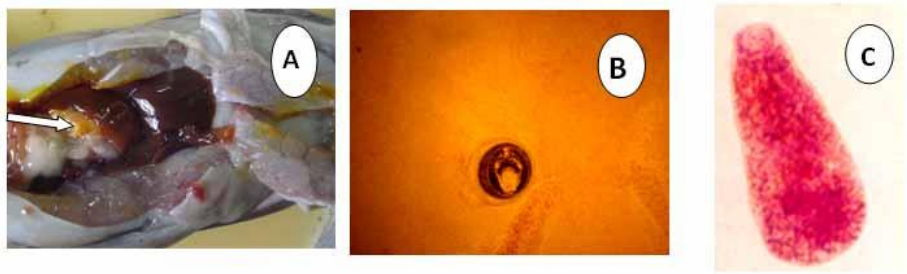

Figure (4): A) *C .gariepinus* liver infested with encysted metacercariae showing pin point white cysts. B) *Haplorichiaed* encysted metacercaria (x10).C) *Haplorichiaed* excysted metacercaria (x10)

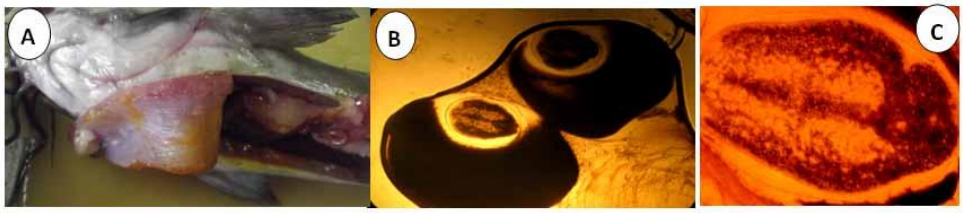

Figure (5): A) *C. gariepinus* musculature infested with encysted metacercariae showing large macroscopic white cysts. B) *Cyanodiplostomatid* encysted metacercariae. (x10). C) *Cyanodiplostomatid* excysted metacercariae. (x10).

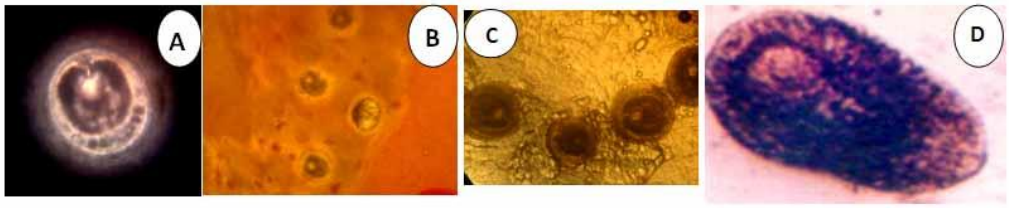

Figure (6): A) *Prohestomatide* encysted metacercariae obtained from kidney of *C .gariepinus*, (x10). B) *Prohestomatide* encysted metacercariae which obtained from liver of *C .gariepinus*, (x10). C) *Prohestomatide* encysted metacercariae which obtained from musculature of *C .gariepinus*, (x10. D) *Prohestomatide* excysted metacercariae. (x10)

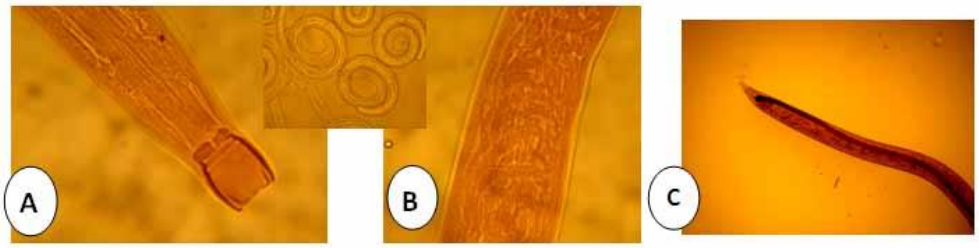

Figure (7): A) *Procamallanus laeviconchus* was isolated from stomach of *C. gariepinus* (anterior end), (x10). B) *Procamallanus laeviconchus* (middle part) larvaeparous nematode. (x10). C) *Procamallanus laeviconchus* (posterior end), (x4)

Figure (8)A: *Paracamallanus cyathopharynx* which was isolated from intestine of *C. gariepinus* (anterior part), (x10). B: *Paracamallanus cyathopharynx* male (posterior part), (x10). C: *Paracamallanus cyathopharynx* female (posterior part), (x10)

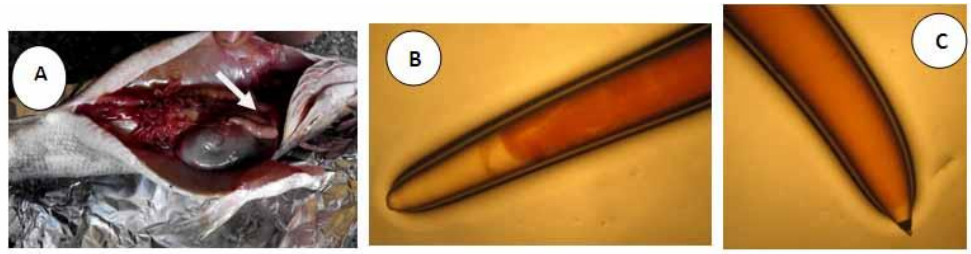

Figure (9): A) *Lates niloticus* abdominal cavity infested with *Contracaecum* species.
B) *Contracaecum* spp. (anterior end), (x4). C) *Contracaecum* spp. (posterior end), (x4)

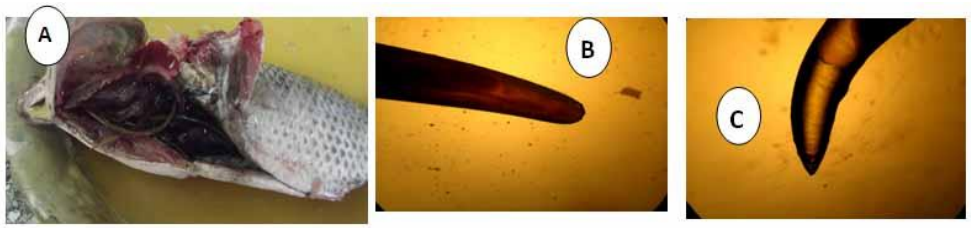

Figure (10): A) Body cavity of *O. niloticus* infested with *Contracaecum* spp. B) *Contracaecum* spp. (anterior end), (x4) .C) *Contracaecum* spp. (posterior end) (x4)

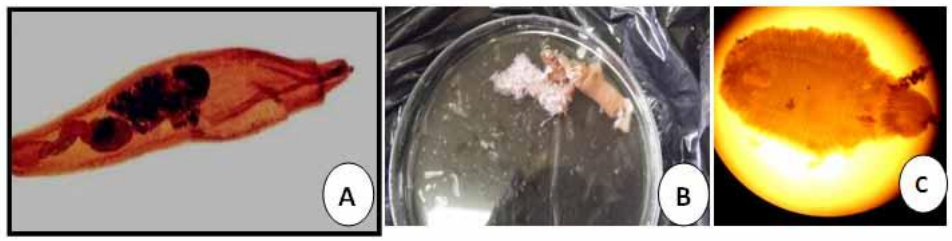

Figure (12): A) *Acanthosentis tilapiae*, (stained with Acetic acid alum carmine (x4). B) *C. gariepinus* intestine (longitudinal incision) which infested with acanthocephalans spp. C) Acanthocephalans spp. of *C. gariepinus*. (x4)

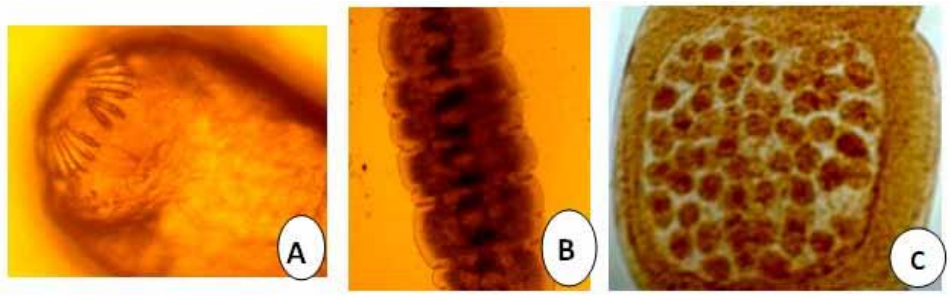

Figure (11) A: *Polyonchobothrium clarias* was isolated from stomach and intestine of *C. gariepinus* (rostellum), (x40. B: *Polyonchobothrium clarias* (mature segments), (x10). C: *Polyonchobothrium clarias* (gravid segment), (x40)

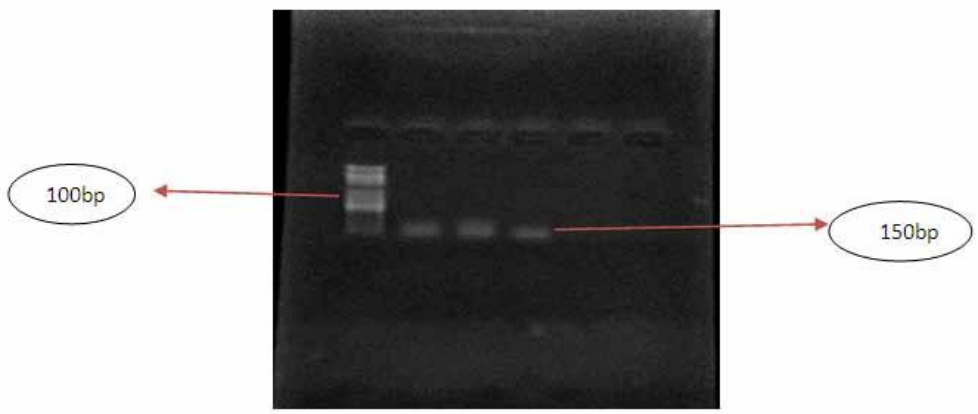

Figure (13): Showing Lanes 2, 4: 100 bp PCR products amplified from DNA of *Contracaecum spp* in *Lates niloticus*. Lane 3: 150 bp PCR products amplified from DNA of *Contracaecum spp* in *Oreochromis niloticus*.

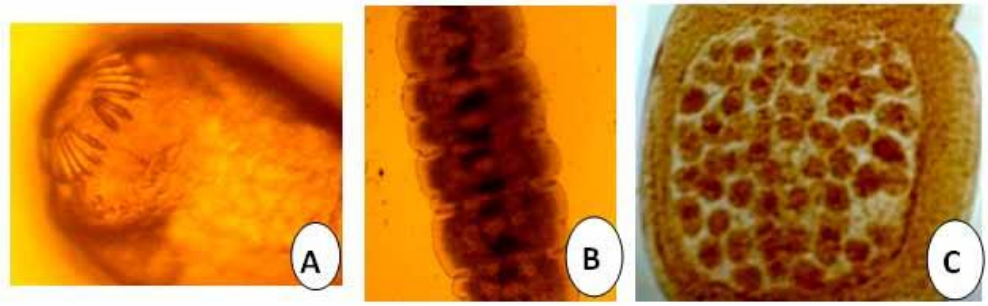

Figure (14): A) *Acanthosentis tilapiae*, (stained with Acetic acid alum carmine (x4). B) *C. gariepinus* intestine (longitudinal incision) which infested with acanthocephalans spp. C) Acanthocephalans spp. of *C. gariepinus*. (x4)

التعرف على بعض الطفيليات الداخلية المرتبطة بأمراض أسماك المياه العذبة

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تم اجراء هذه الدراسة على اجمالى ع دد ٦٤٣ سمكة منها ٣٤٨ بلطى نيلى (برى ومستزرع)، ٢١٩ قرموط افريقى و ٧٦ قشر بياض ، تم تجميعها خلال الفترة من فبراير الى نوفمبر ٢٠١٣ من اماكن مختلفة من الرياح التوفيقى وفروعه و مزراع سمكية من محافظة كفر الشيخ . وأظهر الفحص الخارجى لمعظم أنواع الأسماك المصابة بالأ مراض الطفيلية الداخلية عدم وجود علامات مرضية مميزة أو تشوهات ماعدا وجود انتفاخ طفيف في البطن . تم عزل حويصلات ميتاسركاريا تنتمى الى الكلينوستومم و اليوكلينوستومم. اما بالنسبة للديدان المعوية فى كلا من البلطى النيلى والقرموط الافريقى فقد لوحظ وجود طفيل الاكانثوسيفلا فى الامعاء. الديدان الخيطية مثل بروكمالينس ليفيكونشس وباراكمالينس سيازوفارنكس فى معدة و امعاء القرموط الافريقى. سجل القرموط الاقريفى وجود ديدان شريطية مثل بوليانكوبوثريم كيلاريس. تم عزل يرقات الكونتراسيكم من التجويف البطنى لاسماك البلطى النيلى وقشر البياض وكان أعلى معدل الاصابة بالطفيليات الداخلية في فصلي الربيع والصيف يليه الخريف والشتاء .تم استخدام التقنية الجزيئية لتعرف على الكونتراسيكم التى سجلت فى كلا من البلطى النيلى وقشر البياض . وقد اظهر تفاعل البلمرة التسلسلى للحمض النووي للكونتراسيكم باستخدام بادئات عامة لعائلة الانيساكيدى اختلافا ملحوظا. ففى البلطى النيلى اظهر تفاعل البلمرة التسلسلى للحمض النووى شريطا عند **bp** ١٥٠ بينما فى قشر البياض سجل تفاعل البلمرة التسلسلى شريطا للحمض النووى عند **bP** ١٠٠.