

Prevalence of nematodes infestation in *Clarias gariepinus* from El-Burullus Lake and Lake Nasser, Egypt

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Abstract

A total number of 400 *Clarias gariepinus* were collected alive from several and various locations at El-Burullus Lake and Lake Nasser, to investigate the prevailing nematodes that infect this fish species from the two locations. Nearly all the examined fish were positive for one or more nematodes; four spp. of two families were identified from *Clarias gariepinus* at El-Burullus Lake: *Procamallanus laevisconchus*, *Paracamallanus cyathopharynx*, *Neocamallanus* spp. (recovered from the gall bladder for the first time) and *Terranova* spp. larvae (recorded for the first time in Kafr El-Sheikh Governorate representing new locality record), one nematode from *Clarias gariepinus* at Lake Nasser: 3rd stage larvae (L₃) of *Contracaecum* spp. (has zoonotic importance). The prevalence of *Procamallanus laevisconchus*, *Paracamallanus cyathopharynx*, *Neocamallanus* spp. and *Terranova* spp. larva were 37.5%, 44%, 0.5% and 10% respectively, meanwhile the prevalence of L₃ larvae of *Contracaecum* spp. were 100%. This study was planned to compare between the nematodes infecting *Clarias gariepinus* from the two lakes evaluating clinical signs, postmortem examinations, parasitological examinations, seasonal prevalence and histopathological investigations of infected fish with different nematodes infestation. *Clarias gariepinus* must be eviscerated as soon as possible after catching to prevent 3rd stage larvae of *Contracaecum* spp. in the abdominal cavity from liberating from their sheaths and attacking the fish musculature (the edible part in the fish) as well as, prevent *Terranova* spp. larvae in the gills from attacking another parts of the fish and thus prevent the transmission of the nematodes of zoonotic importance to the consumers.

Keywords: Catfish, *Terranova* spp., *Neocamallanus* spp., *Contracaecum* spp., Pathology

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الإصابات السائدة بالديدان الاسطوانية في القرموط الأفريقي من بحيرة البرلس وبحيرة ناصر، مصر

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الخلاصة

تم في هذه الدراسة استبيان معدل انتشار النيماطود (الديدان الاسطوانية) في عدد ٤٠٠ من اسماك القرموط الأفريقي من بحيرة البرلس وبحيرة ناصر بمصر وتم تسجيل الاعراض المرضية و الصفة التشريحية للامراض الناتجة عنها و تم التعرف على الديدان الاسطوانية من اسماك القرموط الأفريقي بحيرة البرلس وهى كالتالى: بروكاميلينس ليفيكونكس و باراكاملينس ثياسوفرنيكس و نيوكاملينس سبيشس (تم عزلها من الحوصلة المرارية لأول مرة) و يرقات تيرانوفا سبيشس (لأول مرة بمحافظة كفر الشيخ) بينما انفرد القرموط الأفريقي بحيرة ناصر بالطور الثالث من يرقات كونتراسيكم سبيشس (لها القدرة على عدوى الانسان). سجلت الدراسة نسب اصابة بالديدان الاسطوانية كالتالى: ٣٧,٥% بروكاميلينس ليفيكونكس و ٤٤% باراكاملينس ثياسوفرنيكس و ٥,٥% نيوكاملينس سبيشس و

١٠% يرقات تيرانوفا سبيشس بينما سجل الطور الثالث من يرقات كونتراسيكم سبيشس نسبة إصابة ١٠٠% تم دراسة التغيرات النسيجية المرضية المصاحبة للإصابة بالديدان الأسطوانية المختلفة. يجب نزع أحشاء القرموط الإفريقي عقب الصيد مباشرة لمنع يرقات كونتراسيكم سبيشس من مهاجمة عضلات الأسماك (وهو الجزء المأكول من السمك) وكذلك منع يرقات تيرانوفا سبيشس من مهاجمة أجزاء أخرى في السمكة غير الخياشيم وبالتالي منع نقل العدوى بالديدان الأسطوانية إلى المستهلكين.

Introduction

The African catfish; *Clarias gariepinus* is a widely distributed fish food in Africa (1) of high growth rate and excellent meat quality (2). It has a great potential as a protein and energy sources in human nutrition and its lipids are a good source for polyunsaturated fatty acids, especially what is known as omega 3 fatty acids (3).

Clarias gariepinus in many African countries is an important solution to the increasing demand for fish and fish products in response to rapid human population growth and the fear of animal-related disease outbreaks e.g., avian and swine influenza (4). With the growing of the economic value of *Clarias gariepinus*, the interest in its parasitic loads and its impact on the fish increased (5).

Fish diseases constitute one of the most important challenges confronting fish industry in both natural and culture environments especially parasitic one which has a serious impact on the fish as they cause mortality, low growth rate, lower food conversion rates and decreased marketability as well as it may have zoonotic threats, to human consumers (6-8).

About 650 species of nematodes parasitize fish as adults and many others nematodes use fish as intermediate hosts (9). The nematodes cause damage to the hosts by depriving them of digested food and by feeding on host tissues, sera, or blood. In some cases, direct mechanical damage results from their fixing to host tissues and developing or migrating in them (10,11). Small numbers of nematodes often occur in healthy fish, but high numbers cause illness or even death (12).

Contracaecosis and pseudoterranovosis are parasitic diseases caused by infestation with larval nematodes of the taxonomic genus *Contracaecum* and *terranova* respectively which belong to the family, Anisakidae (13,14). They are zoonotic diseases with medical and economic importance and can result in considerable costs to the fishing industry. Humans are accidentally infected by consuming raw, poorly cooked (15), cold smoked or lightly salted fish (16).

The aim of the present study is to carry out as a reconnaissance survey of the nematodes infestation in *Clarias gariepinus* from both El-Burullus Lake and Lake Nasser, which can be used in the fish health assessment recording the clinical signs, the postmortem changes, the total and the seasonal prevalence, as well as the histopathological alterations in the infected fish.

Material and methods

Study areas

El-Burullus Lake is a brackish water lake and is the second largest Egyptian northern lake along the Mediterranean coast. It is located in the central part of the northern shoreline of the Nile Delta. The lake is shallow, with a maximum depth of 175 cm in the middle and western parts. The lake surface area is 410 km².

Lake Nasser, together with Lake Nubia, is the second largest man-made lake in Africa created as a result of the construction of the Aswan High Dam in the 1960's on the River Nile. Lake Nasser extends 300 km within the Egyptian borders and is now one of the most important sources of freshwater fish in Egypt.

Fish samples

A total number of 400 *Clarias gariepinus* of different weights and sizes were collected, randomly and alive, from two different ecosystems; 200 *Clarias gariepinus* from El-Burullus Lake and 200 from Lake Nasser during 2017. The collected fish were transferred alive and kept in prepared glass aquaria in the laboratory of Parasitology, Faculty of Veterinary Medicine, Kafrelsheikh University and the laboratory of Fish Diseases, Faculty of Fish and Fisheries Technology, Aswan University respectively.

Clinical and postmortem examinations

The fish was subjected to full clinical examination; the external and internal gross lesions were recorded immediately according to the method described by (17).

Parasitological examinations

Parasitological examinations were performed and then the collected parasites were washed, fixed and mounted according to the methods described by (17,18).

Identification of collected helminthes

The collected nematodes were identified according to the identification keys of (19-22).

Histopathological examination

After necropsy, sections of stomach, intestine, kidney and muscles (parts of abdominal and trunk muscles closely situated to 3rd stage larvae of *Contracaecum* spp. in the abdominal cavity of infected fish) were fixed immediately

in 10% formalin and processed for histopathological evaluation as described by (23).

Results and discussion

Clinical and postmortem examinations

The fish infested with small numbers of nematodes showed no pathognomonic lesions while the severely infected ones showed emaciation and this may be due to the adult nematodes in the stomach and intestinal tract damage its lining and rob the nutrients of the fish (12), as well as the mechanical pressure along the gastrointestinal tract in which the parasites attached from outside; causing a wasting effect. Dark or pale body coloration could be recorded and hemorrhagic patches may be found on some infected fish.

The gills of fish naturally infected with the microscopic nematodes showed paleness with excessive mucus which may be a defensive mechanism to diminish the irritant effect of the pathogenic nematodes.

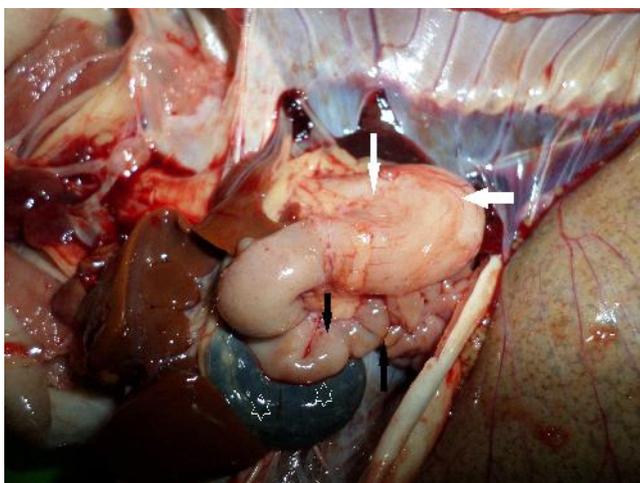


Figure 1: *Clarias gariepinus* from El Burullus Lake infected with *Procamallanus laevisconchus* and *Paracamallanus cyathopharynx* showing: Congestion and hemorrhages of the stomach (white arrows) and intestine (black arrows) as well as distended gall bladder (white stars).

Stomach and intestine were congested (Fig. 1, Fig. 2) and this may be attributed to the firm attachment of the nematodes (*Procamallanus laevisconchus* and *Paracamallanus cyathopharynx*) to them; embedding their buccal capsule between the villi of the lining mucosa; causing local damage and possibly peritonitis in addition to 3rd stage larvae of *Contracaecum* which attached along the alimentary tract causing tissue destructions or alterations. Moreover, proteolytic enzymes discharged from adult

worms may be degrading the gastric and intestinal tissues (24). Helminthes produce toxic metabolic by-products which harm the infected host by causing occlusion of blood vessels, intestine and other ducts and resulting in the inflammation and congestion described in the internal organs. Visible nematodes could be seen by the naked eye in the abdominal cavity (Fig. 2) and this result is similar to that recorded by (25), these nematodes will liberate from their fibrin sheaths and invade the internal organs and the musculature if the fish do not eviscerate as soon as possible after catching. Also the gall bladder, the stomach and the intestine of fish are infected with smaller visible nematodes as that recorded in the stomach and the intestine of *Clarias gariepinus* by (26).

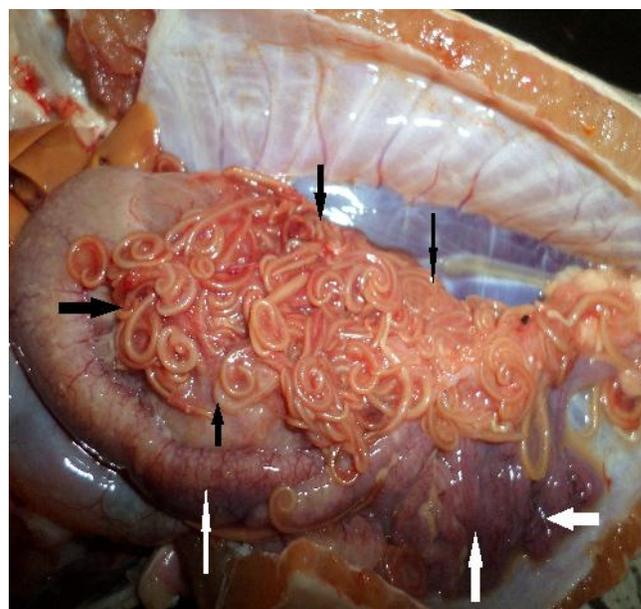


Figure 2: *Clarias gariepinus* from Lake Nasser infected with 3rd stage larvae of *Contracaecum* spp. showing: 3rd stage larvae of *Contracaecum* spp. attached to the alimentary canal and the mesentery (black arrows) and congested intestine (white arrows).

Parasitological examinations

Based on the morphological examination, the following nematodes were identified from *Clarias gariepinus* at El-Burullus Lake, Kafr El Sheikh Governorate.

Procamallanus laevisconchus (Fig. 3: a, b, c, d) was isolated from the stomach and the intestine of *Clarias gariepinus*. It is small ovoviviparous nematode, yellowish in color with continuous buccal capsule, not separated into paired lateral-valves, the wall may be smooth or with spiral markings internally. The uterus of mature female is filled with larvae. Such adult nematodes are belonged to family Camallanidae (27-29).

Paracamallanus cyathopharynx (Fig. 4: a, b) was isolated from the stomach and the intestine of *Clarias gariepinus*. It is cylindrical, yellowish brown in color with transverse annulations and the buccal capsule is consisting of two lateral valves with a large chitinous buccal cavity or pharynx behind the valves; the trident is present. The tail is tapering in female and curved in male. Such adult nematodes are belonged to family Camallanidae (27,29).

The microscopic larval nematode, *Terranova* sp. (Fig. 5: a, b) isolated for the first time from the gills of *Clarias gariepinus* in Kafr El Sheikh Governorate and considered as a new locality record. The genus *Terranova* was erected by Leiper and Atkinson (30). *Terranova* was later considered as a synonym of *Phocanema* and *Pseudoterranova* (20). Our *Terranova* sp. larva is very small and cannot be seen by the naked eyes. Their lips are with dentigerous ridges. The ventriculus and the intestinal cecum are present with no ventricular appendix. Such larval nematodes are belonged to family Anisakidae (22).

The nematode *Neocamallanus* spp. (Fig. 6) isolated for the first time from the gall bladder of *Clarias gariepinus*. It is transparent with buccal capsule consisting of two lateral valves, but without tridents or rods associated with it. A strongly developed chitinous is present behind the buccal valves. Such adult nematodes are belonged to family Camallanidae (19).

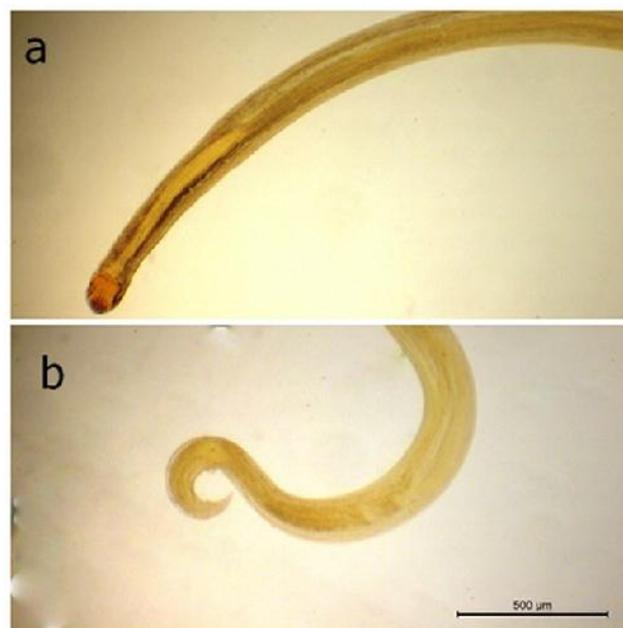


Figure 4: *Paracamallanus cyathopharynx* isolated from stomach and intestine of *Clarias gariepinus* of El-Burullus Lake. (a): Anterior end x 500. (b): Male posterior end x 500.

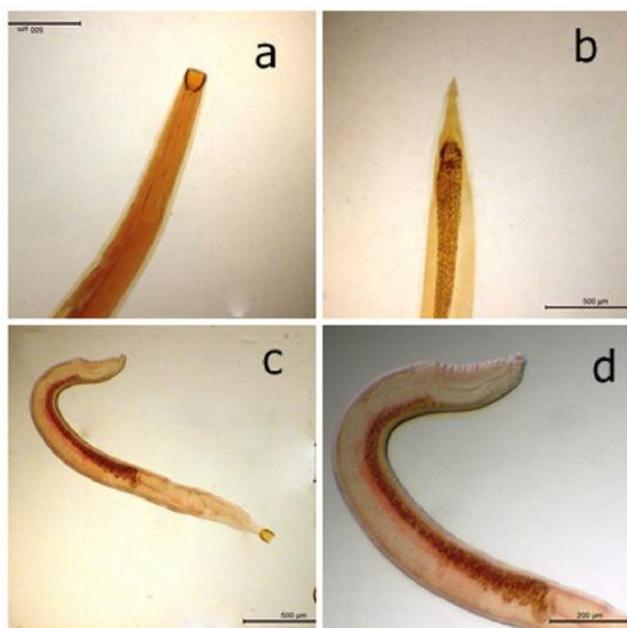


Figure 3: *Procamallanus laeviconchus* isolated from stomach and intestine of *Clarias gariepinus* of El-Burullus Lake. (a) Anterior end x 500. (b) Female posterior end x 500. (c) Male whole worm x 500. (d) Male posterior end x 200.

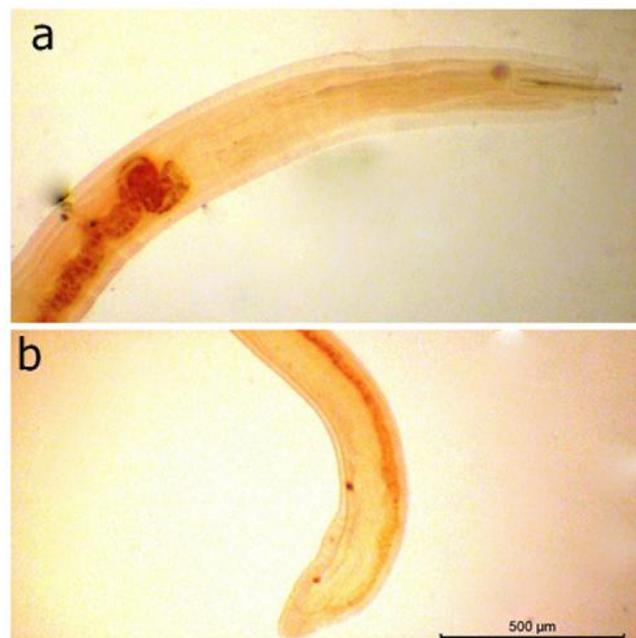


Figure 5: *Terranova* spp. isolated from the gills of *Clarias gariepinus* of El-Burullus Lake (a): Anterior end x 500. (b): Posterior end x 500.



Figure 6: Anterior end of *Neocamallanus* spp. isolated from the gall bladder of *Clarias gariepinus* of El-Burullus Lake x 400.

The only nematode isolated from *Clarias gariepinus* at Lake Nasser, Aswan Governorate was: The 3rd stage larva of *Contracaecum* spp. (Fig. 7: a, b) in the abdominal cavity encapsulated in a fibrin sheath and attached to the alimentary canal. It is yellowish-red in color with long, cylindrical body. The cuticle is smooth and transversely striated with regular and irregular ring forms. The anterior end is rounded, while the posterior one is pointed. The mouth is surrounded by three small lips with a prominent papilla. The oesophagus is narrow and long. Two blind caeca are branching off from the intestinal tract at the junction of the oesophagus and the midgut. The ventricular appendix is shorter and pointing posteriorly while the intestinal caecum is longer and pointing anteriorly. The anus is located near the posterior end. The tail is conical in shape with a tapered spine. Such larval nematodes are belonged to family Anisakidae (25,28).

The total and seasonal prevalence of detected parasites:

Clarias gariepinus from El Burullus Lake revealed a nematodal infection rate with prevalence of 62% of the examined fish.

The total prevalence of *Procamallanus laeiviconchus* and *Paracamallanus cyathopharynx* are 37.5% and 44% (Table 1) showing high prevalence in summer (70, 80%), spring (36, 40%), autumn (28, 28,23%) then winter (16, 24%) respectively (Table 2) and this result is higher than that recorded by (29) who isolated *Procamallanus laeiviconchus* and *Paracamallanus cyathopharynx* with prevalence of 26% and 31% showing high prevalence also in summer (48%, 56%) and low prevalence in winter (4% and 8%) respectively at Ismailia Governorate, Egypt. Also our results are higher than that recorded by (31) who detected *Procamallanus laeiviconchus* from *Clarias gariepinus* with prevalence of 23.33% in Lake Manzala, Egypt. *Procamallanus laeiviconchus* and *Paracamallanus*

cyathopharynx were isolated from *Clarias gariepinus* with prevalence of 8.74% and 55.33% respectively in Nigeria (32) which differ completely with our results. This variation may be attributed to the different ecological factors in the different localities which usually enhance or limit the parasites spread as well as, the different sources of the examined fish, the type, the age and the sex of the examined fish.



Figure 7: 3rd stage larva of *Contracaecum* spp. (a): Anterior end x 500. (b): Male posterior end x 500.

Increase infestation in summer may be due to increase the feeding activity of the fish at high temperatures and the intermediate host is more abundant during these warmer months; increasing the chances of infection via copepods ingestion and vice versa (33).

The life cycle of *Paracamallanus cyathopharynx* and *Procamallanus laeiviconchus* in Egypt were studied previously by (21,34) and found that the Copepoda; *Mesocyclops leukarti* harbors the first 3 larval stages of both these nematodes. The copepod must then be ingested by *Clarias gariepinus* to continue the life cycle. This assumption is strengthened by the feeding habits of catfish which is a voracious omnivore, with predator tendencies, eating animal matter of any sort which in turn assists the transmission of more helminthes through their feeding on aquatic animals harboring the infective stages of these parasites or even the young infected fish. Moreover the leathery skin of *Clarias gariepinus* may be attacked easily by leeches and copepods and this explain the recorded hemorrhagic lesions on skin and fins of some infected fish (35).

The microscopic larval nematode; *Terranova* spp. in the gills of *Clarias gariepinus* with an overall prevalence of 10% (Table 1), a higher infection rate is in summer 22%, spring 8%, winter 6% and then autumn 4% (Table 2).

The *Neocamallanus* spp. isolated from the gall bladder of *Clarias gariepinus* is only one and recovered in summer (Table 1, 2).

To our knowledge, no one recovered *Terranova* sp. from *Clarias gariepinus* in Kafr El Sheikh Governorate before although (36) listed seven marine fish species parasitized by *Terranova* sp. in the Red Sea fish in Egypt. It is safe to assume that *Terranova* can be found wherever suitable hosts live and favorable environmental conditions permit (14).

3rd stage larvae of *Contracaecum* spp. are found throughout the period of study in *Clarias gariepinus* of Lake Nasser with a prevalence of 100% (Table 1). This makes *Contracaecum* the most prevalent fish nematodes in Lake Nasser and the fact that its life cycle involves migratory bird species (as cormorants) can justify this observation as well as, in Aswan the high temperature is maintained nearly throughout the year; this favors the development of both the invertebrate hosts and the final hosts throughout the year around the Lake and increase the plankton production, which is the source of nutrition of

intermediate hosts. This result is similar to that recorded by (25) from *Clarias gariepinus* of Lake Nasser and higher than that recorded by (37) who found 42.6%. of *Clarias gariepinus* infected with 3rd stage larvae of *Contracaecum* spp. from Lake Chivero, Zimbabwe as well as, (28) who declared that *Clarias gariepinus* infected with 86% *Contracaecum* spp. in South Africa and (38) who observed *Contracaecum* spp. from 9.9% of the examined *Clarias gariepinus* in Cameroon.

The nematodes recorded in *Clarias gariepinus* of El Burullus Lake, kafr El Sheikh Governorate are completely different from that of Lake Nasser, Aswan Governorate this may be due to that the climate change might have a direct effect on the parasite species and also have indirect effects through changes in the distribution and abundance of their intermediate and final hosts (39) in addition to the different ecological factors in the two localities.

Histopathological examination

The histopathological examination of *Clarias gariepinus* infested with *Procamallanus laevisconchus*, *Paracamallanus cyathopharynx* revealed multifocal lymphocytic enteritis as well as intestinal atrophied crypts (Fig. 8-A). This result is nearly similar to that reported by (29).

Table 1: Prevalence of nematodes among the examined *Clarias gariepinus* from El Burullus Lake and Lake Nasser at 2017

Fish species (location of the fish)	No. examined fish	Nematodes									
		<i>Procamallanus laevisconchus</i>		<i>Paracamallanus cyathopharynx</i>		<i>Terranova spp. larva</i>		<i>Neocamallanus spp.</i>		3 rd stage larva of <i>Contracaecum</i> spp.	
		Infected No.	% of infections	Infected No.	% of infections	Infected No.	% of infections	Infected No.	% of infections	Infected No.	% of infections
<i>Clarias gariepinus</i> (El Burullus Lake)	200	75	37.5	88	44	20	10	1	0.5	0	0
<i>Clarias gariepinus</i> (Lake Nasser)	200	0	0	0	0	0	0	0	0	200	100

Table 2: Seasonal prevalence of different nematodes in *Clarias gariepinus* from El Burullus Lake during 2017

Season	No. examined Fish	Nematodes							
		<i>Procamallanus laevisconchus</i>		<i>Paracamallanus cyathopharynx</i>		<i>Terranova</i> spp. larva		<i>Neocamallanus</i> spp.	
		No. of infected fish	% of infection	No. of infected fish	% of infection	No. of infected fish	% of infection	No. of infected fish	% of infection
Spring	50	18	36	20	40	4	8	0	0
Summer	50	35	70	40	80	11	22	1	2
Autumn	50	14	28	16	32	2	4	0	0
Winter	50	8	16	12	24	3	6	0	0
Total	200	75	37.5	88	44	20	10	1	0.5

Clarias gariepinus infested with 3rd stage larvae of *Contracaecum* spp. showed varying degrees of pathological changes including lymphocytic enteritis with presence of dead cyst (Fig. 8-B) like that recorded by (40) who declare the effect of L3 of *Contracaecum* spp. on *Lates niloticus*. The kidney showed interstitial lymphocytic nephritis

accompanied with severe diffuse lymphocytic infiltration (Fig. 8-C) and multiple interstitial hemorrhages (Fig. 8-D). The skeletal muscle showed myositis associated with perivascular lymphocytic and plasma cells infiltration (Fig. 8-F).

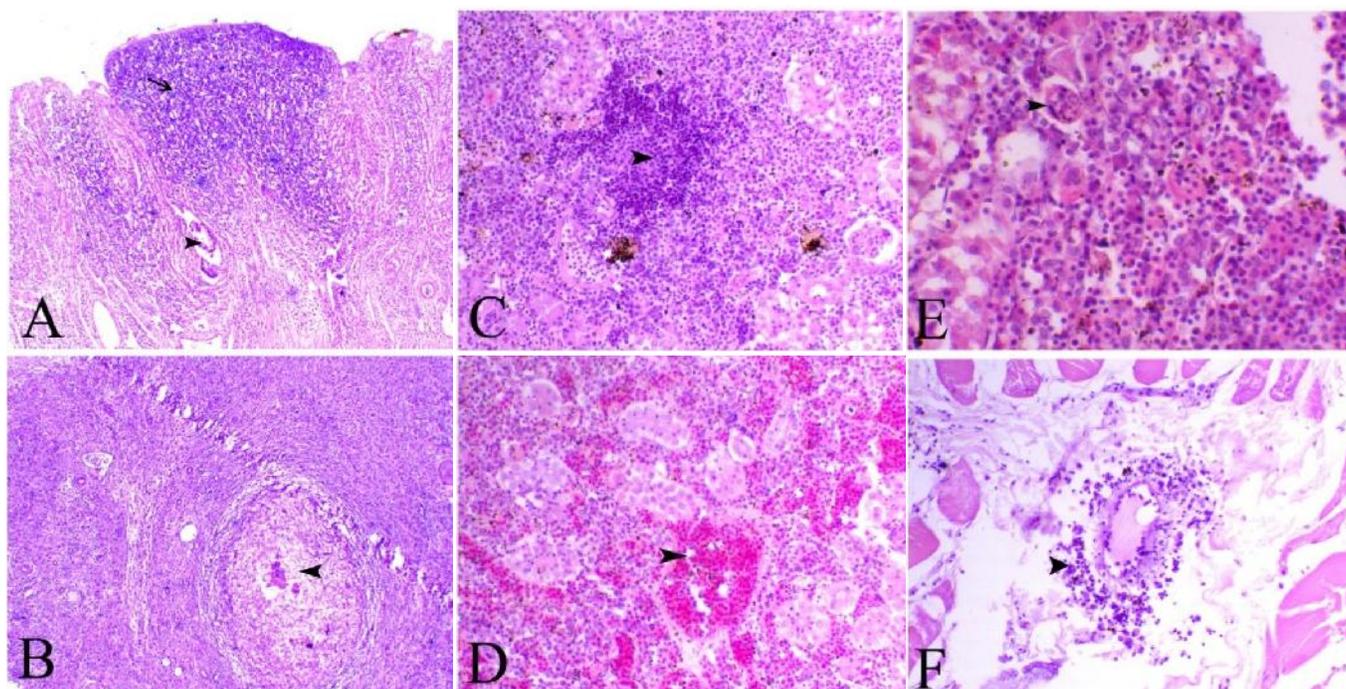


Figure 8. *Clarias gariepinus* infected with *Procammallanus laeiconchus*, *Paracamallanus cyathopharynx* or 3rd stage larvae of *Contracaecum* spp. from El Burullus Lake and Lake Nasser during 2017 showing: (A): Intestine infected with *Procammallanus laeiconchus* and *Paracamallanus cyathopharynx* showing multifocal lymphocytic enteritis (arrow) (arrowhead indicates atrophied crypts) H&E, x 100. (B): Intestine infected with L3 of *Contracaecum* spp. showing lymphocytic enteritis with presence of dead cysts (arrowhead) H&E, x 40. (C): The kidney of *Clarias gariepinus* infected with L3 of *Contracaecum* spp. showing features of interstitial lymphocytic nephritis (arrowhead) accompanied with severe diffuse lymphocytic infiltration, H&E, x 100. (D): Kidney of *Clarias gariepinus* infected with L3 of *Contracaecum* spp. with interstitial haemorrhage (arrowhead) H&E, x 100. (E): Kidney of *Clarias gariepinus* infected with L3 of *Contracaecum* spp. with parasitic cyst (arrowhead) H&E, x 200. (F): The musculature of *Clarias gariepinus* infected with L3 of *Contracaecum* spp. showed myositis associated with perivascular lymphocytic and plasma cells infiltration (arrowhead) H&E, x 200.

Conclusion

Nematodal infection of *Clarias gariepinus* is an important factor affecting wild populations of *Clarias gariepinus* in El-Burullus Lake and Lake Nasser. Therefore, extended investigations about their effects on the survival, the growth and the fecundity of this fish species are recommended to ensure successful fishery management plans.

Clarias gariepinus must be eviscerated as soon as possible after catching to avoid the attack of 3rd stage larvae of *Contracaecum* spp. in the abdominal cavity to the fish

muscle (the edible part in the fish) and disrupt the life cycles of the other detected nematodes. The disposal of viscera or infected fish parts in water should be strenuously prohibited. Regular monitoring of the fish in both lakes is a must. Further studies on *Terranova* spp. in *Clarias gariepinus* (new locality record) are needed.

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