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PARASITES OF PUBLIC HEALTH IMPORTANCE IN NILE AND CULTURED FISH IN EL-MINYA GOVERNORATE

SHIMAA MOHAMED AHMED ¹; YEHIA ABD ELBADEE HEFNAWY ²; MOHSEN IBRAHIM ARAFA ³ AND ASHRAF MOHAMED ABD EL-MALEK⁴

¹ Department of Food Hygiene Assistant Researcher at Animal Health Research Intitute, El-Minia ²⁻⁴ Prof. of Meat Hygiene, Department of Food Hygiene, Faculty of Veterinary Medicine Assiut University ³ Prof. of Parasitology, Animal Health Research Institute, Assiut

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ABSTRACT

The present work aimed to investigate the prevalence of zoonotic parasites in the Nile and cultured freshwater fish in El-Minya Governorate, Egypt. A total of 200 fish samples (100 Nile and 100 cultured) included 50 for each; Tilapia nilotica (Oreochromis niloticus), catfish (Clarias gariepinus), bajad (Bagrus bajad) and carp fish (Cyprinus carpio) were randomly collected from markets and some fish farms from El-Minya city. Out of 200 examined fish 187 samples were infested by one or more zoonotic parasites with a prevalence of 93.5%, highest infestation rate was detected in Nile fish 100 % compared to 87 % in farmed ones. The total prevalence of Encysted metacercaria (EMC) among examined fish was 86.5%; it was 88% and 85% in Nile fish and farmed fish, respectively. Microscopic (EMC) was detected in 83 and 73% of Nile fish and farmed fish, respectively, while macroscopic (EMC) was detected in 5 and 12% of farmed fish and Nile fish, respectively. Microscopic (EMC) was identified as: Cynodiplostomum (EMC) and Prohemistomum (EMC) while, macroscopic (EMC) was identified as Clinostomum phalacrocoracis. On the other hand, the total prevalence of *Ichanthochephala* among examined fish was 8 %, it was 13 and 3 % in Nile fish and farmed respectively, while the total prevalence of Cryptospordium spp and Capillaria spp. among examined fish was 39 and 14.5 %, respectively. The current study revealed a variable prevalence of different zoonotic parasites in different species of examined fish, which represents a potential risk to public health if consumed raw or improperly cooked.

Keywords: Fish, Encysted metacercariae, Ichanthochephala, Capillaria, Cryptospordium

INTRODUCTION

Fish is considered one of the most nutritive and easily digested proteins (El-Naffar *et al.*, 1985). Fish demand as human food is increasing especially in the

Corresponding author: Shimaa Mohamed Ahmed E-mail address: shimaamohammed2016@yahoo.com Present address: Department of Food Hygiene Assistant Researcher at Animal Health Research Intitute, El-Minia

developing countries where people income is low leading to expansion in the production of fishes from both natural water resources and fish cultures to compensate the shortage of good quality animal protein (Pillay, 1990). Fish can serve as intermediate, transport or definitive host for various stage of parasites which may be harmful for human or fish eating mammals if it is consumed raw or improper cooked (Koyunce and Toksen, 2010).

Heterophyiasis is considered a zoonotic problem in Egypt which may be present with diarrhea and abdominal pain (Mahmoud et al., 2002). Their minute eggs may enter nearby lymphatic or blood vessels by the action of their proteolytic enzymes and be transported to other organs, such as the heart, spinal cord or brain, lungs, liver and spleen which is potentially life-threatening (Chai and Lee, 1990). Prohomistomum vivax is of zoonotic importance and may cause death (Williams and Jones, 1976). Five species of Haplorchid are acknowledged as the species responsible for human infection (Yu and Mott, 1994). Clinostomum complanatum is an important zoonotic agent of human infection that causes Yellow grub disease which is transmitted to humans leading to laryngyopharyngitis (Hefnawy et al., 2019).

Ichanthochephalan infections could negatively impact individual health and population recovery as they have significant effects on host metabolism, digestion, nutrient absorption, and energetics (Lotfy, 2020). Cryptosporidium infection in immunocompromised humans causes chronic diarrhea, cachexia, lack of appetite and malnutrition (Xiao et al., 2012).

Intestinal capillariasis is an important fish zoonotic disease. Patients present clinical symptoms of borborygmus, chronic diarrhea, intermittent abdominal pain, weight loss, and several degrees of painless lower-leg edema (Limsrivilai *et al.*, 2014).

Therefore the present study aimed to investigate the prevalence of the different zoonotic parasites in the Nile or cultured freshwater fish at El-Minya Governorate and also to investigate the infectivity of EMC through mice bioassay.

MATERIALS AND METHODS

1- Collection of samples:

From March 2021to February 2022, a total of 200 fish samples (100 wild and 100 cultured) were collected randomly from different

localities and some fish farms at El-Minya Governorate. The obtained samples included 50 of each; tilapia (*Oreochromis niloticus*), catfish (*Clarias gariepinus*), bajad (*Bagrus bajad*) and carp fish (*Cyprinus carpio*). Fish samples were kept in an ice box and examined directly at El-Minya regional Lab Animal health research institute.

2- Parasitological examination:

2.1 Examination of fish for encysted metacercaria

-Macroscopic examination:

The specimens under investigation were carefully examined by the naked eye for the detection of macroscopic metacercaria in musculature and gills (Mahdy *et al.*, 1995).

Microscopic examination for detection of encysted metacercariae:

From each fish, snips (about one gram each) were taken from different muscles of the fish, especially the head, near dorsal fins, and tail regions. Muscle snips were compressed between two glass microscopic slides and examined under a binocular dissecting microscope to detect EMC and to determine viability by observing their characteristic rotator movement (Sohn *et al.*, 2005).

2.2 Examination of fish for *Ichanthochephala* and *Capillaria spp*

Each fish was dissected and their stomach and intestines were removed, and put in small Petri dishes containing a small amount of saline. The stomach and intestine were teased apart with a fine needle and examined by inverted microscope using 4X objective lens and 10X eye lenses for the presence of Ichanthocephala and Capillaria spp adult worms. The collected Ichanthochephala and adult Capillaria spp were washed in saline solution, refrigerated in cold water for relaxation and then fixed in AFA (Alcohol 90% 125 ml, formalin 10% 50ml and acetic acid 25 ml), dehydrated by exposure to ascending grades of ethyl alcohol (30%, 50%,70%, 80%, 90%, 95%) and lastly to absolute alcohol (Pritchard and Kruse, 1982). 2.3Examination of fish for Cryptospordium

2.3Examination of fish for *Cryptospordium spp.*:

Mucosal scrapings of the intestine were collected from examined fish and centrifuged for concentration of *Cryptosporidium* oocysts. Thin smears were done from the sediment and stained with a modified Zeil-Neolsen acid-fast, Hefnawy (1988). Detected parasites were photographed using a Leica microscope (Leica DM 1000, Germany).

2.4 Identification and evaluate the infectivity of detected metacercariae:

For identification and insure of infectivity of detected EMC mice bioassay was performed. Highly positive fish samples were digested as described by Garcia (2001) to obtain the suitable number of EMC that is used in mice bioassay.

Bioassay testing was designed to inoculate mice with a suitable number of EMC (250) orally (Dubey, 2010 and Jennes *et al.*, 2017). Ten albino mice weighing 200-300 gm were

reared for one week and their feces were examined daily for any parasitic infestation to ensure it is free from parasites then divided into two groups:

- The first group was infected with the detected EMC.
- The second group of mice was left uninfected as the negative control group.

Each mouse was infected experimentally with (250) encysted metacercaria, in accordance with the local Institutional Animal Care and Ethics Committee (Assiut University, Egypt).

Recovery of adult worms from the experimentally infected mice was done after egg detection in the fecal examination. Positive infected mice were sacrificed within 10 days from the day of infection to collect and counted under a microscope, as in previous studies (Hefnawy *et al.*, 2019).

RESULTS

Table 1: Prevalence of parasitic infestation in examined cultured and Nile fish.

Cultured fish N=100			e fish =100	To N=2		
Inf.	%	Inf.	%	Inf.	%	
87	87	100	100	187	93.5	

Table 2: Prevalence of different parasites in examined Cultured and Nile fish.

Parasite _		Cultured fish		Nile fish		Total		
		NO	%	NO	%	No	%	
EMC	Microscopic	73	73	83	83	156	78	
	Macroscopic (Clinostoum sp.)	12	12	5	5	17	8.5	
	Total EMC	85	85	88	88	173	86.5	
	Capillaria sp.	ND	_	29	29	29	14.5	
	Cryptospordium sp.	39	39	39	39	78	39	
	Ichanthocephala sp.	3	3	13	13	16	8	

Table 3: Prevalence of differ	ant norgeitag datagtad	in dittorant and	are at avaminad tich
Lable 3: Flevalence of unite	THE DATASHES DETECTED	III OHTELEHI SDE	CIES OF EXAMINED HISH

				50 S	ample	for Ea	ch			Total	
Parasite		Carb		Catfish		Tilapia		Bajad			
		no	%	no	%	no	%	no	%	no	%
EMC	Microscopic	48	96	20	40	49	98	39	78	156	78
	Macroscopic (Clinostoum sp.)	ND	_	ND	_	17	34	ND	_	17	8.5
	Capillaria sp.	ND	_	ND	_	ND	_	29	58	29	14.5
	Cryptospordium sp.	9	18	32	64	24	48	13	26	78	39
Id	chanthocephala sp.	ND	_	ND	_	16	32	ND	_	16	8





Fig 1. Capillaria spp **a:** -Their uterus female; contained both thin and thick-shelled eggs (×40)., **b:** Translucent and barrel-shaped Capillaria spp eggs with rounded mucoid plugs protruding from both poles (×400).



Fig. 2: Adult male of Ichanthochephala spp. $(\times 100)$ stain (100).



Fig. 3: Cryptosporidium oocysts stained with modified Ziehl-Neelsen

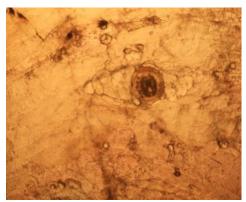


Fig .4: *Prohemistomumspp*.E.M.Cx40

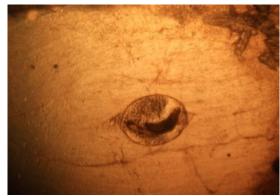


Fig. 5: *Cynodiplostomum spp.* E.M.C.x40

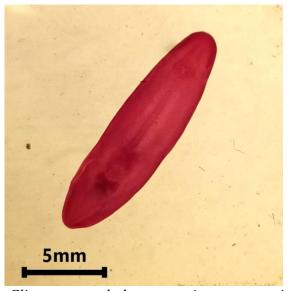


Fig. 6: Clinostomum phalacrocoracis metacercariae x40

DISCUSSION

Parasitic infestation of fish in tropical and subtropical countries represents a serious problem for aquaculture as a result of their huge economic losses (Borji *et al.*, 2012). They affect the growth rate especially for young fish as well as they lose their market value (Rim *et al.*, 2008). The presence of metacercariae is very common, especially in freshwater fish and they may cause public health problems (Eissa, 2002).

Out of 200 examined fish samples, 187 were infested by one or more zoonotic parasites with a prevalence of 93.5%, highest infestation rate was detected in wild Nile fish 100% compared to 87 % in farmed one (table1).

This result was much higher than Khalil *et al.* (2014) in Saudi Arabia, who found that 10.4% of fish were to be infested with zoonotic helminthes parasites. This may be mainly attributed to the locality from which the fish were caught; the degree of water pollution due to human, animal, and bird excreta.

The overall infestation rate of encysted metacercariae in examined fish was 86.5% (173/200), infestation rate of cultured fish with microscopic metacercariae was 73% (73/100) and in Nile fish was 83% (83/100). The infestation rate of EMC in Nile fish and cultured fish was 88% & 85%, respectively. Macroscopic metacercaria was detected only in *Tilapia nelotica*, it was 12% (12/100), and 5 % (5/100) of cultured and Nile fish,

respectively. Microscopic EMC was detected in 78% (156/200) and macroscopic EMC was detected in 8.5% (17/200) of examined fish. The total infected rate of *Cryptospordium* among examined fish samples was 39% and was the same percentage in Nile and cultured fish 39%. *Capillaria spp* was only found in Nile fish with 29%. The total infection rate of *Ichanthochephala* was 8% and it was 13% and 3% in Nile and cultured fish, respectively (Table 2), (fig 2.).

The high prevalence of encysted metacercariae detected in the present study in different species of examined fish reflected the presence of intermediate and definite hosts in our study region. These results were relatively similar to that obtained by previous researchers as Taher (2009) in Assiut Governorate and Sahar et al. (2009) in Sharkia Governorate (Egypt) and Saad et al. (2019) in Giza Governorate, who reported infection rates with different encysted metacercariae was 84.75%, 84.8% and 82.8%, respectively. On the other hand, the present results are considered higher than those reported by Chi et al. (2008) they recorded that the prevalence of EMC was 44.6% in fish species collected from grow-out ponds, and cultured in nurseries was 43.6%. Also El-Sheikh and El-Shazly (2008), their prevalence rate of EMC was 23.2%. Such variations in the frequency EMC may be attributed to various factors including the locality from which fish were caught and fish species of examined samples and their feeding habit. In addition to the degree of water pollution with human, animal and birds excreta. In the current study, the infection rate of microscopic encysted metacercaria was relatively higher in Nile fish than cultured fish; it was 83% & 73% respectively (Table 2). High prevalence in Nile fish was recorded also by Ammar and Arafa (2013) who mentioned that the prevalence rate of microscopic EMC was 88.3 % and 26.7% in Nile fish and cultured fish respectively in Assiut Governorate. The difference in percentage between Nile and farmed fish may be attributed to fish raised in rivers, canals, streams and lakes may be more exposed to

infected snails/cercariae, and reservoir hosts, compared to fish raised in farms, where the pond environment may not favor the presence of snails (Nguyen *et al.*, 2007).

Contrary to this, the result was recorded by Eissa et al. (1996) who reported that the infection rate in farmed and Nile fish in Sharkia Governorate was 67.33% and 42.33% respectively. Also, Ibrahim (2012) recorded that the infection rate in farmed fish was 63.51% and in wild fish was 44.37% in Ismailia Governorate. On the other hand, macroscopic encysted metacercaria was however identified as Clinostomum phalacrocorais. It was detected only in Tilapia niloticus and not detected in other fish species. The total infection rate was 34%, it was 12% and 5% in cultured and Nile Tilapia respectively.

Cl.Phalacrocoracis metacercaria recorded as a parasite only for Nile tilapia (Caffara et al., 2019). It was found previously in Egypt by Ammar and Arafa (2013) who detected it in 19.2 % of examined Nile O. niloticus and they did not detect it in farmed Tilapia. Manal et al. (2018) described it as yellow to orange in color cysts in 39.16% of O. niloticus from the River Nile in El-Minya District. Also, Mai et al. (2021) recorded the infection rate of Cl. Phalacrocoracis EMC among the investigated O. niloticus in Egyptian water was 60.93%. Regarding the morphological characters, the description of C. phalacrocoracis detected in the present work agrees with Thabit (2004) and Mai et al. (2021).

The protozoan parasite Cryptospordium is regarded as producing emergent zoonosis as producing clinical diseases which have been recorded in numerous host species including man (O'Donoghue, 1985). Infection of man can occur through contaminated hands with intestinal contents during fish evisceration and cleaning or through eating insufficiently cooked fish such as grilled ones. Spreading of infection is the result of mature Cryptospordium oocysts which are strongly resistant to many disinfectants, but their infectivity can be destroyed by exposure to ammonia with 10% formal saline, freezing or heating to 65°C for 30 minutes (Hefnawy 1988). Table (2) and fig (3), showed the overall infection rate of Cryptospordium spp in examined fish was 39 %. This result was relatively similar to that obtained by Certad et al. (2015) as overall prevalence (36.6%) and Hefnawy (1988)recorded the Cryptosporidium prevalence in Nile Tilapia was (30%). But this result is higher than Ammar and Arafa (2013) who recorded that 23% of examined fish were infected with Cryptospordium spp. On other hand, in the current study, there is no difference in infestation rate between Nile and cultured examined fish, the infection rate in both was 39 %. The present result is considered higher than those reported by Ammar and Arafa (2013) they mentioned that Cryptosporidium infection was 15.0 % and 23.3% in Nile and cultured O. niloticus respectively and higher than Reid et al. (2010) they observed a prevalence of only 2.4% (6/255) in Nile fish. Also Morine et al. (2012) found that the prevalence of infection of freshwater ornamental fish collected from 8 retail outlets across Perth was 3.5%.

The infestation of intestinal rate Ichanthochephala was higher in Nile fish than in the cultured where it was 13% and 3%, respectively. A lower infection rate with Ichanthochephala in both Nile and cultured fish was reported previously by several authors: Eissa et al. (2011) who found the infection rates of in cultured and wild O. niloticus were 5.2% and 1.5%, respectively. Mathenge (2010) reported that the prevalence of Ichanthochephala in Nile fish was 10% and 15.5% in cultured fish. While a higher infection rate was reported by Eissa et al. (2010) was 41% in Nile O. niloticus and 50.4% in cultured O. niloticus, Akoll et al. (2012) as the infectivity rate was 44.3% in cultured fish. These variations might be attributed to the differences in locations and variations in climate conditions and higher exposure to intermediate hosts. Where, the fish encounters the infective larval stage after

feeding on an infected crustacean (Amin, 1998).

Microscopic EMC was found in between the muscle fibers of infected fish; they were heavily distributed at the posterior part of the body. The highest prevalence rate microscopic EMC was showed in 98 % (49/50) of examined Tilapia followed by Carb spp. 96% (48/50), in Bajad samples was 78% (39/50), while the lowest infestation rate of microscopic EMC 40% (20/50) was found examined Catfish. Morphologically **EMC** differentiated microscopic Prohemistomum EMC, Cynodiplostomum EMC (fig.4& 5). The macroscopical EMC was found in the branchial and pharyngeal regions of Tilapia spp. only and their infestation rate was 34% (17/50).Morphologically macroscopic **EMC** differentiated Clinostomum into **EMC** phalacrocoracis (fig. 6). Ichanthochephala is only found in Tilapia fish, the rate was 32% (16/50). Capillaria spp was only found in Bajad samples, the rate was 58% (29/50). The highest prevalence rate of Cryptospordium spp showed in Catfish at 64% (32/50) followed by Tilapia with an infection rate of 48% (24/50), while the lowest infection rate of Cryptospordium spp was 18% (9/50)in carb fish (Table 3), fig(3).

As shown in Table 3, the prevalence of microscopic encysted metacercaria varied according to fish species. Their highest infestation rate was detected in Tilapia "O. niloticus" 98%. This result cleared that tilapia "O. niloticus" thought to be the main second intermediate host for trematodes EMC in Egypt. These are nearly similar to those given by Sahar et al. (2009) and Taher (2009). On other hand, the lowest infection rate with EMC was detected in cat fish "C. garpienus" 40%. This result not agree with previous studies Sahar et al. (2009) and Hefnawy et al. (2019). Low infection rate in catfish in the present study may as a result relative resistance of catfish to infection.

Differences in infection rates between fish species may be attributed to the difference in

the habitat, food supply and abundance of both aquatic snails and aquatic birds which play the main role to complete the life cycle of some trematodes and climatic variations of the fish sampling areas (Taher, 2009).

Microscopic metacercaria recovered in the present work were identified as *Prohemistomatide* and *Cynodiplostomatide*, their morphologically coincided with that previously reported by Mahdy *et al.* (1995), Mousa *et al.* (2000) and Saad *et al.* (2019).

Few human cases of *Ichanthochephala* have been reported worldwide; and it is common in certain foci in China (Taraschewski, 2000). The overall infection rate of *Ichanthochephala* (*Acanthocentius tilapae*) in the present work was 8%, Table (3) and fig (2). This result was partially similar to Ammar and Arafa (2013) who detected it in 9.2% of Tilapia. But the present data was lower than Bayoumy *et al.* (2006) in Giza, Egypt and Paller *et al.* (2016) in a Philippine, they found it in 49.3% and 74 %, in Nile and Cultured fish respectively.

Intestinal capillariasis is a fish-borne nematodiasis, it is an important emerging zoonotic disease. Capillariasis, mostly caused by eating raw fish, is the most common disease that appeared repeatedly all over the world starting in Philippines, Iran, Taiwan even in Egypt (Cross, 1992). Diagnosis of the disease in infected man is difficult as its symptom appear atypically and which make confusion with other causes of diarrhea as GIT cancer, thyroid gland disease, besides the absence of Capillaria when the stool is examined, however, serology can help in diagnosis (Intapan et al., 2017).

Egypt was considered a non-endemic area of capillariasis but now the issue is not and annually there is a considerable number of cases reported. Moreover, many cases passed unnoted. It has been clearly demonstrated that the severity of intestinal capillariasis has grown in Egypt and the number of reported cases increasing which can be explained by improvement in diagnosis also clinicians become more aware of parasites and indicate

that we have natural intermediate host, freshwater fish (Attia *et al.*, 2012; El Dib *et al.*, 2015).

In the present results, adult worm of *Capillaria spp*. and its larvae were found only in *Bagrus Bajad*. *Capillaria spp* was detected previously in the same fish species (*Bagrus Bajad*) by Mohamed *et al.* (2003), Khalil *et al.* (2014), Khalil *et al.* (2016) also Okpasuo *et al.* (2016).

In this study, the overall infection rate of *Capillaria* in examined fish was 14.5% from all examined fish samples, whereas in Bajad species was 58%. The present results are considered higher than those reported by Thilakaratne *et al.* (1994) and Khalil *et al.* (2014) who recorded that the prevalence of *Capillaria spp* was 4% and 15 %, respectively, in Bajad at southern Saudi Arabia. Also, Okpasuo *et al.* (2016) reported that the infectivity rate of *Capillaria spp* in *Bargus Bajad* was 25%.

On contrary, the present study results are considered lower than those reported by Shehzad and Ansari (2018) and Abdel-Rahman *et al.* (2019) who determined the *Capillaria* infection in all examined Bajad samples (100%). This cleared that Bajad is thought to be the main second intermediate host for *Capillaria spp.* in the examined area.

In the current study, infection rate of *Capillaria* is detected only in Nile fish (Bajad) and not detected in cultured fish. This result agree with Abdel-Rahman *et al.* (2019) who recorded that the *Capillaria* in nearly all collected Nile fresh water, *Bargus Bajad*.

On the other hand, the infection with *Capillaria spp*. in the present work was not detected in other species of examined fish (Bolty, Catfish and Carp fish). This result did not agree with previous studies of Abdel-Rahman *et al.* (2019), who detected it in Bolty and with Khalil *et al.* (2016) who detected it in Catfish.

The prevalence of *Cryptospordium* infection varied according to fish species. Their highest infection rate was detected in Catfish (Clarias gariepinus) at 64% Table (3). This result agreed with Saad-Alla et al. (2022) who mentioned that the overall prevalence of Cryptosporidium in Clarias gariepinus fish was 64% in Giza Governorate, using a modified Ziehl-Neelsen staining technique. The high infection rate of *C. gariepinus* could be due to being omnivorous in its feeding habits and such species of fish will utilize all the available potential food resources of the water bodies. In addition to the sanitary condition of the area, the location of the drainage canals from living areas and biological pollution Ammar and Arafa (2013).

CONCLUSION AND RECOMMENDATION

This calls for raising awareness of fish health management and the application of appropriate control measures. Therefore public awareness creation activities should be conducted on the zoonotic nature of fish parasites and the danger of consumption of raw or undercooked fish.

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الطفيليات ذات الأهمية الصحية في الأسماك النيلية والمستزرعة في محافظة المنيا

شيماء محمد أحمد ، يحيى عبد البديع حفناوى ، محسن ابر اهيم عرفة ، أشرف محمد عبد المالك

أجرى هذا العمل بهدف تحديد نسبة الإصابة بالطفيليات المشتركة بالأسماك المستزرعة والنيلية بمحافظة المنيا بمصر. أجريت الدراسة على عدد ٢٠٠ سمكة (٢٠٠ سمكة من كلا من النيلي والمستزرع) لعدد ٥٠ سمكة من كلا من البلطي , القراميط , البياض والمبروك وقد تم تجميع العينات عشوائيا من الاسواق والمزارع المختلفة بمدينة المنيا. وقد بلغ عدد الأسماك المصابة ١٨٧ سمكة من ٢٠٠ سمكة تم فحصها بنوع واحد أو أكثر من الطفيليات وبلغت نسبة الاصابة ٥,٩٣ وكانت أعلى نسبة اصابة في الأسماك النيلية بنسبة ١٠٠ ٪ مقارنة بالأسماك المستزرعة التي بلغت ٧٨٪. متوسط نسبة الاصابة الكلية بالميتاسيركاريا المتحوصلة ٥,٦٠ ٪ بنسبة ٨٨٪ في السمك النيلي و٥٠٪ في الأسماك المستزرعة وقد بلغت نسبة الاصابة الحسابة الميتاسيركاريا المجهرية ٥ ٢٠٪ في الأسماك النيلية و ٧٣٪ في الأسماك المستزرعة ود ٢٠٪ في الأسماك المستزرعة و ٢٠٪ في الأسماك النيلية و ٢٠٪ في الأسماك المستزرعة وكانت نسبة الاصابة الكلية بالايكانسوكيفالا ٨٪ حيث بلغت ٣٪ في الأسماك النيلية و ١٣٪ في الأسماك المستزرعة وكانت نسبة الاصابة الكلية بالكريبتوسبوريديوم والكابيلاريا ٩٣٪ و ٥,٤١٪ على الترتيب وهذه الدراسة أوضحت نسب اصابة متعددة بأنواع مختلفة من الطفيليات المشتركة في أنواع مختلفة من الأسماك والتي تشكل خطرا على صحة الانسان عند استهلاك الأسماك النيئة أو الغير مطهية جيدا .