

PREVALENCE AND MORPHOLOGICAL CHARACTERIZATION OF PARASITIC INFECTION IN FRESH WATER FISH IN SOHAG PROVINCE, EGYPT

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ABSTRACT

Although fish are a valuable source of animal protein for human, their production is reduced sometimes by parasitic diseases. The purpose of this study was to ascertain the frequency and importance of possible parasites that could infect freshwater fish in Sohag governorate. A total of 150 samples [100 Nile tilapia (*Oreochromis niloticus*) and 50 catfish (*Clarias gariepinus*)] were chosen at random from various farms in the Sohag Governorate for this purpose. According to the parasitological analysis of the collected fish, *Clarias gariepinus* had the highest infection rate 64% (32/50) compared to *Oreochromis niloticus* 56% (56 / 100). *Quadriacanthus* spp. (50%), *Contracecum* spp. (50%), *Trichodina* spp. (56%), *Myxobolus* spp. (55%), *Cryptosporidium* spp. (50%), and encysted metacercaria [microscopic (55%), and macroscopic (35%)] were the parasites found in Nile tilapia fish. In contrast, the identified parasites in catfish included microscopic encysted metacercaria (60%) and *Quadriacanthus* spp. (54%), *Trichodina* spp. (64%), *Myxobolus* spp. (44%), *Henneguya* spp. (40%), and *Cryptosporidium* spp. (56%). It is evident from the current study that a significant frequency of numerous zoonotic parasites were found in the fish under examination. As a result, control measures in fish farms are required to prevent the spread of these parasites, which pose a risk to public health.

Keywords: Fresh water fish, Nile tilapia, Cat fish, *Oreochromis niloticus*, *Clarias gariepinus*, parasites, Sohag governorate, Egypt.

INTRODUCTION

One of the best and least expensive sources of protein is fish. About a quarter of

All animal protein consumed worldwide comes from fish, which also provides many people with jobs and money at the household and national levels. To address the needs of fish as a source of protein, fish farming in natural and aquaculture systems has greatly improved (Mgbemena *et al.*, 2020), (Abd-Elrahman *et al.*, 2023).

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Because of its omnivorous diet, hardiness, and quick growth and reproduction rates, the Nile tilapia (*Oreochromis niloticus*) is farmed in aquaculture throughout Asia, Africa, and America (Pinto *et al.*, 2014). On the other hand catfish are bottom feeders that consume both dead and living animals. In order to escape from captivity, find food, or overcome desiccating conditions, they can also crawl on dry ground. It turned out to be a profitable endeavor with few expenses, rapid returns, and low investment (Ikechukwu *et al.*, 2017).

However, there are some obstacles to fish production, such as pollution and fish infections. The accumulation of fish construction and the absence of health management measures have resulted in the formation of bacteria, viruses, fungi, and parasites. Approximately 80% of fish illnesses in Egypt are parasitic. Changes in the distribution and abundance of the parasite species' intermediate and ultimate hosts can have an indirect impact on them in addition to direct ones (Lima dos Santos & Howgate, 2011)

Any kind of aquatic or cultural system can harbor parasites, which can infect any kind of fish. They can be either protozoan or metazoan, and include nematodes, myxozoans, trematodes, cestodes, acanthocephalans, and crustaceans (Mitiku *et al.*, 2018). Furthermore, some parasites have intermediate hosts in freshwater fish, which increases the possibility of transmission to humans after consuming raw or undercooked fish items (Ronald and Campus 2018).

Fish parasites typically result in economic losses because of tissue damage and mortality (Asely *et al.*, 2015). Additionally, it can cause the host's intestinal tissue to completely deteriorate. It is important to note that the detrimental effects of the parasite grow more serious in cases of severe infestation (El-Mansy *et al.*, 2011). In Sohag Governorate, Upper Egypt, the current study sought to determine the frequency of

parasitic infection and the morphological identity of isolated parasites in freshwater fish.

MATERIAL AND METHODS

Ethical Considerations:

This work was approved with ethical approval number 06/2024/0199 by the research ethical committee of the Faculty of Veterinary Medicine, Assiut University, Egypt.

Study Region and Sample Gathering:

150 freshwater fish samples in total (100 fingerling Tilapia and 50 catfish) were collected from several farms in Sohag Governorate, Upper Egypt. After that, the fish were brought to the Animal Health Research Institute's Sohag branch's parasitology lab in separate, labeled plastic bags for a thorough parasitological examination and description of each fish (Pinto *et al.*, 2014).

Inspection of fish for different parasites:

Macroscopic Examining: Each fish was weighed in the lab and then placed on a dissection plate with its right side toward the dissector's abdomen. Two slits were made in the body cavity using a pair of scissors. The external surface, gills, fins, intestine, muscles, and body cavity were examined with a dissecting microscope and the naked eye to look for any macroscopic parasites. The first cut ran from the rectal opening up to the branchial cavity, and the second cut followed an arch from the anus up to the spine and further to the upper side of the branchial cavity (Mdegela *et al.*, 2011).

Microscopic examination:

Examining the intestinal tract: The gastrointestinal tract (GIT) was taken out and put in a black tray. It was then straightened, opened, and the contents were emptied into a petri dish and diluted with physiological saline. The films were then made and analyzed. After that, the gut was thoroughly cleaned with distilled water,

carefully shaken, and examined under a lab lamp with a magnifying lens to check for parasites. Using plastic forceps, the observed parasites were removed and placed in universal bottles containing 15 milliliters of 70% alcohol so they could be identified under a microscope (Abd El-Lateif 2004).

Examining the respiratory organs: To reveal the gills and auxiliary respiratory organ, the operculum was cut off using scissors. The gills were examined to check for hemorrhages, excessive mucus, parasite cysts, and pigmentation. A wet mount was made from the gills and other respiratory organs, and it was inspected under a microscope to check for parasites (Abd El-Lateif, 2004).

Modified protocol for Zeihl-Neelsen staining: Ziehl-Neelsen staining and methanol fixation were applied to prepared smears derived from the intestinal and stomach epithelium of separated samples (Henriksen *et al.*, 1981). This process was modified for staining *Cryptosporidium* spp. oocysts, which show up as green-blue fecal detritus, tissues, or yeasts on a brilliant red background (Casemore *et al.*, 1985).

Examination of fish muscle: To find any macroscopic cysts, the specimens of fish muscle that were gathered underwent examination. A tiny sample of muscle from the head, trunk, and tail areas of each fish was broken up between two glass slides and

examined under a microscope (Aly *et al.*, 2005). The infected samples are then artificially digested.

Artificial Tissue Digestion of Infected Samples: After cutting the infected fish muscles into smaller slices, the samples were processed with artificial gastric juice (7.5 mg Pepsin powder, 10 mL hydrochloric acid 37%, and 1000 mL distilled water). They were then placed on a magnetic stirrer and allowed to completely dissolve before being filtered and allowed to settle for a short while. After the supernatant was poured off, the sediment was put into Petri dishes and looked at under a dissecting binocular microscope to see if any tiny encysted metacercaria were present (Abd-Elrahman *et al.*, 2023).

RESULTS

Prevalence of different parasites in examined catfish in relation to body weight (gram):

The overall prevalence of parasitic infection was 64% (32/50) in 50 examined catfish weighing between 100 and 750 grams. The parasites that were isolated were microscopic encysted metacercaria, *Quadriacanthus* spp., *Trichodina* spp., *Myxobolus* spp., *Henneguya* spp., and *Cryptosporidium* spp. Table 1 shows these parasites.

Table 1: Prevalence of different parasites in examined catfish in relation to body weight (gram).

Variable Weight (g)	No Ex	Helminthes						Protozoa					
		Mic. ency. metacercaria		<i>Quadriacanthus</i>		<i>Trichodina</i>		<i>Myxobolus</i>		<i>Henneguya</i>		<i>Cryptosporidium</i>	
		No. inf	%	No. infec	%	No. inf	%	No. inf	%	No. inf	%	No. inf	%
100-300	33	22	66.7	17	51.5	20	60.6	15	45.5	13	39.4	12	36.4
301-400	10	7	70	7	70	9	90	5	50	6	60	9	90
401-750	7	1	14.3	3	42.9	3	42.9	2	28.6	1	14.3	7	100
Total	50	30	60	27	54	32	64	22	44	20	40	28	56

Prevalence of different parasites in examined tilapia fish in relation to body weight (gram): The overall prevalence of parasitic infection was 56% (56/100) in the 100 Nile tilapia fish with variable weight (100 g to 750 g). Table 2 shows that the

parasites that were isolated were *Clinostomum* spp. (macroscopic encysted metacercaria), Microscopic encysted metacercaria, *Quadricanthus* spp., *Contracecum* spp., *Trichodina* spp., *Myxobolus* spp., and *Cryptosporidium* spp.

Table 2: Prevalence of different parasites in examined tilapia fish in relation to body weight (gram).

Variable Weight (gram)	No. examined	Helminthes								Protozoa					
		Mic. ency. metacer.		Mac. ency. metacer.		<i>Quadricanthus</i>		<i>Contracecum</i>		<i>Trichodina</i>		<i>Myxobolus</i>		<i>Cryptosporidium</i>	
		No. inf	%	No. inf	%	No. infec	%	No. inf	%	No. inf	%	No. inf	%	No. inf	%
100-300	67	45	67.2	30	44.8	35	52.2	30	44.8	47	70.1	43	64.2	40	59.7
301-400	13	7	53.8	4	30.8	12	92.3	2	15.4	10	76.9	11	84.6	9	69.2
401-750	20	3	15	1	5	3	15	18	90	3	15	1	5	1	5
Total	100	55	55	35	35	50	50	50	50	56	56	55	55	50	50

Morphological characterization of isolated parasites infecting tilapia and catfish:

The collected parasites were morphologically identified according to (Ali *et al.*, 2002, Elseify *et al.*, 2015, Abd-Elrahman *et al.*, 2023).

Nematodes (*Contracecum* third stage larva):

Isolated from the body cavity of the examined fish, their cuticle was sharply serrated, it revealed teeth that were boring (Fig. 1) and an anterior intestinal caeca. The posterior end has a subterminal anal aperture and a conical tail with a retractile tip that bears tiny spines (Fig. 2).

A-Helminthes



Fig.1.Anterior region of larval stage of *Contracecum* ×100, show boring teeth and oesophagus.



Fig.2. Posterior region of larval stage of *Contracecum* ×100, show conical tail.

Macroscopic encysted metacercaria (*Clinostomum*): taken from the skin, muscles, and fins of the fish that were studied. Its body is elongated, with the ventral sucker appearing in the third of the parasite and the oral sucker on the anterior

portion. There was a short oesophagus. The oesophagus gave rise to the bifurcate intestinal caeca, which split laterally and terminated along the body. The ovary is situated between the two testes, which were positioned in tandem (Fig. 3).



Fig. 3. Wet mount of excysted *Clinostomum* sp.X40.

Microscopic encysted metacercaria:
A double-walled, spherical or sub-

spherical cyst lodged in the muscle of an infected fish (Fig. 4).



Fig. 4. Microscopic encysted metacercaria within the muscle of infected fish 40X (arrow)

B- Protozoa

***Trichodina* sp.:** Its circular body surrounded by many rows of cilia, and

its adhesive is shaped like a saucer (Fig.5).



Fig. 5. *Trichodina* sp. 400x (arrow)

***Henneguya* sp.:** It resembles an oval spore with two long caudal appendages and two anterior polar capsules (Fig. 6)

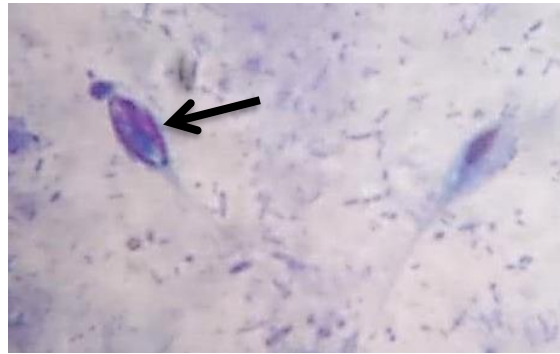


Fig. 6. *Henneguya* sp. 1000x (arrow)

***Myxobolus* sp.:** Ovoidal spore featuring internal polar filaments and a polar capsule (Fig.7).

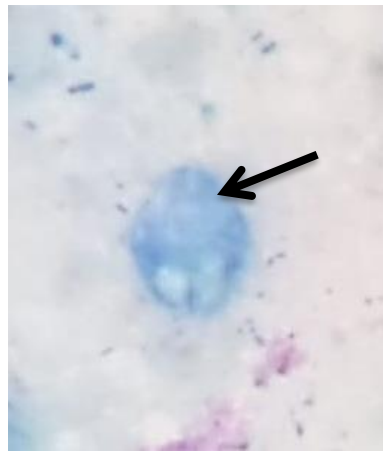


Fig. 7. *Myxobolus* sp. 1000x (arrow).

***Cryptosporidium* spp.:** Oocysts having a smooth wall, spherical to ovoid in shape, and an acid-fast reddish pink appearance (Fig. 8)

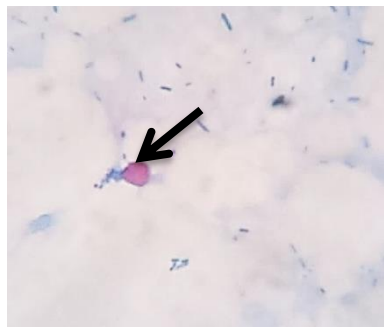


Fig. 8. *Cryptosporidium* spp. 100x (arrow).

DISCUSSION

The current study found that the prevalence of parasitic infection in the fish under examination was 58.7% (64% in the case of

Clarias gariepinus and 56% in that of *Oreochromis niloticus*). These findings are less than those reported by Juntaban et al. (2021) in Thailand, who found 75.69%, Monir et al. (2015) in Bangladesh, who

found 94.54%, and Sahar et al. (2009) in Egypt, who found 84.8%. On the other hand, Oso et al. (2017) found that 30.9% in Nigeria.

Microscopic encysted metacercaria 60% (30/50), *Quadriacanthus* spp. 54% (27/50), *Trichodina* spp. 64% (32/50), *Myxobolus* spp. 44% (22/50), *Henneguya* spp. 40% (2/50), and *Cryptosporidium* spp. 56% (28/50) were the parasites found in the catfish under examination. While Mahmoud et al. (2018) in Assiut (Egypt) observed *Trichodina* sp. in 14.1% of the catfish, El-Seify et al. (2013) in Egypt found *Henneguya* sp. (1.9% and 4.11%), Hefnawy et al. (2019) in El Minia found tiny encysted metacercaria in 70.0% of the fish. Abrunhosa et al. (2017) discovered *Myxobolus* in 20% of the Brazilian Amazon region, Onojafe et al. (2021) in Nigeria who examined *Quadriacanthus* in 2.1%, and Shaapan et al. (2022) in Egypt who examined *Cryptosporidium* spp. in 68.3% of examined cat fish.

Quadriacanthus spp. (50%), *Contracaecum* spp. (50%), *Trichodina* spp. (56%), *Myxobolus* spp. (55%), *Cryptosporidium* spp. (50%), and encysted metacercaria [microscopic (55%), and macroscopic *Clinostomum* (35%)], were the identified parasites in the 100 tilapia fish that were tested. While Hussein et al. (2019) in the Qena governorate mentioned *Quadriacanthus* spp. (30%), Abd-ELrahman et al. (2023) in Upper Egypt display *Contracaecum*. 2%, *Myxobolus* 2%, macroscopic EMC 37% and microscopic EMC 58%, and El-Ghaysh and El-Mahdy (1998) in Egypt exhibited *Cryptosporidium* in 20% of examined fish.

These discrepancies in the results could be caused by differences in the water's depth and current, the oxygen content of the surrounding air, the immune system, water pollution, sampling, the location of the intermediate host snails, and the species of fish under investigation (Biu, et al, 2014), (Paperna, 1996).

In terms of size, fish weighing between 100 and 300 grams were shown to be more susceptible to parasite infection than larger fish weighing between 401 and 750 grams. This finding is consistent with other research conducted in the Giza Governorate (Egypt) and Upper Egypt (ELrahman *et al.*, 2023) (Saad *et al.*, 2019). The underdeveloped immune systems of smaller fish could be the cause of this (Magnadottir, 2010).

CONCLUSION

The recent study found that many zoonotic parasites (such as EMC, *Clinostomum*, and *Cryptosporidium*) were highly prevalent in the fish that were investigated. If these parasites are consumed raw or cooked incorrectly, there may be a health risk to the population. Therefore, control measures should be considered carefully in order to prevent further dispersion.

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انتشار العدوى الطفيلية وتوصيفها المظهري في أسماك المياه العذبة في محافظة سوهاج، مصر

مروة السيد على ، احمد كمال دياب ، محسن ابراهيم عرفة ، مديحة درويش ، احمد جارح

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الأسماك هي أحد أهم مصادرنا من البروتين. يحصل الناس في جميع أنحاء العالم على ٢٥% من البروتين الحيواني عن طريق الأسماك و المحار. أيضا توفر صناعة الاسماك العديد من فرص العمل و كذلك الدخل على مستوى الاسره و المستوى القومي.

لقد ادى زيادة تكثيف انتاج الاسماك و نقص اجراءات الاداره الصحيه الى العديد من مشاكل الامراض البكتيرييه والفيروسيه والفطريه و الطفيليه .حوالي ٨٠% من مشكلات الاسماك المرضيه هي طفيليه خصوصا في اسماك المياه الدافئه

ويمكن اعتبار طفيليات الاسماك كمؤشرات بيولوجيه للتاثيرات والتغيرات البيئيه و قد يكون لتغير المناخ تاثير مباشر على انواع الطفيليات و ايضا تاثيرات غير مباشره من خلال التغيرات في توزيع ووفرة عوائلهم الوسيطة و النهائيه فمن الواضح ان الاسماك تعتبر أرخص مصدر للبروتين للانسان و الماشيه الاخرى ولكن مزارعو الاسماك يقيدهم معدل النفوق الهائل للزريعة و الاصبغيات نتيجة غزو الديدان الطفيليه

يواجه انتاج الاستزراع المائي عددا من التحديات بما في ذلك امراض الاسماك و التلوث.و تعد أسماك البلطي النيليه هي الانواع الاصليه في افريقيا وذلك بسبب خصائصها كمعدل التكاثر و النمو السريعين و التغذية النهمة و الصلابه و قد استخدمت في الاستزراع المائي في جميع انحاء العالم بامريكا و افريقيا و اسيا

واسماك البلطي هي اهم ثاني مجموعه من الاسماك المستزرعه بعد اسماك المبروكه مع انتاج تربية الاحياء المائيه العالميه اكثر من ٣ ملايين طن في عام ٢٠١٢

الطفيليات مكونات مهمه لبيولوجيا العائل المضيف و البنيه السكانيه في واقعيه عمل النظام البيئي. حيث توجد الطفيليات المختلفه من اوليات و ديدان شريطيه و اسطوانيه و ورقيات في مختلف انواع الاسماك و نظم تربية الاحياء المائيه.بالاضافه الى كون اسماك المياه العذبه عوائل وسيطه لبعض الطفيليات و التي تشكل خطرا بان انتقالها للانسان من خلال تناول المنتجات السمكيه النيئه أو الغير مطهيه جيدا .

لا خلاف ان ديدان الاسماك الطفيليه هي من اهم مسببات أمراض الاسماك و قد تضر عوائلهم بطرق عده . فهذه الطفيليات قد تسبب تهيج و اصابة او ضمور الانسجه و انسداد القناه الهضميه و الاوعيه الدمويه و القنوات الاخرى و قد يؤدي وجودهم الى تغيرات معينه في انشطه الانزيمات و الفيتامينات و هرمونات عوائلهم المضيفه و ايضا قد يؤدي ادخال منتجات التمثيل الغذائي السامه الى حرمان الاسماك من التغذية الطبيعيه

معظم القشريات الطفيليه لاسماك المياه العذبه يمكن رؤيتها بالعين المجرده حيث تعلق على الخياشيم و جسم و زعانف العائل المضيف و تقضي جزء كبير من حياتها على جسم السمكه حيث تمتلك اعضاء لاصقه و اجزاء فمويه تكيفها لثقب الانسجه و امتصاص دماء الاسماك . فالطفيليات احادية و ثنائيه العائل تسبب اضرار فادحه لخياشيم الاسماك و تؤدي رؤوس الديدان الشريطيه الطفيليه الى تغلغلها العميق في انسجه امعاء الاسماك المصابه بها . و ايضا يسبب طفيل اكانثوس تمزق شديد في الانسجه المصابه بواسطه مخالب خرطوميه في موضع الالتصاق. فالطفيليات مسؤولة عن خسائر اقتصادية سنويه هائلة في عمليات الاستزراع المائي في كلا المياه العذبه و المالحة. وقد أدت هذه الخسائر إلى انتشار واسع النطاق للعلاجات الكيماوية في محاوله للسيطره على العدوى من هذه الطفيليات و بسبب الآثار الجانبية البيئية المرتبطة بهذه العلاجات قد انتشرت في السنوات الاخيره النباتات الطبيه بما في ذلك اكليل الجبل و الثوم و الزنجبيل و الشيح و النعناع مظهرين نشاطاً و اعدا مضافاً للديدان حيث ان غالبية النباتات المذكوره اعلاه لها بعض التاثيرات المناعيه على الاسماك وقد تم تحسين وظائف المناعه غير النوعيه مثل نشاط الجراثيم و كرات الدم البيضاء لدى تلك الاسماك. و قد استخدم الثوم و اللوز الهندي لعلاج طفيليات سمك البلطي الخارجيه.

في هذا البحث تم فحص ١٠٠ من اسماك بلطي المزارع و ٥٠ من اسماك قراميط المزارع في محافظة سوهاج تم قياس وزن و طول كل سمكه على حده و بفحص الطفيليات الموجوده في كل من العضلات و الأمعاء و الجهاز التنفسي في كل سمكه تبين انتشار مرتفع للعديد من الطفيليات الحيوانية المنشأ (القابله للانتقال من الاسماك للانسان) كالكليستومام و الكريبتوسبورديوم و الذي يمثل خطر محتمل على الصحة العامه اذا تم استهلاكها نيئه أو مطبوخه بشكل غير صحيح لذلك ينبغي اتخاذ تدابير الرقابه في المداولات لتجنب مزيد من الاصابات.