



Comparative studies on incidence of *Cryptosporidium spp.* in wild marine and fresh water fish at Alexandria and Behera Provinces, Egypt

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Cryptosporidium is a widespread pathogen that affects a wide-ranging of vertebrate hosts including humans, in which it is one of the main causes of diarrhea. Marine and fresh water fishes also harbor *Cryptosporidium* species. Relatively little is known about the epidemiology and taxonomy of *Cryptosporidium* in fish. In the current study three hundreds and ten fishes were collected from Mediterranean sea as a group one representing wild marine fish (n=130) and River Nile as a group two representing wild fresh water fish (n=180). Intestinal smears were stained by modified Ziehl–Neelsen stain then microscopically examined using objective lenses 10x, 40x and 100x. the infection rate was 25.48%, *Cryptosporidium* recorded higher prevalence in wild fresh water fish (30.5%) than wild marine water fish (18.46%), with significant difference (P=0.0159). In wild marine fish *Cryptosporidium* infection was higher in Sea bass (*Dicentrarchus labrax*) (22.8%) than Sea bream (*Sparus aurata*) (13.33%), which does not show any significance (P=0.1629). On the other side for fresh water fish the infection was higher in *Tilapia nilotica* (38.8%) than *Mugil cephalus* (22.2%), with significant difference (P=0.0152). The present study provides the first assessed data on the prevalence of *Cryptosporidium spp.* in wild marine and fresh water fish in Egypt, with future prospective to detect genetic characterization and zoonotic relationship of different *Cryptosporidium* genotypes in different types of fish.

Key words:

Cryptosporidium spp., wild marine fish, Wild fresh water fish

Introduction

Cryptosporidium (phylum Apicomplexa) is a protozoan parasite that affects the epithelium of the gastrointestinal tract in a variety of vertebrate hosts, including humans and domestic and wild animals. There are now 44 known *Cryptosporidium* species, which can infect fish, amphibians, reptiles, birds, and mammals, (Chalmers et al., 2018; Holubová et al., 2020; Ježková et al., 2021; Zahedi et al., 2021). *Cryptosporidium* is one of the pathogens most frequently found in water, which has

been recorded in a variety of water types around the world (rivers, recreational, consuming, and sewage). The fecal-oral mode of transmission for *cryptosporidium* makes excellent use of water as a medium for their spread. (Omarova et al., 2018; Vermeulen et al., 2019). Considering the recorded morbidity and mortality rates in some fish species, *Cryptosporidium* infections in cultured fish could have a considerable negative economic impact on the aquaculture sector. Usually, no obvious clinical signs observed on fish infected

with cryptosporidium, eventhough some clinical manifestations like, anorexia, listlessness, ascitia, emaciation and low growth rate can be described (Murphy et al., 2009; Gabor et al., 2011; Nematollahi et al., 2016).

Many studies on *Cryptosporidium* in aquatic hosts has expanded recently, reiterating the parasite's ubiquity. Infection has been found in numerous free-living, domesticated, and ornamental fish species from both marine and freshwater settings. *Cryptosporidium* was firstly described in fish by Hoover et al. (1981) who confirmed the presence of *Cryptosporidium* species (*C. nasoris*) in the intestine of the tropical marine fish naso tang (*Naso lituratus*). Then it was detected in many other types of fish which elucidated in cryptosporidial infection of 14.3 % carp fish (Pavlásek, 1983), in addition to 58.8 % of cichlid fish (Landsberg and Paperna, 1986). In black Nile catfish and North African catfish prevalence was 10% and 20% respectively, while in *Tilapia nilotica* prevalence was 30% (Hefnawy, 1989), in brown trout prevalence was 38.9% (Rush et al., 1990), in red drum 21.7% infection detected (Camus and López, 1996). After that Álvarez-Pellitero and Sitjá-Bobadilla (2002) detected a new species of *Cryptosporidium* which called *C. molnari* in stomach of farmed marine fish mainly in sea bass. Then another species *Cryptosporidium scopthalmi* was identified in the intestine of cultured turbot Álvarez-Pellitero et al. (2004).

The majority of research on piscine *Cryptosporidium* has focused on aquarium or farm fish, while information on *Cryptosporidium* in wild fish is rare. Consequently, this study aims to elucidate the prevalence of *Cryptosporidium* species in wild marine and fresh water fish in Alexandria and Behera Provinces, Egypt. Future research will broaden the range of hosts that are fish, and new *Cryptosporidium* species and genotypes will be suggested. To enable agreement on

the nomenclature used to define new piscine species/genotypes, the taxonomy and evolutionary connections within the genus must first be clarified.

Material and methods

Sample collection:

A total of 310 wild fish representing two groups were collected from Mediterranean Sea, Alexandria province for wild marine fish and Nile River, Rasheed branch, Behera province for wild fresh water fish. In Group One, 130 marine fish were collected {Sea bream (*Sparus aurata*) 60; Sea bass (*Dicentrarchus labrax*) 70}, Group Two with 180 fresh water fish {*Tilapia* (*Oreochromis niloticus*) 90; Mullet (*Mugil cephalus*) 90}. Fish were immediately transferred in Ice box to the laboratory of Parasitology Department Faculty of Veterinary Medicine, Alexandria University for dissection and examination.

Laboratory Examination:

Sharp scissors were used for opening fish abdomen. Fine smears from the stomach and intestine epithelial layers of fish were fixed with methanol and stained with a modified Ziehl-Neelsen stain (MZN) according to (OIE, 2008) In brief, Intestinal smears were air dried and fixed with little drops of absolute methanol for 5 minutes. The slides were then stained with carbol fuchsin for 15 min, and the extra stain was washed with tap water. 1% acid alcohol was used as decolorizing agent for 15 seconds. The slides were washed again with tap water then counterstained with 0.4% methylene blue for 1 min. finally slides were washed with tap water and air dried. The MZN stained smears were immersed by thin transparent oil immersion. This technique will increase the resolving power of microscope. The *Cryptosporidium* oocysts will be good seen while using 10x as a red dot like pinhead. It became clear at 40x (this objective lens do not touch the oil thin layer), and immersing both the objective lens 100x and the specimen in the oil

immersion Higher-magnification to detect and measure *Cryptosporidium* spp., a 100X objective lens with a stage micrometer and an eyepiece micrometer can be used under a light microscope. (Xiao et al. 2001).

Statistical analysis:

Statistical analyses were performed by Chi square analysis using SAS,2004 software, the data are significant at $p < 0.05$ to compare diversity of *Cryptosporidium* from marine and fresh water fish, moreover the diversity between different species of marine fish and different species of fresh water fish. A significance level of

Results

The oocysts of *Cryptosporidium* spp. were spherical to ovoid shapes with smooth walls, an incomplete suture line along the oocyst wall, and an acid-fast (red-pink) appearance on a blue background. The oocysts' diameter ranged from 3.3-4.5 x 3.8-6.04 μ m, with a mean (3.9 x 5) μ m, Fig (1). Regarding to *Cryptosporidium*

prevalence it was detected in 79 out of 310 fish sample representing 25.48 %. Prevalence was higher in fresh water (30.5 %) than marine fish (18.46 %), with statistically significant difference ($P=0.0159$) (Table ,1), Chart 1. In Group one demonstrating marine fish prevalence was 18.46 % (24 out of 130), with higher prevalence in Sea bass 22.8 % (16 out of 70) than Sea bream which show 13.33 % prevalence (8 out of 60), with No significant difference ($P=0.1629$) (Table,2), Chart,2. Pertaining to Group Two, *Cryptosporidium* prevalence was elucidated in fresh water fish as 30.5 % (55 out of 180) with higher prevalence in Tilapia fish 38.8 % representing 35 out of 90 fish, than Mugil revealing 22.2 % (20 out of 90). Statistical analysis confirm a significant difference ($P=0.0152$) (Table,3), Chart, 3 .

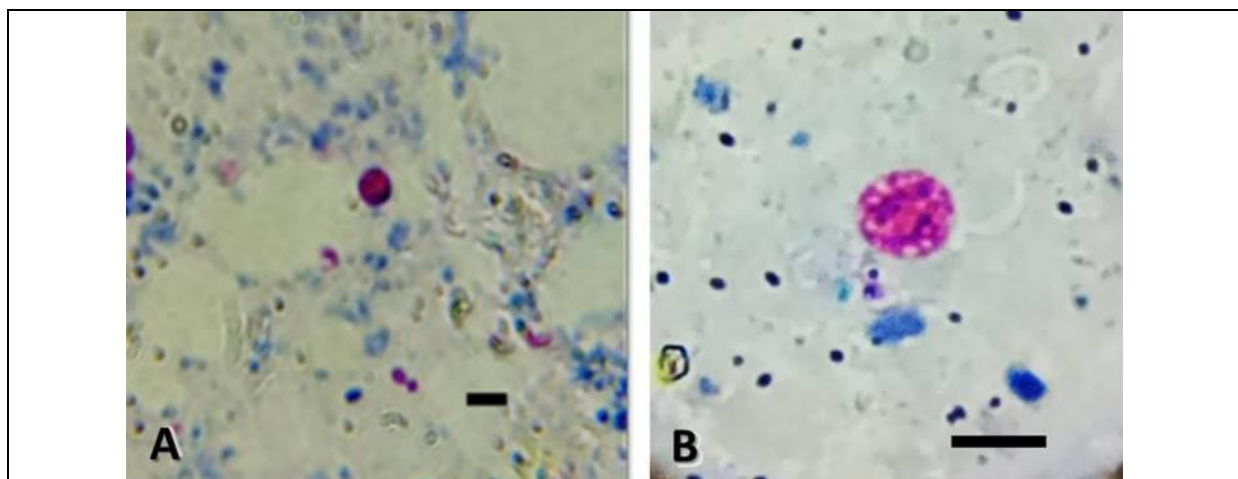


Fig. 1 *Cryptosporidium* spp. oocysts from wild marine and fresh water fish in the intestinal smears, modified Ziehl-Neelsen staining. **A)** Microscope magnification 40x, clear image when using thin oil immersion, scale bar 5 μ m; **B)** Microscope magnification of 100x. scale bar 5 μ m.

Table (1): Prevalence of *Cryptosporidium* in total number of examined marine and fresh water fish.

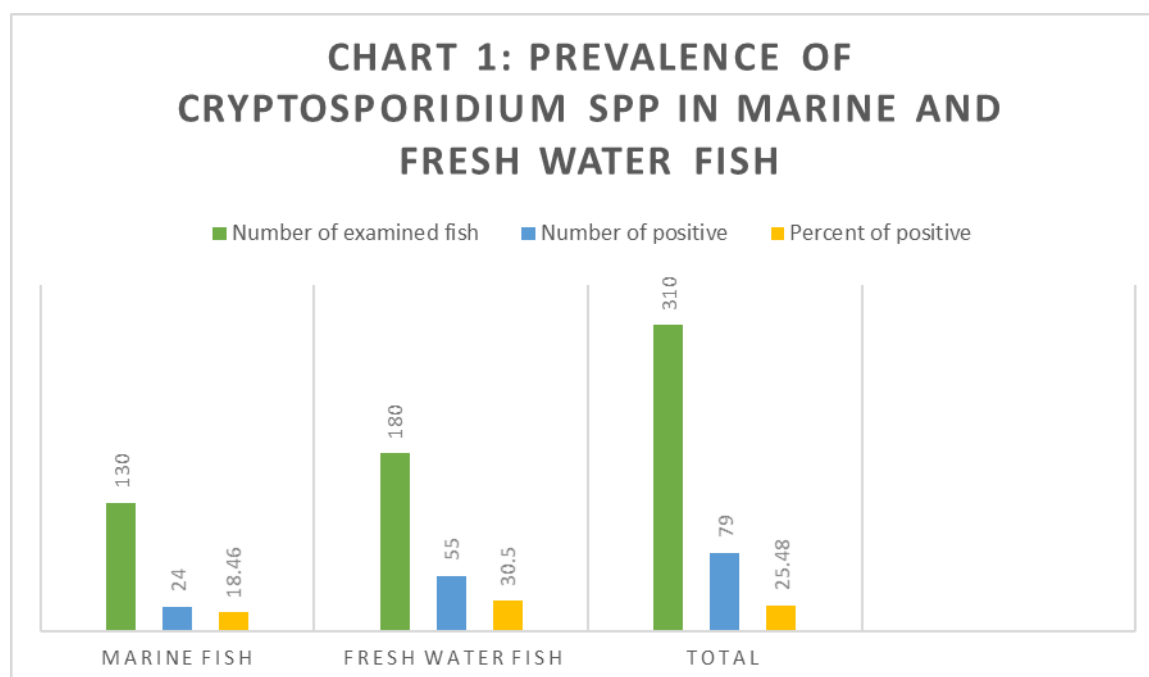
Fish type	Number	Positive (n)	Positive (%)	Chi-square and P-value
Marine fish	130	24	18.46	5.8141* P=0.0159
Fresh water fish	180	55	30.5	
Total	310	79	25.48	

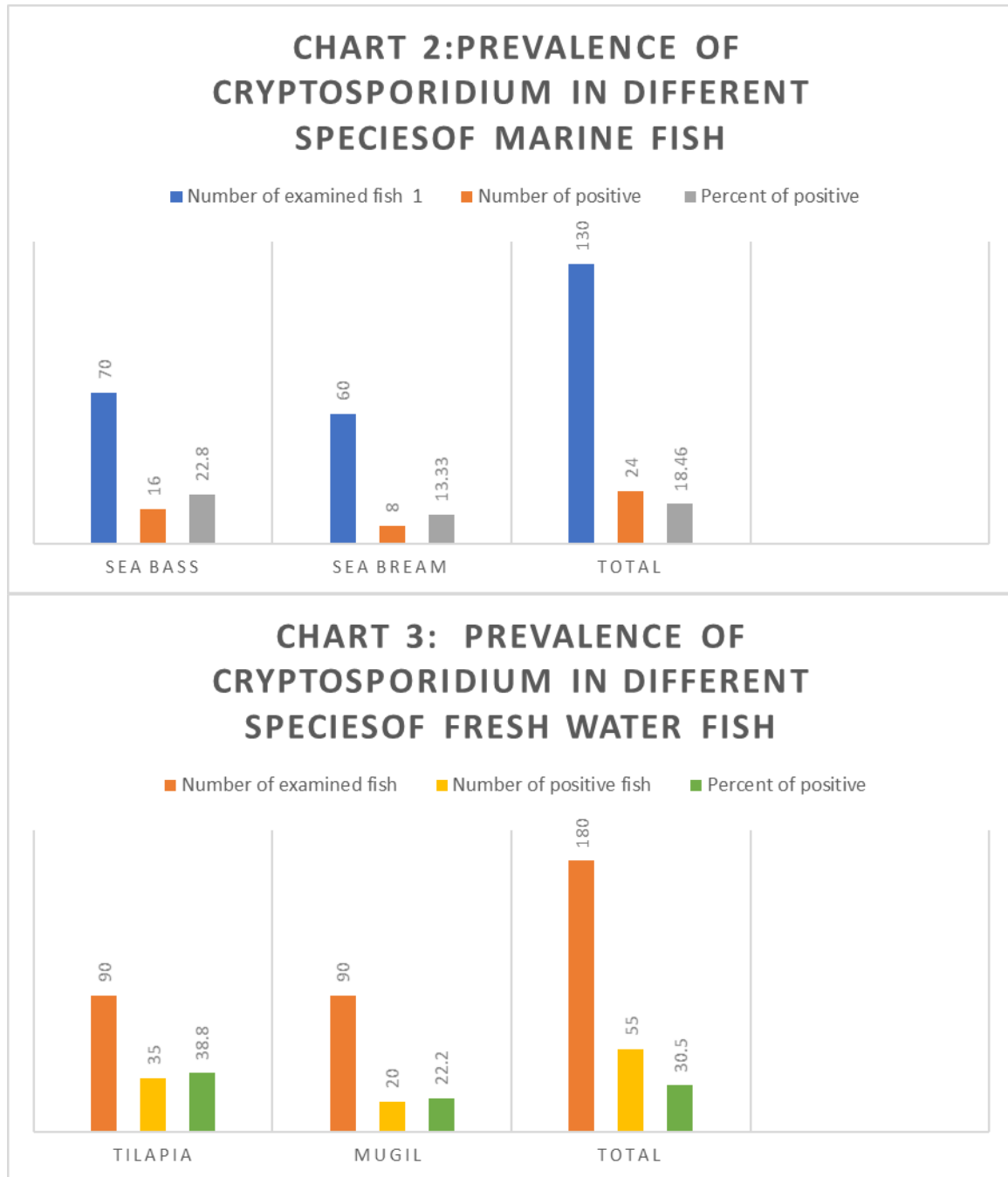
Table (2): Prevalence of *Cryptosporidium* in different species of marine water fish

Fish species	Number	Positive (n)	Positive (%)	Chi-square and P-value
Sea bass	70	16	22.8	1.9467 NS P=0.1629
Sea bream	60	8	13.33	
Total	130	24	18.46	

Table (3): Prevalence of *Cryptosporidium* in different species of Fresh water fish

Fish species	Number	Positive (n)	Positive (%)	Chi-square and P-value
Tilapia	90	35	38.8	5.8909* P=0.0152
Mugil	90	20	22.2	
Total	180	55	30.5	





Discussion

In the present study, the overall prevalence of *Cryptosporidium* in fish was 25.48 %. It was obvious that higher infection recorded in fresh water (30.5 %) fish than marine fish (18.46%). Moreover, in fresh water fish higher infection rate was recorded in Tilapia fish (38.8%) than Mugil fish

(22.2%). In marine fish a higher prevalence in seabass (22.8%) then sea bream (13.33%). Fishes can have prevalences of *Cryptosporidium spp.* that range from 0.8% to 100%, particularly in juvenile fish. (Certad et al., 2015; Yang et al., 2015) Previously, other studies

described a high prevalence of *Cryptosporidium spp.* in marine fish, but mainly in juveniles. In cultured marine fish, the prevalence of *C. molnari* in *Dicentrarchus labrax* and *Sparus aurata* was 50 and 95% in hatcheries and 58 and 65% in the on growing systems, respectively (Sitjà-Bobadilla et al., 2005), Prevalence rates of up to 100% for *C. scophthalmi* were also reported in juvenile turbot (*Psetta maxima*) (Alvarez-Pellitero et al., 2004). This higher prevalence can be explained due to higher population densities in the surroundings of aquaculture facilities, attracted by the abundance of food (Uglen et al, 2014). Furthermore, high prevalence of *cryptosporidium* was recorded in *Clarias gariepinus* fishes from drainage canal and River Nile in Giza Province (69.3%) by ELISA and (64%) by modified zhiel nelson test (Saad-Alla et al,2022). The location's hygienic conditions may be to blame for the percentage variation. On the other side our results differ from Moratal et,al 2022 who detect *cryptosporidium* prevalence 4.2 % in wild and cultivated marine seabass and seabream fish in Eastren Spain this due to different geographic area. Ammar and Arafa (2013) declared that 23 fish (19.2%) tested positive for *Cryptosporidium spp* when MZN stained intestinal contents smears were examined. Infection rates ranged from 15.0% in wild fish to 23.3% in farmed fish. In Assiut, 30% of the tilapia samples that Hefnawy (1989) examined contained *Cryptosporidium*. In the fish from Mosul that were investigated, Al-Tae (2008) detected the infection in 28.97% of the fish. In Zagazig Abd-Allah (2009) declared that infection rate of *cryptosporidium* was 13.75 % in crayfish. It was also found by Mahmood (2012) in 16.9% of Iraqi carp fish. The species and

quantity of fish, the moment the samples were taken and examined, the frequency with which the water was contaminated, the level of stress that the fish were exposed to, as well as the diagnostic techniques employed, could all contribute to variations in the rate of infections. (Ammar and Arafa ,2013)

In order to better understand the zoonotic potential of this parasite between piscine hosts, it will become increasingly crucial in the future to identify the implicated *Cryptosporidium* species using molecular techniques and to look into any potential evolutionary relatedness that could be inferred from phylogenetic analysis.

.Conclusion:

This work confirmed the presence of *cryptosporidium* infection in wild marine and fresh water fish in Egypt with higher incidence in wild fresh water fish than marine fish, Moreover infection was higher in wild sea bass than wild sea bream fish, in the same way infection was higher in wild Tilapia than wild Mugil fish. . It is worthy to mention that thin smear of oil immersion on the MZN stained intestinal scrapes give good clearances of *Cryptosporidium* oocysts it will be easily and rapid detected firstly at 10x and confirmed with 40x and also at 100x.

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الملخص العربي

دراسة مقارنة عن طفيل الكريبتوسبوردييوم في الأسماك البرية في المياه المالحة و المياه العذبة

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يعتبر طفيل الكريبتوسبوردييوم من الطفيليات واسعة الانتشار في العديد من العوائل بما فيها الانسان وتعتبر مسبب رئيسى للاسهالات في هذه العوائل. كما تصيب أيضا اسماك المياه العذبة والمالحة. ونظرا لقله الدراسات على نسبة انتشار هذا الطفيل في الأسماك وخصوصا البرية تم عمل هذه الدراسة حيث تم تجميع 310 سمكة في صورة مجموعتين, المجموعة الأولى تم تجميعها من البحر الأبيض المتوسط في محافظة الإسكندرية وتمثل اسماك برية للمياه المالحة وبها 130 سمكة, والمجموعة الثانية تم تجميعها من نهر النيل فرع رشيد في محافظة البحيرة وتمثل اسماك برية للمياه العذبة وعددها 180 سمكة. تم فتح الأسماك وعمل مسحة من الأمعاء وصبغها بصبغة موديفيدزيل نيلسون, وبعد الفحص تبين ان نسبة الاصابه بطفيل الكريبتوسبوردييوم كانت 25.48% وكما تبين ان نسبة الإصابة كانت اعلى في اسماك المياه العذبة البريه 30.5% من اسماك المياه المالحة البرية 18.46%. بالنسبة لاسماك المياه المالحة في المجموعة الأولى كانت نسبة الإصابة اعلى في سمك القاروص 22.8% من سمك الدنيس 13.33%, بينما في اسماك المياه العذبة البريه كانت نسبة الإصابة اعلى في سمك البلطى (38.8%) من سمك البورى (22.2%). وتمثل هذه الدراسة اول دراسة تقييمية لنسبة انتشار طفيل الكريبتوسبوردييوم في الأسماك البرية للمياه المالحة والعذبة في مصر, مع التطلع لتحديد الوصف الجينى للأنواع المختلفة من هذا الطفيل وقدرته على الانتقال للإنسان من أنواع الأسماك المختلفة