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Prevalence of Protozoal Parasites in Fish in Mosul City, Iraq

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ABSTRACT

The current study aimed to determine the prevalence of *Cryptosporidium* sp, *Eimeria* sp., *Entamoeba* spp. and *Giardia* sp. A total of 200 pieces of five different species of fresh fish were sampled. The study was conducted from August 2023 -February 2024. Microscopic examination was done by stomach and intestine direct smears stained Lugol iodine and floatation technique. Results showed that fish were infected with *Cryptosporidium* sp. 26.5%, *Eimeria* spp. 26.5%, *Entamoeba* spp. 1 % and *Giardia* sp. 3.5% . with no significant differences between them. As for months of the study, there were no significant infection rates where the rates ranged between 34.28% and 14.28% at $p \leq 0.05$. Microscopically, *Cryptosporidium* oocysts were spheroidal to ovoidal , 4.6 - 5.5 X 3.8 - 4.7 μ m while *Eimeria* oocysts were spherical to ovoid, 12-19 μ m. The cysts of *Entamoeba* were spherical, 10 to 18 μ m. while *Giardia* cysts were oval with four nuclei, 8-12 μ m in size. Intestinal protozoa recorded in fish species in the current study may be due to age factor and water contamination.



Introduction

Fish is considered important as food for human consumption because they contain essential nutrients including taurine, omega 3 fatty acids, vitamin D and selenium that are essential for human health. Fish consumption had increased by 3.1% from the annual growth rates compared to other proteins of animal source, and in 2017 consumption of fish increased 17% of total animal proteins [1, 2, 3]. Parasitic diseases represent 80% of fish diseases, most common are internal and external parasitic protozoa [4]. Internal parasitic protozoa are found in the internal organs of fish, including the gastrointestinal tract which is characterized by a direct life cycle, causing economic losses, especially when the fish are in a crowded environment, which leads to weight loss, retarded growth especially for smaller fish, effect on fish behavior as well as secondary infections by inhibition of fish immunity and mortality [5, 6]. The parasites, *Cryptosporidium* spp. and *Giardia* sp. recently have been discovered to contaminate aquatic environments as a new possible risk of fish borne diseases [7]. Parasitic infections of various roundworms, tapeworms, trypanosomes and arthropods were recorded in carp fish in most parts of Iraq, including Mosul, Thi-Qar, Diwaniyah and Erbil, but there are still few studies on the internal parasitic infections that affect fish [8, 9, 10, 11] and therefore this study was conducted to investigate prevalence of internal parasitic protozoa infecting fish.

Materials and methods

Sample collection

A total of 200 pieces of five different species of fresh fish (54 *Cyprinus carpio*, 56 *Arabibarbus grypus*, 30 *Chondrostoma regium*, 35 *Mesopotamichthys sharpeyi* and 25 *Luciobarbus xanthopterus*) from Mosul city markets were collected by 2-3 visits, at a rate of 6-9 fish per week, between August 2023 and February 2024 samples were collected in a cool box and then transported to the parasitology Lab. Of the College of Veterinary Medicine at University of Mosul for parasitic protozoa detection (Figure 1).

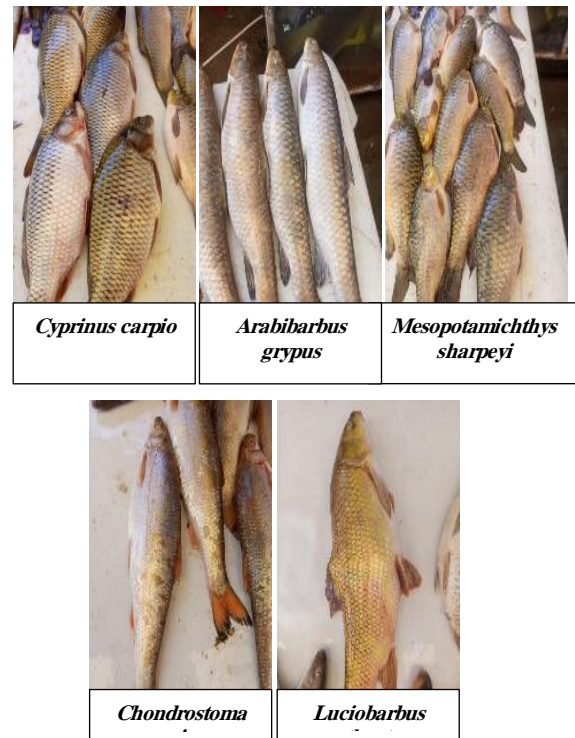


Figure 1. Types of fish examined.

The fish were dissected longitudinally in the ventral midline, extends from the beginning of the fish head to the end of the outlet opening, and then another incision was made from the end of the first incision to the lid of the gastrointestinal tract. The gastrointestinal tract was separated from its contact area from the oral cavity and the outlet opening, the intestine was opened and put into Petri dish (Figure 2), then floated with saturated sugar solution, the stomach and intestine were scrapped and dyed with Giemsa and lugal iodine and then examined using an optical microscope with magnification 40X then identifications of the parasites base on morphology were done according to [12,13].



Figure 2. Dissecting fish and isolation of stomach and intestine.

Results

About 200 of fish samples belonging to different species were examine. Study results showed that the fishes were infected with *Cryptosporidium* sp. 26.5%, *Eimeria* spp. 26.5%, *Entamoeba* spp. 1 % and *Giardia* sp. 3.5% with no significant differences between them as in (Table 1).

Table 1. Infection rates with internal fish protozoa

Internal Protozoa	No. of fish exam.	No. of infested animals	No. of fish inf.
<i>Cryptosporidium</i> sp.	200	53	%26.5 a
<i>Eimeria</i> spp.	200	43	%21.5 a
<i>Entamoeba</i> spp.	200	2	%1 b
<i>Giardia</i> sp.	200	7	%3.5 b

Different letters between groups indicate the existence of significant differences

As for months of the study, there were no significant infection rates where the rates ranged between 34.28% and 14.28% at $p \leq 0.05$. (Table 2).

Table 2. Prevalence of internal parasites according to months

Month	No. of fish Exam.	No. of fish Inf.	Percentage of Inf.
August	37	10	%27.02 a
September	31	9	%29.03 a
October	35	12	%34.28 a
November	27	8	%29.62 a
December	33	8	%24.24 a
January	23	4	%17.39 a
February	14	2	%14.28 a
Total	200	53	%26.5

Different letters between groups indicate the existence of significant differences

Microscopic examination results revealed that the morphological characteristics by direct smears of stomach and floatation method with lugal iodine revealed that oocysts of *Cryptosporidium* were spheroidal to ovoidal, 4.6 - 5.5 X 3.8 - 4.7 μ m. as in (Figure 3). The *Eimeria* oocysts were spherical to ovoid, 12-19 μ m in size as in (Figure 4). The *Entamoeba* cysts were spherical, 10 to 18 μ m as in (Figure 5). While *Giardia* cysts were oval with nuclei with protective wall , 8-12 μ m in size (Figures 6).

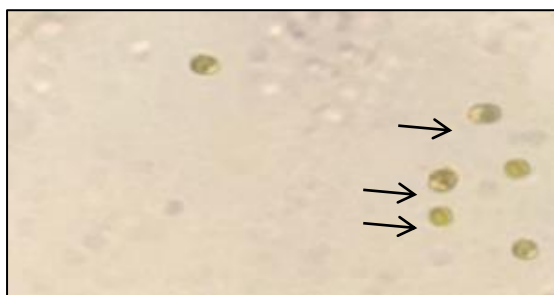


Figure 3. Oocysts of *Cryptosporidium* sp. in the intestinal content of infected fish using floatation method with lugal iodine, 40X.



Figure 4. Unsporulated oocyst of *Eimeria* sp. using floatation method, 40X.

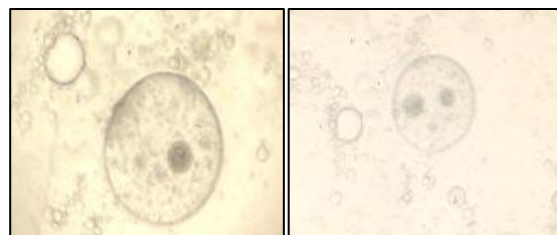


Figure 5. Cyst of *Entamoeba* spp. using floatation method, 40X.

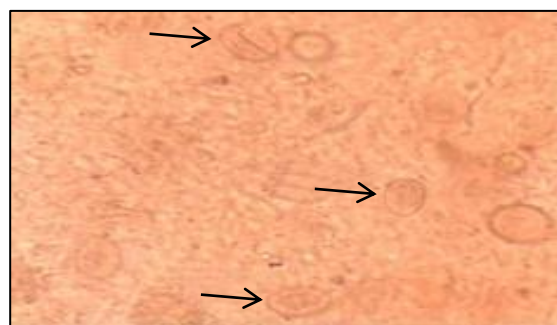


Figure 6. Cyst of *Giardia* sp. using floatation method, 40X.

Discussion

Prevalence of *Cryptosporidium* sp., *Eimeria* spp., *Entamoeba* spp. and *Giardia* sp. was observed in this study revealing higher infection rates during hot months while the lowest rates were observed in cold months. This agreed with the results of [14]; and [15] who recorded higher infection rate 87.5% in July but the lowest 44.4% in January. This may be attributed to different factors such as influence of physiology of the host, immunity and feeding also *Cryptosporidium* is characterized by the shedding of high numbers of oocysts that remain for long periods in water, in addition their ability to cause infection immediately after shedding in feces. Resistance of *Cryptosporidium* oocysts to most of the sterilizers used in water sterilization is due to the thickness of oocysts wall, and their viability in water for 66 days [12, 14]. Intestinal protozoa causes serious diseases especially in crowded populations due to direct lifecycle and rapid reproduction mainly by binary fission. Some species of protozoa are pathogenic to fish, while other protozoa are zoonotic [7]. Fish may act as a carrier for zoonotic protozoan such as

Cryptosporidium and *Giardia* to human or get infected with them [7, 14]. Our results recorded infection with four intestinal protozoa, *Cryptosporidium* spp., *Eimeria* spp., *Entamoeba* spp. and *Giardia* sp. with infection rates 26.5 %, 21.5%, 1% and 3.5% respectively. High infection rate 26.5% with the zoonotic parasite *Cryptosporidium* sp. This result is similar to [8] who recorded 28.97% in *Liza abu* fish in Mosul, but disagree with another study in Thi-Qar 12.02% infection rate [9]; and in Al-Diwaniya 6.18% infection rate [10]. The study also recorded infection rate with the zoonotic parasite *Giardia* sp. 3.5% which mean that contamination of water environment through water drainage and bad sanitation also contribute to the infection rate. The researcher [16] mentioned that *Giardia duodenalis* is the most prevalent waterborne parasite and species of zoonotic importance from fish which has been identified in wild tilapia, mullets and the aquatic environment. The most common host for *Giardia* parasite is wild fishes and consumption of such from contaminated waters always poses a risk. Other zoonosis intestinal protozoan parasite is *Entamoeba* sp. which recorded 1% in our study although is low, but is considered important for being and indicator for insufficient sanitation and hygiene. The species *Entamoeba* is considered as an important fish borne parasitic zoonosis because of recent outbreaks in Southeast Asian nations such as Vietnam and Thailand [17, 18]. Consumption of undercooked fish from contaminated sewage-fed or waste-water cultures and traditional cooking methods may be the main factors behind these outbreaks. High infection rate with *Eimeria* 21.5% was recorded in this study which agreed with another study in Mosul [8] who recorded 23.29% and also is similar to [19] who recorded 20.6% using fish fecal disposals and intestinal scrapings in wet samples method. However, its presence in fish species may result from the age of the fish, species of the fish and water contamination.

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Competing Interests

Researchers declare that they have no conflicts of interest regarding the publication of this research.

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