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INVESTIGATION OF EXTERNAL PROTOZOA IN FISH IN MOSUL CITY

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ABSTRACT

Two hundred samples of five fish species were taken from local marketplaces in Mosul from Aug. 2023 – Feb. 2024. Results revealed that fish were infected with four types of external protozoans, *Ichthyophthirius* sp., *Trichodina* spp., *Piscinoodinium* sp., as well as *Chilodonella* spp. The highest infestation rate was recorded in *Trichodina* was 19.5%, then *Chilodonella*, *Ichthyophthirius* were 7.5%, 6.5%, respectively, while the lowest infestation rate was recorded in *Piscinoodinium* was 3%. No significant differences were recorded during the months of the study. The location of the external protozoa varied in the skin, gills and fins. The ectoparasite *Trichodina* was isolated from different places in fish skin, gills, in addition to fins. *Ichthyophthirus* was found on fins and in gills, whereas *Chilodonella* and *Piscinoodinium* were found on the skin and in gills of fish. This study also detected for the first time the presence of the ectoparasite *Piscinoodinium pillulare,* which was in a round shape, golden to brown and of different sizes.

Keywords: *Trichodina, Ichthyophthirus*, *Chilodonella, Piscinoodinium*, fish, Mosul.

INTRODUCTION

Fish belong to the phylum Chordata and are the most diverse group of known aquatic organisms (Tedesco *et al.,* 2017). Fish are a vital source of human food and therefore play a significant role in the economy of various countries (Andronova and Yakimovich, 2019). Parasitic infections represent 80% of fish diseases (Eissa and Gharib, 2005).

Protozoan parasites are either external or internal. Ectoparasites are commonly seen in farmed fish, and feed on the upper layer of the skin (Faruk, 2018). However, some species have a host-independent cyst shape. These parasites usually appear in large numbers on the surface of the fish, inside the gills, or in both. The general effect of these parasites is irritation of the epithelial surface, which leads to an increase in the production of mucous (Faruk, 2018; Hoffman, 2019).

Parasitic infections affect fish, leading to growth retardation, particularly in immature fish, and increase the likelihood of secondary infection by suppressing the immunity of fish (Elamie, 2001). Ciliates as well as flagella parasites are among the most important external parasitic protozoa that cause fatal diseases that lead to weight

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loss, wasting and mortality, especially in intensive fish farms (Lerssutthichawal *et al.,* 2016; Gomes *et al.,* 2017; Wang *et al.,* 2019). External protozoa are present in large numbers on the surface of skin. The life cycle of the parasite is direct, which leads to the accumulation of very large numbers in a short time, especially when the fish are in a crowded environment (Lerssutthicha-wal *et al.,* 2016).

Various parasitic infestations of roundworms, tapeworms, trematodes and arthropods have been recorded in carp fish in most parts of Iraq; in Mosul, Salah al-Din, Babylon, Diwaniyah, Erbil and Najaf (Ryan *et al.,* 2014; Thompson and Ash, 2016; FAO, 2020; Chalmers *et al.,* 2020). However, there are still a few studies on the infection of internal and external parasitic protozoa that affect fish. So, this study was proposed in order to provide information on the infection of external parasitic protozoa and find out their prevalence for the correct treatment and control to eliminate such infections.

MATERIALS AND METHODS

Five types of fish samples were collected between 100-859 g. Two hundred samples were distributed as follows: 65 *Arabibarbus grypus*, 35 *Mesopotamichthys sharpeyi*, 54 *Cyprinus carpio*, 30 *Chondrostoma regium* and 25 *Luciobarbus xanthopterus.* They were brought from local markets in the city of Mosul with 2-3 visits, at a rate of 6-9 fish per week, for the period from August 2023 to February 2024, and transported in an iced box to the parasitology laboratory at the College of Veterinary Medicine at the University of Mosul. Wet mounts were made from different areas of fish skin, both fins and gills were separated and placed in petri dishes to make direct smears, as shown in Figure (1). Some of the slides were dyed with Giemsa stain and Lugol iodine and the smears were examined using a 40X light microscope to detect external protozoa (Shuaib and Osman, 2015). After that, important measurements were taken into account for each type of parasitic protozoa using an optical micrometer.

Figure 1: Method for wet mount smears of skin, fins and gills.

Statistical analysis

Data in this research were statistically calculated and were confirmed using the Chi-square test at the significant value $P<0.05$ (Petrie and Watson, 2006).

RESULTS

This study detected four types of ectoparasites in fish, including *Ichthyophthirius* sp., *Trichodina* sp., *Chilodonella* spp. and *Piscinoodinium* spp. The highest infestation rate was recorded in *Trichodina* was 19.5%, followed by the parasites *Chilodonella* and *Ichthyoph-thirius* of 7.5% and 6.5%, respectively and the lowest was in *Piscinoodinium,* with an infection rate of 3% (Table 1).

Table 1: The infection rates with external parasitic protozoa in fish.

Protozoa	Number of fish examined	Number of fish infected	Infection rate %
Trichodina sp.	200	39	19.5 a
Ichthyophthirius sp.	200	13	6.5 b
Piscinoodinium sp.	200	6	3 b
Chillodenella spp.	200	15	7.5 b

Different letters between the percentages of infection indicate significant differences at P≤0.05.

No significant differences were recorded in the infection rates with ectoparasites during the months of the study at P≤0.05, and rates of infection ranged from 32.43% to 14.28%. The highest infection rate recorded during August was 32.43% and the lowest was in February, 14.28% (Table 2).

Table 2: Number of ectoparasitic protozoa in fish according to study Months

Months	No. of examined fish	No. of infected fish	Infection rate %
August	37	11	32.43 a
September	31	7	29.03 a
October	35	8	25.7 a
November	27	6	23.07 a
December	33	7	21.21 a
January	23	4	17.39 a
February	14	2	14.28 a
Total infestation rate %	200	45	22.5

Similar letters between the percentages of infection indicate no significant differences at P≤0.05.

Also, the study revealed that through wet mounts of skin, fins and gills, the site of parasites varied, in *Trichodina* was in the skin, fins and gills, and in *Ichthyophthiruis* was on fins and in gills, while both *Chilodonella* and *Piscinoodinium* were located on the skin and gills of fish Table (3).

Table 3: Shows the site of external parasitic protozoa in the body of fish.

Protozoa	Infection sites		
	skin	gills	fins
***Trichodina* sp.**	+	+	+
***Ichthyophthyrus* sp.**	+	_	+
***Piscinoodinium* sp.**	+	+	_
***Chillodenella* spp.**	+	+	_

Morphologically, *Trichodina* appeared pear or round in shape between 37-60 µm, golden to brown in color, with large and small nuclei with cilia surrounding the outer surface of the parasite with an adhesive fixing disc, the outer ring is called the serrated ring (Figure 2). While *Ichthyophthirius* tomont, the fourth stage was pear-shaped 0.03 - 1 mm, containing large numbers of theronts (Figure 3). The parasite *Chillodenella* was oval-leaf shaped 50 - 70×30 - 40 µm, flat dorsally and ventrally with large nucleus as in (Figure 4).

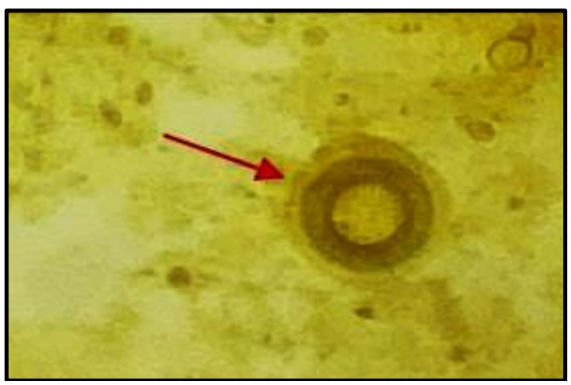

Figure 2: *Trichodina* parasite in wet mount smear of skin with lugol-iodine 40X.

Figure 3: *Ichthyophthyrus* spp. in wet mount smear in gills 40X.

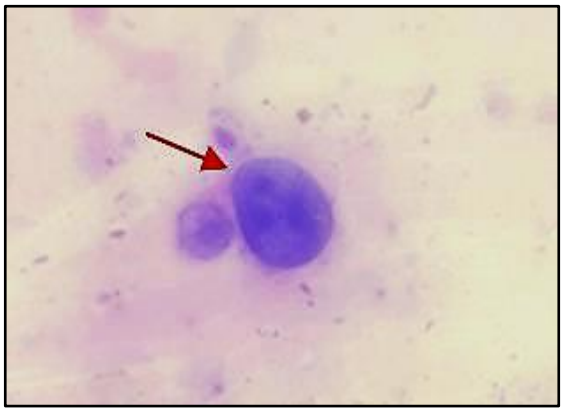

Figure 4: *Chillodenella* spp parasite in wet mount smear in gills 100X.

While the parasite *Piscinoodinium* sp. appeared in the skin pear to round, golden to brown, with different sizes and using anatomical microscopy, a lot of parasites were detected in colonies (Figure 5).

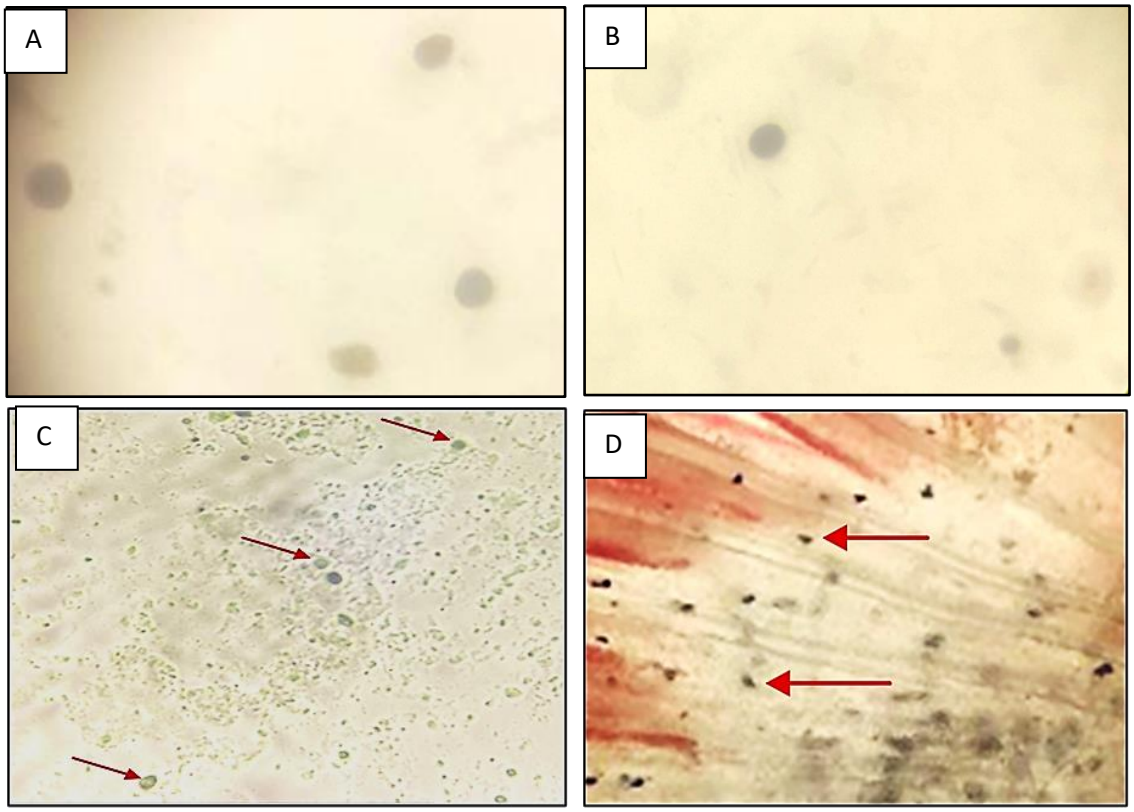

Figure 5. *Piscinoodinium* sp. parasite *(*a, b) parasite in the gills wet mounts 40X (c) Skin parasite 10X wet mount (d) Hundreds of parasites attached to the glaucoma filaments using an anatomical microscope 25X.

DISCUSSION

External parasites are considered significant parasites that parasitize in or on fish with direct lifecycle and do not require intermediate hosts to accomplish its full lifecycle. These protozoa are easily transmitted between fish and direct contact is one of the most important ways of transmission of infection, especially in crowded farms. It is considered the most

pathogenic fish parasite where mortality occurs in heavy infections, as well as external protozoa that parasitizes on the gills and skin is responsible for weight loss, change in fish behavior, abnormalities in the gills and their effect on reproduction (Aksoy *et al.,* 2006; Nematollahi *et al.,* 2013).

The current study investigated the prevalence of infection of external protozoa in 200 samples of five species of fish mostly consumed in the city of Mosul, and the results of microscopic examination detected four types of ectoparasites on fish: *Ichthyophthirius* sp., *Trichodina* sp., *Chillodenella* spp. and *Piscinoodinium* sp. The highest rate was recorded in *Trichodina* sp., 19.5% with a significant difference, *Ichthyophthirius* sp. 6.5%, *Piscinoodinium* sp. 3%, and *Chillodenella* spp. 7.5%. This variation may be due to many causes, including the age and host type, food, health, immune status, the life of the host in different types of fish, as well as the life of the parasite in terms of its life cycle and reproduction in addition of the impact of environmental factors (seasons, geographical location, depth of water) that can also explain the variation of different species in the types and numbers of parasites to the different numbers of fish examined during the study period. The current study results agree with the researchers Hussein *et. al.* (2011) in the province of Babylon, where they recorded infection with the parasite *Ichthyophthirius multifiliis* and *Trichodina cottidarum*.Also, Salah al-Din, Muhammad *et. al.* (2013) recorded an infection rate of the parasite *Trichodina mutabilis* 25%, but disagree with Zangana (2008), who recorded an infection rate 31%. The reason for the difference in infection rates by *Trichodina* sp. may be attributed to the fish species (Mudeed *et al.,* 2020), where the recorded infection rates were 13.8% and 8.7% in catfish and *Mesopotamichthys sharpeyi* fishes, respectively.

The incidence of *Icthyophthirius multifiliis* was 6.5% in fins and gills, and this is much less than what Obaid, *et al.* (2021) recorded in Erbil governorate within carp fish 50% in each of the fins, gills and skin, as well as with the study of Zangana (2008), where it recorded 28.6% in the city of Mosul. In other studies, this parasite was recorded in nineteen different fish species from many fish farms in central and southern Iraq (Mhaisen, 2012) and from the small Zab River and Lake Darbandikhan in the Kurdistan Region (Abdullah, 2002; Abdullah, 2005), respectively. Obaid *et al*. (2021) recorded an infection rate with *Icthyophthirius* spp. of 5.6% in the skin and gills is identical to the infection rate in the current study, and they also recorded an infection rate with the parasite *Trichodina* sp. 17.2%, which is close to the infection rate in the current study, 19.5%.

The current study recorded an infection rate of 7.5% with the *Chilodonella* parasite in the skin and gills, which is similar to what Obaid, *et al.* (2021) reported in Erbil governorate, at a prevalence rate of 9.5% for the parasite in the skin only. This parasite was first recorded in Iraq by Ali *et al.* (1987) in freshwater fish, *Mystus pelusius,* from the Tigris River in Baghdad and carp in Zafaraniyah, Baghdad (Sadek, 1999). Mudeed *et al.* (2020) recorded this parasite on the skin and gills of carp and *Barbus xanthopterus, 7*.9% and 7.5%, respectively, which is identical to the results of this study. Skin is considered the first defensive barrier for aquatic animals to encounter infections of external pathogens and different environmental conditions. Our study, in agreement with most previous studies, indicated that skin is the main preferred site for external protozoa, then the gills and the fins (Gonzalez *et al.,* 2007).

In this study, the parasite *Piscinoodinium pillulare* was detected for the first time in Iraq, specifically in the city of Mosul, where it appeared in pear and circular shapes and brownish to black in colour, indicating

different developmental stages of the parasite, and this is consistent with Martins and his group (2001).

The results revealed no significant differences in infection with external protozoa according to the months. The highest infection rates were recorded during summer months, and the lowest were recorded during cold months. These results are consistent with Certad *et al.* (2015) and Saad-Alla *et al.* (2022), who recorded the highest rates of infection was 87.5% in June and the lowest at 44.4% in January. This may be due to many factors, including host physiology, immunity and nutrition (Certad *et al.,* 2015; Taylor *et al.,* 2015).

CONCLUSION

Routine wet mount smear examination of the protozoa using optical microscopy, is the first step in diagnosis. The study concluded that fish are infested with four ectoparasites, *Trichodina* sp., *Ichthyophthirius* sp., *Chillodenella* spp. and *Piscinoodinium* sp.. Higher infection rate was recorded with *Trichodina* was 19.5%, then by *Chillodenella*, *Ichthyophthirius* 7.5%, 6.5% respectively, while a lower infestation rate was recorded with the *Piscinoodinium* 3%. *Piscinoodinium* sp. is registered for the first time in Mosul, Iraq. No significant differences in infections were found according to the months of the study.

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التحري عن الأوالي الخارجية في الأسماك في مدينة الموصل

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تم جمع ٢٠٠ عينة من الاسماك الطازجة من خمسة انواع هي سمك الكارب وسمك الشبوط الاعتيادي وسمك البني وسمك الزولي وسمك الكطان من عدة اسواق في مدينة الموصل من شهر آب ٢٠٢٣ الى شباط ٢٠٢٤. اوضحت نتائج الفحص المجهري بالمسحات الرطبة المباشرة ان الاسماك خمجة بالاوالي الخارجية التالية: Trichodina sp. بنسبة ١٩,٥ % وهي الاعلى وتلاها *Chilodonella* ٧,٥٪ و*Ichthyophthirius* ٦,٥٪ واقلها بالطفيلي *Piscinoodinium* ٣ %. في حين لم تسجل فروق معنوية في نسب الخمج بالاوالي الخارجية خلال أشهر الدراسة اذ تراوحت نسب الاصابة ما بين ٣٢,٤٣٪ الى ١٤,٢٨٪ وبلغت اعلى نسبة خمج في شهر آب ٣٢,٤٣٪ واقلها في شهر شباط ١٤,٢٨٪.

تواجدت الاوالي الخارجية في مواقع مختلفة، حيث عزل طفيلي الـ *Trichodina* من الجلد والزعانف والغلاصم في حين ظهر طفيلي *Ichthyophthirius* في الزعانف والغلاصم، في حين كان تواجد الطفيليان *Chilodonella* و*Piscinoodinium* في الغلاصم والجلد.

مجهريا، ظهر طفيلي *Trichodina* بأشكال مستديرة الى كمثريه ما بين ٣٧-٦٠ ميكروميتر، حاوي على نواة كبيرة ونواة صغيرة، ذهبي إلى بني، وحاوي على قرص، حافته الخارجية بارزة جداً اما طفيلي *Ichthyophthirius* ظهر بشكل كمثري ٠,٠٣ -١ ملم. بينما *Chilodonella* ظهر على شكل ورقة بيضاوية ومسطحة ظهرياً بطنياً ٥٠-٧٠×٣٠-٤٠ ميكروميتر، يحتوي على نواة كبيرة. وسجلت الدراسة ولأول مرة الطفيلي *Piscinoodinium* sp. في المسحات الرطبة للغلاصم والجلد بأشكال كمثريه - مستديرة وبأحجام مختلفة، ذهبي إلى بني اللون، وكما لوحظ اتجمع العديد منها على شكل مستعمرات.