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# Study of parasitic infection in ornamental fish farms in Qom province, Iran

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## Abstract

Ornamental fish culturing has been altered into one of the most vital and profitable industries in numerous countries all over the world. The cultivation and propagation of ornamental fish have been increasing in the last few years in Iran. Qom is one of the provinces where this industry has expanded significantly. The purpose of this study was to investigate the parasitic infections in 14 ornamental fish farms, where diseases and death had been reported in different fish species. This research was carried out for seven months from November 2021 to May 2022 on 15 different species of referred ornamental fish. Ornamental fish species including *Gymnocorymbus ternetzi*, *Poecilia reticulata*, *Poecilia sphenops*, *Carassius auratus*, *Hyphessobrycon eques*, *Pterophyllum scalare*, *Danio rerio*, *Betta splendens*, *Puntius tetrazona*, *Hemigrammus ocellifer*, *Ancistrus* sp., *Heros severus*, *Andinoacara rivulatus*, *Cyprinus rubrofuscus*, and *Melanotaenia praecox* were sampled and examined for internal and external parasitic infestations by light microscopy. In the results, external parasites including *Trichodina* sp., *Gyrodactylus* sp, *Lernaea* sp, *Ichthyobodo* sp., *Tetrahymena* sp., *Oodinium* sp., *Dactylogyrus* sp., and *Ichthyophthirius multifiliis* and internal parasites including *Capillaria* sp., and *Hexamita* sp. were detected. The highest prevalence of parasites was related to *Capillaria* sp. (17.69%) and the lowest prevalence was related to *Lernaea* sp. and *Ichthyobodo* sp. (0.77%). It was concluded that these findings can help to develop a program for the prevention and treatment of parasitic diseases in freshwater aquarium fish.



## Introduction

The aquarium fish business is an important part of the world trade (Winfree, 1989) and freshwater aquarium fish are the biggest part of this industry (Helfman, 2007). In Iran, the export of ornamental fish has been raised as an economic activity with significant foreign exchange earnings over the past few decades (Mousavi *et al.*, 2011; Mousavi *et al.*, 2020; Mousavi *et al.*, 2021). Although the freshwater ornamental fish industry in Iran has a high potential for development, one of the most important weaknesses observed in this industry is the lack of sufficient understanding of the problems related to the disease and health status of these fish (Mousavi *et al.*, 2011). This lucrative business has also led to the rapid spread of ornamental fish pathogens around the world. Therefore, to risk analysis and preventive measures, controls of common infectious diseases are very important (Kayis *et al.*, 2013). Environmental fluctuations, inappropriate management practices, handling and transportation, overcrowding, drug treatment, improper feeding or malnutrition, temperature fluctuations, and poor water quality affect fish in intensive culture and can cause significant stress to impose on the homeostatic mechanisms of fish and make them sensitive to a wide range of parasites (Subasinghe, 1997). Thus, to be successful in the ornamental fish business, it is very important to have adequate information about the parasites that infect them. Besides, having information about the epidemiology of parasites and their control strategies is essential to have therapeutic and preventive applications (Santos *et al.*, 2017; Hoshino *et*

*al.*, 2018; Ferreira *et al.*, 2019). In general, younger fish are more susceptible to parasites than mature ones. The number of parasites required to cause damage and effect is different in hosts with low body mass than in hosts with large body mass. Also, the immune responses of adult fish against subclinical contamination may be more developed; in addition, the function of the immune system plays a fundamental role in the relative resistance of fish against parasite species (Gratzek, 1988).

Although Qom province is one of the hot and dry regions of the country, which suffers from a lack of water resources, this province currently has nearly 100 ornamental fish breeding ponds (Khan, 2009). At present, due to the expansion of people's desire to keep ornamental fish in Iran and the world, attention to the health and diseases of aquarium fish has also expanded. Fish species face more parasitic diseases than viral, bacterial, and fungal agents. Parasites can be found everywhere and on any living organism. The severity of the prevalence of parasitic diseases depends on several environmental factors, including the conditions of the host, age, size, and density of the population. For this reason, although diseases caused by parasites have been reported in some fish species, mass death do not usually occur in nature (Adel *et al.*, 2015).

The purpose of this study was to investigate the parasitic contamination of ornamental fish farms, which had reported losses in different fish species.

## Materials and Methods

In this study, samples of sick and moribund fish from 14 different farms in Qom province, Iran were examined over seven months, from November 2021 to May 2022. These samples were referred to the laboratory in the fish's aquarium water and aerated plastic bags.

Appearance symptoms included various lesions of the skin and gills such as erosion, ulceration, hemorrhage, excess mucus, darkening, discoloration, white spots, and a tendency to whiteness (Rahmati-Holasoo *et al.*, 2022). Also, some behavioral changes such as weakness, depression, anorexia, and emaciation were observed in the macroscopic examination of infected fish (Rahmati-Holasoo *et al.*, 2022).

The samples, in the first minute after death or after euthanizing, were evaluated in terms of infection with external parasites, by wet mounts of scrapings of the skin and gills, and internal parasites, by dissection and tissue sampling. First, live fish were examined for skin lesions and parasites visible to the naked eye (Thilakaratne *et al.*, 2003). The mucus was scraped from the skin and gills, and fresh wet mounts were taken. After examining each sample, in terms of ectoparasites, each fish was dissected under sterile conditions and wet mounts were prepared from their internal organs. Fifteen ornamental fish species include *Gymnocorymbus ternetzi, Poecilia reticulata*, *Poecilia sphenops, Carassius auratus*, *Hyphessobrycon eques*, *Pterophyllum scalare*, *Danio rerio*, *Betta splendens*, *Puntius tetrazona*, *Hemigrammus ocellifer*, *Ancistrus* sp., *Heros severus*, *Andinoacara rivulatus, Cyprinus rubrofuscus,* and *Melanotaenia praecox* were sampled and their wet mounts were examined by light microscopy at ×10 and ×40 magnifications.

## Results

In total, 96 out of 130 examined fishes (equal to 73.85%) were infected with different parasites and the rest had no parasite infestation. External parasites including *Trichodina* sp. (3.85%), *Gyrodactylus* sp. (13.08%) (Fig. 1), *Lernaea* sp. (0.77%) (Fig. 2), *Ichthyobodo* sp. (0.77%), *Tetrahymena* sp. (13.85%) (Fig. 3), *Oodinium* sp. *(*2.31%), *Dactylogyrus* sp. (5.38%) (Fig. 4), *I. multifiliis* (2.31%) (Fig. 5), and internal parasites including *Capillaria* sp. (17.69%) (Fig. 6), and *Hexamita* sp. (13.85%) were detected. The lowest percentage of contamination was related to *Lernaea* sp. and *Ichthyobodo* sp. (0.77%), and the highest percentage was related to *Capillaria* sp. (17.69%). The most referred patient samples were *P. reticulata*, which showed involvement with four different parasites in different aquariums and farms. *G. ternetzi* has shown the highest diversity of parasitic infections (5 parasite species out of 10 species found in this study) among the studied fish species (Table 1). *Capillaria* sp. was found in seven different species and *Tetrahymena* sp. was found in six different species, so they were the most common parasites among the studied species respectively. *Lernaea* sp. and *Ichthyobodo* sp. showed contamination in only one species and had the lowest prevalence among the species (Table 1). In addition, simultaneous infection with two or more parasites was not observed in any of the samples. Since in this study, only sick fish were sampled, and parasites can be both the main cause and the secondary cause of disease in fish, in the samples of all 14 investigated farms, at least one parasitic species was found. The status of involvement with different parasites in the investigated ornamental fish species, and the number of involved cases, were given in Table 1 and the percentage of each infection was given in Table 2.

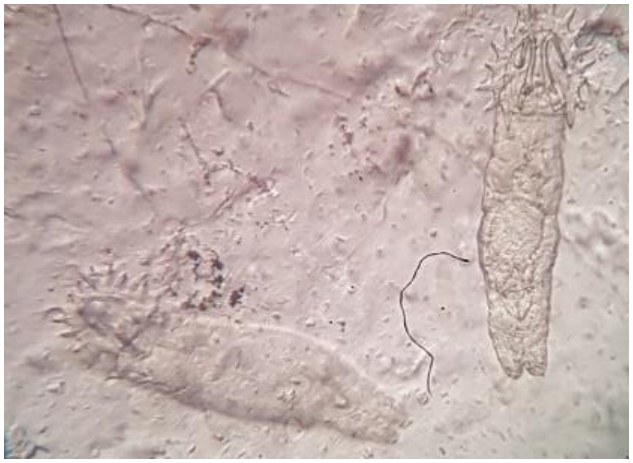

**Fig. 1.** *Gyrodactylus* sp.

**Fig. 2.** *Lernaea* sp.

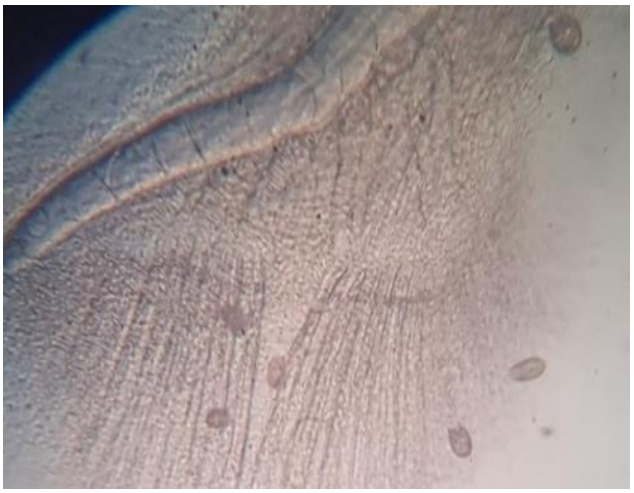

**Fig. 3.** *Tetrahymena* sp.

**Fig. 2.** *Dactylogyrus* sp.

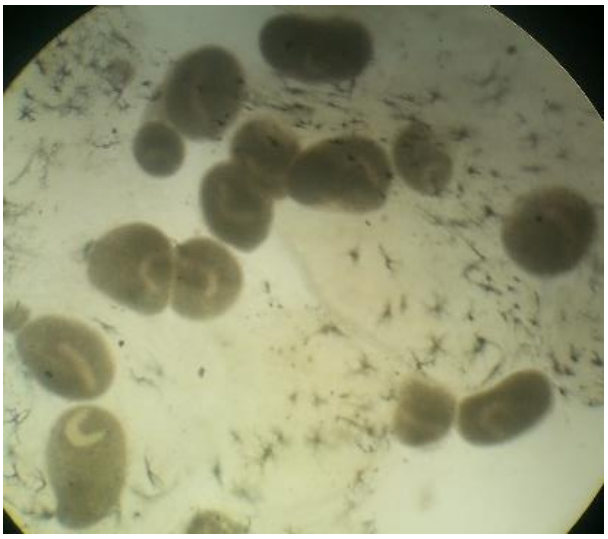

**Fig. 1.** *Ichthyophthirius multifiliis*

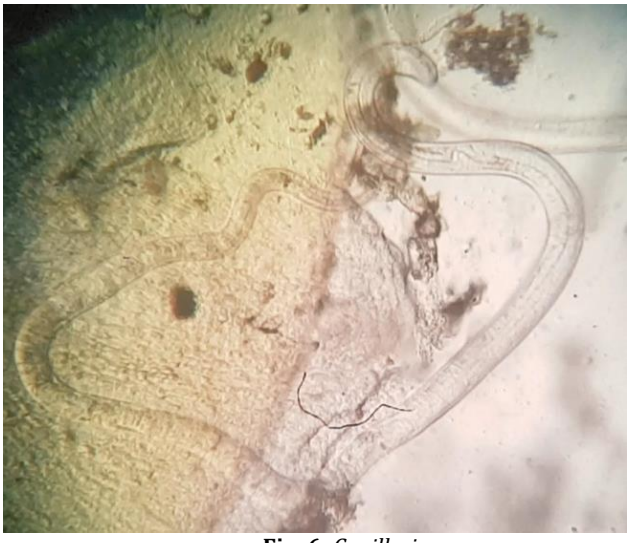

**Fig. 6.** *Capillaria* sp.

**Table 1.** Parasitic infection status of fish.

| Fish species | *Dactylogyrus* | *Ichthyobodo* | *Tetrahymena* | *Gyrodactylus* | *Lernaea* | *Trichodina* | *Oodinium* | *Ichthyophthirius* | *Hexamita* | *Capillaria* | Negative | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Gymnocorymbus ternetzi* | 4 | 1 | 2 | 5 | - | - | - | - | - | 4 | 5 | 21 |
| *Poecilia reticulata* | | | 3 | 7 | | | | | 3 | 5 | 4 | 22 |
| *Poecilia sphenops* | - | - | - | 3 | 1 | - | - | - | - | - | 2 | 6 |
| *Carassius auratus* | | | | 2 | | 2 | | | | | 4 | 8 |
| *Hyphessobrycon eques* | - | - | 5 | - | - | - | - | - | - | - | 3 | 8 |
| *Pterophyllum scalare* | - | - | 1 | - | - | - | - | - | 2 | 2 | 3 | 8 |
| *Danio rerio* | - | - | - | - | - | - | 1 | - | - | 4 | 4 | 9 |
| *Betta splendens* | - | - | - | - | - | - | 2 | - | - | - | 2 | 4 |
| *Puntius tetrazona* | - | - | 5 | - | - | - | - | - | 5 | 3 | 2 | 15 |
| *Hemigrammus ocellifer* | - | - | - | - | - | - | - | - | 2 | - | 1 | 3 |
| *Ancistrus* sp | - | - | - | - | - | - | - | - | - | 3 | 0 | 3 |
| *Heros severus* | - | - | - | - | - | - | - | 2 | - | 2 | 0 | 4 |
| *Andinoacara rivulatus* | - | - | - | - | - | - | - | 1 | - | - | 2 | 3 |
| *Melanotaenia praecox* | - | - | 2 | - | - | - | - | - | - | - | 0 | 2 |
| *Cyprinus rubrofuscus* | 3 | - | - | - | - | 3 | - | - | 6 | - | 2 | 14 |
| Total | 7 | 1 | 18 | 17 | 1 | 5 | 3 | 3 | 18 | 23 | 34 | 130 |

**Table 2.** Parasitic infection percentages.

| Parasite species | Total infected Fish | Percentage |
|---|---|---|
| *Dactylogyrus* sp. | 7 | 5.38% |
| *Ichthyobodo* sp. | 1 | 0.77% |
| *Tetrahymena* sp. | 18 | 13.85% |
| *Gyrodactylus* sp. | 17 | 13.08% |
| *Lernaea* sp. | 1 | 0.77% |
| *Trichodina* sp. | 5 | 3.85% |
| *Oodinium* sp. | 3 | 2.31% |
| *Ichthyophthirius multifiliis* | 3 | 2.31% |
| *Hexamita* sp. | 18 | 13.85% |
| *Capillaria* sp. | 23 | 17.69% |
| Total | 96 | 73.85% |

## Discussion

Over the past decades, the identification of fish parasites has become increasingly noticeable due to the growth of the freshwater ornamental fish industry worldwide (Jalali, 1997). Parasitic diseases cause economic losses to the ornamental fish industry by affecting physiological and biological characteristics and causing mechanical damage (Jalali, 1997; Adel *et al.*, 2015).

In this study, we found a total of 10 parasite species among 15 ornamental fish species.

A significant part of the world trade of aquatic animals is the trade of tropical aquarium fish (Evans and Lester, 2001). Therefore, Quarantine measures should be implemented during the trade of live animals, including aquatic animals, to prevent the transmission of pathogens, which lead to disease outbreaks and economic losses (Helfman, 2007). The risk of parasites entering other countries and their further spread in the future is minimized by treating infected fish before export or after entering the importing country (Koyuncu, 2009).

In most farms, long-term salt (sodium chloride) immersion is routinely used as a suitable preventive measure. However, given that many aquatic parasites tolerate low salt concentrations (0.5 ppt), the use of higher salt concentrations (1 ppt or more) seems necessary for the effective prevention of parasitic infections (Rahmati-Holasoo *et al.*, 2022). In addition, some owners of ornamental fish farms in Qom province, when faced with diseases and losses in their fish, try to solve the problem by using various drugs and disinfectants experimentally and after not getting results from these methods, they refer to the aquatic veterinarian. At this time, as a result of ineffective treatments, the fishes suffer from weakness, excessive stress, and even conflict with secondary infections. Moreover, excessive or unwanted use of some anti-parasitic compounds has caused some parasites to become relatively resistant to them (Rahmati-Holasoo *et al.*, 2022). Therefore, informing the owners of ornamental fish farms about irreparable damages and economic losses of arbitrary treatment is important to reduce parasitic contamination. In addition to direct mortality losses, parasites may have significant effects on fish growth and behavior (Scholz, 1999), which can reduce farm efficiency and production, causing increased costs, reduced profits, and impact on earning foreign currency (Thilakaratne *et al.*, 2003). In the future, with the expansion of natural control, the need for chemical methods to prevent the spread of

parasites will disappear (Barker and Cone, 2000; Garcia *et al*., 2009). The key to successful natural control is a proper understanding of the environmental impact on parasite transmission (Garcia *et al*., 2009).

*Trichodina* sp., *I. multifiliis, Ichthyobodo* sp., *Tetrahymena* sp*., Oodinium* sp*.,* and *Hexamita* sp. are the protozoan parasites that were found in this survey. *Trichodina* sp. can survive for a while, from a few hours to a few days, without fish, and there are also several host species other than fish that can support them temporarily (Lom, 1995).

All freshwater fish are susceptible to ichthyophthiriasis and *Ichthyophthirius* can be considered one of their most common parasites (Woo, 1995). The reason for the high and widespread prevalence of protozoan parasites can be seen as their high compatibility with non-fish hosts, which causes their high survivability in fish-free waters (Lom, 1995).

*Ichthyobodo* sp. is ectoparasitic flagellate that may cause ichthyobodosis in fish, which is a common problem in the aquaculture industry worldwide; and is often associated with a wide range of other pathogens that can make disease diagnosis difficult (Isaksen *et al*., 2012).

*Tetrahymena* sp. can infect poecilid species, especially when water quality is poor and the fish is immunosuppressed (Gratzek, 1993).

*Oodinium* sp. is a genus of parasitic dinoflagellates and its hosts are saltwater and freshwater fish. This parasite causes gold dust disease, which is a velvety disease in fish (Mills and McLean, 1991).

*Hexamita* sp. is a parasite of freshwater fish's intestine and gall bladder, which often infects Salmonidae and is also present in common carp and ornamental fish. In weakened fish, hexamitosis is a common secondary infection (Evans and Loster, 2001).

Monogenic trematodes have high reproduction and under poor management conditions, their effective transmission increases (Soulsby, 1982). They have hooks by which they attach to the opisthohaptor (Wildgoose and Association, 2001) and cause eye problems including increased eye movements, blinking, as well as physical damage such as ulcerations, hemorrhages, and erosions in fish (Roberts, 2001).

The direct and very short life cycle of *Gyrodactylus* sp. and *Dactylogyrus* sp. without the need for an intermediate host, allows them to multiply rapidly to dangerous levels under management conditions prevailing on farms (Citino, 1974). In addition, although *Gyrodactylus* sp. has a specific host, if their environmental conditions are not optimal, they can also infect other fish (Cable *et al*., 1999).

In this study, the prevalence of crustaceans was low, which could be because the life cycle of these parasites depends on the water temperature and the duration of their preservation (Rahmati-Holasoo *et al*., 2022). Most common species of commercial ornamental fish are not kept in farms for more than 2-3 months and are usually sold early, so they have less opportunity to be contaminated with crustaceans (Rahmati-Holasoo *et al*., 2022).

Adhesion of crustaceans to the surface of the body and fins through haptor for feeding causes lesions, wounds, hemorrhages, desquamation, and a way for secondary pathogens to enter (Xu *et al*., 2007). *Lernaea* sp., especially in cases of heavy infestations, can cause severe mortality from haemorrhages and secondary bacterial infections (Gratzek, 1993).

Some nematodes such as *Capillaria* sp. have a direct life cycle and do not need an intermediate host that can be problematic (Wildgoose and Association, 2001), as in this study *Capillaria* sp. accounted for the highest rate of parasitic involvement.

The results of this study are almost similar to the studies of other researchers in terms of parasite identification such as *Dactylogyrus* sp., *Gyrodactylus* sp., *I. multifiliis*, *Trichodina* sp., *Capillaria* sp., and *Lernaea* sp. in the studies of the parasite fauna of different freshwater aquarium fish species by Meshgi *et al.* (2006) and Adel *et al.* (2015). In addition, *D. rotator, Chiloldonella* sp., *Hexamita* sp*., Ictyobodo necator, I. multifiliis, Microsporidium, Myxosporida* sp., *Tricodina* sp., and *L. cyprinicea* from aquarium fishes around Tehran was studied by Meshgi *et al.* (2006). The parasites including *Ichthyobodo* sp*., I. multifiliis, Trichodina* sp*., Gyrodactylus* sp*., Lernaea* sp*., Dactylogyrus* sp*., Chilodonella* sp., *Argulus* sp*.,* and *Capillaria* sp. was reported in ornamental fish in Turkey by Koyuncu (2009).

Considering the level of parasitic conflict in the ornamental fish farms of Qom province, the need to observe the principles of biological security is strongly felt. For example, dividing the farms into isolated units and separating them from each other, adopting specific hygiene protocols for the transfer of fish and equipment between different units, and extending the quarantine period, which provides more opportunities to identify parasites in the isolation unit and prevent their distribution to other farm units (Rahmati-Holasoo *et al*., 2022), and the use of a specific pathogen-free (SPF) water source, if possible, is recommended (Noga, 2010). On the other hand, more use of immune-stimulating compounds such as probiotics and prebiotics in feed formulation to strengthen the immune system of fish (Gatlin, 2003; Barber, 2007), and the use of antiparasitic compounds with appropriate methods and concentrations can play an important role in preventing the occurrence of parasitic

infections.

The results of this study may help to develop a program for the prevention and treatment of parasitic diseases in freshwater aquarium fish (Pereira *et al*., 2019).

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## Conflict of Interest

The authors do not have any potential conflict of interest to declare.

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