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PATHOLOGICAL STUDIES ON EXTERNAL AND INTERNAL PARASITIC AFFECTIONS OF GOLDFISH (*CARASSIUS AURATUS*)

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ABSTRACT

Ornamental fish aquarium globally is considered a key sector of aquaculture and represents a significant portion of the pet industry. Between June 2022 and February 2023, a total of 229 goldfish were examined to investigate the histopathological lesions associated with the parasitic fauna (*Carassius auratus*) collected from Elsaieda Aisha market in Cairo governorate, Egypt and some pet shops in Beni-suef and Elfayoum governorates. The overall prevalence of parasitic infections was 47.2 %. The isolated parasites include protozoan parasites (*Trichodina spp., Ichthyophthirius multifiliis, Chilodonella sp.* and *Icthyoboda necator*), monogeneans (*Dactylogyrus sp.* and *Gyrodactylus sp.),* Encysted meta cercaria of *Centrocestus formosanus*, crustaceans (*Lernaea sp.* and *Argulus sp.*) and one nematode *Capillaria sp.* with incidence rates 27.9%, 15.3%, 2.6%, 12.7% and 0.4% respectively. Gross examinations of the affected fish were varied and appeared as hemorrhage on the body' with excessive mucus production, ulceration, white-colored worm-like copepods protruding on different parts of the body and pale anemic or congested gills. Histopathological changes included vacuolation and necrosis of epidermal cells and hyperplasia and fusion of secondary gill lamellae. In addition to leucocytic cell infiltrations mainly lymphocytes and macrophages, hyperplasia of gill cartilage and parasitic cysts surrounded by several layers of fibroblast cells were noticed. In conclusion, the prevalence rate of parasite infection in goldfish is relatively high as well as the pathological findings induced by these parasites and might negatively affect the proper function of the skin and gills of the host.

***Key words**:* Parasites, Prevalence, Pathology, *Carassius auratus*

INTRODUCTION

Farming of ornamental fish is considered one of the valuable industries in the meantime and one of the important alternative businesses as the demand for this type of fish as pets and decoration is increased. These fishes are often attributed as living jewels and hobbies due to their shape, color, and behavior. They also could be reared in limited spaces (Aly *et al*., 2008). Its business trade is considered a high-income industry. In 2006, the global volume of

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ornamental fish exports generated US $ 283 million in income (FAO, 2007). The majority of the market is made up of the United States, Europe and Japan (Noga, 2010). South East Asian countries provide more than half of the world's ornamental fish (Olivier, 2003). The pet fish trade benefits a variety of countries. However, it may have negative consequences due to the spread of various infections caused by the import of non-native species. The great majority of ornamental fishes sold as pets are farm-raised and of freshwater origin (Andrews, 2006).

Carassius auratus is a freshwater goldfish, in the *Cyprinidae* family and its variations are the most common decorative fish kept as pets (Ahilan *et al*., 2009). There are eleven distinct varieties of goldfish including fantail (Ahilan *et al*., 2009). Over the past few decades, substantial goldfish culture has taken place. Over 2.9 million tons are produced annually (Zhou *et al*., 2018).

Ornamental fish that are farmed under intensive conditions of high-density fish, poor quality of water supply and bad management can be threatened by serious infectious diseases including bacterial diseases (Pate *et al*., 2005), viral diseases (Hedrick and McDowell, 1995) and parasitic diseases (Smith and Roberts, 2010).

In fact, one of the most considerable troubles affecting fish is the parasitic infection (Jerônimo *et al*., 2012). These parasitic infestations is the common diseases in nearly 80% of fish diseases in Egypt (Eissa, 2006). They can extremely hinder the fish industry as they can result in mortality, retarded growth rates, reduced food conversion rates, decreased marketability and also lead to rejection of the fish by the consumers (Buchmann, 2013, Shinn *et al*., 2015, Hamouda *et al*., 2018). A parasite that can enter the aquarium multiplies quickly and causes significant mortality rates in ornamental fish if the stock density of fish in aquariums is large and the water quality is unsuitable (Al.pbaz, 1984). Ectoparasite-related issues have a significant role in aquarium fish infections (Schperclaus, 1992 and Cengizler, 2000). Fish ectoparasites have been identified as trematodes, annelids, crustaceans, mollusks, flagellates, ciliates and amoebae from protozoa parasites. The general impacts of these parasites include irritation of the epithelial surfaces through increased mucus production, the destruction of mucus cells, gill injury, and respiratory discomfort as a result of the parasite load (Southgate, 1993). Therefore, gaining more understanding of the parasites that affect ornamental fish is essential to success in the ornamental fish sector (Hoshino *et al*., 2018).

Even though Egypt's freshwater pet fish business is crucial to the country's development, one of the most significant shortcomings seen is people's ignorance of health issues and disease-related issues. So this work aimed to spotlight on studying some parasitic diseases threatening goldfish (*Carassius auratus*) focusing on the morphological and histopathological features of the detected parasites.

MATERIALS AND METHODS

1. Fish collection

During the period extended from June (2022) to February (2023), a total of 229 goldfish (*Carassius auratus*) specimens were collected from El-Saieda Aisha market in Cairo and some pet shops in Beni-Suef and El-Fayoum Cities with a mean total length **of** 10.75±0.903 cm and a mean total weight 40±38.087 gm. Sampling duration was spread over three seasons i.e. Summer (from June to August), Autumn (September to December) and Winter (from January to February) and the number of fish collected during these seasons were 55, 124, and 50, respectively**.** The collected fish showed signs of diseases such as abnormal swimming behavior; gasping; hemorrhage; ulcers; attached parasites and abnormal coloration. The fish samples are brought alive in polyethylene bags containing two-thirds of air and one-third of aquarium water and kept in an aquarium containing 30 m^3 of aerated water. All sampling information including the site of sampling, species name, sample number, total length, weight and sampling date were recorded and tabulated.

2. Ethical statement

National and international ethical rules governing fish sampling have been completely embraced and authorized by the ethical community of the faculty of veterinary medicine, Beni-Suef University, Egypt (No.022399).

3. Clinical and postmortem examinations

The external examination of the fish body surface and internal organs was done and any abnormal macroscopic observations were recorded according to the method described by **(Noga, 2010)**. Special care was directed to detect any parasite and its associated lesions including erosion, ulcer, cyst formation, hemorrhage and other alterations. Any gross lesion was photographed.

4. Parasitological examinations

4.1. Examination of skin and fins:

Direct smears were taken from the fins and the two lateral sides of the skin. The skin and fin scrapings were combined with a few drops of distilled water on a slide and inspected under a microscope using both low and high magnification levels (**Lucky, 1977**).

4.2. Examination of gills:

The gill arch and filaments were scraped on a slide and mixed with a few drops of distilled water to create a uniform distribution underneath the entire cover slip, and the sample was then inspected under a microscope. Smears were taken then air dried, fixed by methanol then stained by Geimsa stain to investigate protozoa (Lucky, 1977).

4.3. Examination of internal organs

The internal organs such as the intestine, liver, heart, spleen, kidney, swim bladder and musculature were examined for internal parasites and macroscopic lesions. In addition, squash preparations were performed from these organs and examined microscopically.

4.4. Identification of parasites

- Protozoan parasites were stained using Giemsa stain according to the method of Klein (1958) and identified using the method of Basson and Van As (2006).
- Platyhelminths were fixed in formalin 5% and stained using Semichon's acetocarmine and identified as described in the method of Ogawa and Egusa (1979) and Mehrdana *et al*. (2014).
- Crustacea were cleared in lactophenol, mounted in glycerine-gelatin and identified according to Wadeh *et al*. (2008) and Mousavi *et al.* (2011).
- Nematodes were preserved in 10% buffered formalin and 70% ethanol and identified according to Bell and Beverley-Burton (1981) and Ko (2006).

5. Histopathological examination

Tissue samples of skin, gills, fins and most internal organs were taken and preserved in 10% neutral buffered formalin. The preserved tissues were sequentially dehydrated through ascending concentrations of ethyl alcohol, cleared in xylene, fixed in paraffin wax and then blocked. Tissue blocks were cut into serial sections (3-5µm thick) by a rotary microtome. Sections were taken from each tissue block and stained with Haematoxylin and eosin (H&E) and Masson trichrome. The stained sections were examined under a light microscope and the lesions were photographed using camera Olympus XC30 Bancroft (2013).

6. Statistical analysis

Data were analyzed using a Microsoft Excel worksheet for Windows 2013. Data were summarized by descriptive statistics for the overall prevalence in goldfish.

RESULT

1. **Clinical and post-mortem examination of *C.auratus* infected with parasites:**

The macroscopic examinations of fish infected with *Trichodina* spp. revealed lesions in the skin and gills such as red elevated areas or spots of hyperemia **(Fig. 1A)** and haemorrhage on the fish body with excessive mucus production, pale body coloration, and Scale loss were also noticed. Some of the examined fish showed ulceration together with a pale colouration of the gills. Also, macroscopic examinations of fish infected with *Ichthyophthirius multifiliis* showed only isolated foci of haemorrhage (petichiation), particularly near the scale root **(Fig. 1B)**. Occasionally, the hemorrhagic patches were enlarged and mucous-tinged. In addition, the gross appearance of infected fish by *Ichthyobodo necator* showed *a* round ulcer oozing fluid exudate **(Fig. 1C)**.

Moreover, the infested fish by monogenea *Dactylogyrus* sp. showed pale anemic gills or congested and tumid gills with considerable mucus discharge **(Fig. 1D)**. The infected fish by encysted metacercaria of *Centrocestus formosanus* revealed red-colored inflamed gills were detected in highly affected fish. The infested fish by crustacean, *lernaea* sp. showed white-colored worm-like copepods that appeared as a short piece of thread and protruded on different parts of the body where lernaea penetrated appeared inflamed and swollen with severe lesions such as hemorrhagic nodules or confined ulcers with extensive mucous production and haemorrhages causing erosions of the wounded areas **(Fig. 1E)**. The infested fish by crustacean *Argulus sp.* revealed the characteristic shape of the parasite attached to the surface of the skin, excessive mucous production, haemorrhages and erosions of the skin **(Fig. 1F)**.

2. Morphological identification of isolated parasites from the examined fishes:

Trichodina sp. is a round-shaped parasite with an adhesive disc that looks like a saucer. Denticles (24-29) are carried by the denticular ring. The macronucleus has a horseshoe form **(Fig. 2A)**. *Ichthyophthirius multifiliis* is an oval or round holotrichus ciliate with a huge horseshoe-shaped macronucleus and a small micronucleus **(Fig. 2B)**. *Ichthyobodo necator* is oval or pyriform in shape, has one flagellum, and a single nucleus on one side The parasite has corkscrew swimming pattern **(Fig. 2C)**. *Chilodonella sp.* is oval to bear-shaped and dorsoventrally flattened **(Fig. 2D)**. *Dactylogyrus sp.* have two pairs of eye spots on the front opisthaptor is located at the posterior end of the body and consists of two giant hooks surrounded by fourteen smaller hooks **(Fig. 2E)**. *Gyrodactylus sp.* are provided with a V-shaped head and a fusiform body shape. It possesses two anchor rods, two backers, and sixteen marginal hooks, but no eyespots **(Fig. 2F)**. Encysted metacercaria of *Centrocestus formosanus* is semispherical to oval in shape with an X-shaped excretory bladder **(Fig. 2G)**. *Lernaea sp.* have a long, cylindrical, rod-shaped and non-segmented worm-like body with two egg sacs in the posterior end **(Fig. 2H)**. *Argulus sp.* have a head, a thorax, and an abdomen. A flattened horseshoe-shaped carapace, maxillipeds, a peroral sting, and basal glands cover the head **(Fig.2I)**. *Capillaria* possess barrel-shaped eggs with polar plugs.

3. Prevalence of parasitic affections in *Carassius auratus*:

The current study revealed that one hundred and eight fish out of the investigated clinically abnormal 229 *Carassius auratus* were found infected with parasites with an infection rate (47.2%). The peak of infection with parasites was recorded in Autumn (54.8%) followed by Summer (54.5%) while the lowest rate was in Winter (20%) **Table (1).**

Regarding the prevalence of parasites among clinically abnormal *Carassius auratus*, it was noticed that the prevalence of protozoan parasites, monogeneans and crustacea was (27.9%), (15.3%) and (12.7%). The nematode parasites recorded the lowest prevalence rate (0.4%).

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parasites, monogeneans and crustacea was (27.9%), (15.3%) and (12.7%). The nematode parasites recorded the lowest prevalence rate (0.4%). The recorded prevalence of different parasite species was varied. *Trichodina species* showed the highest rate (15.3%) followed by *Dactylogyrus* species (12.2%). On the other hand, it was found that *Capillaria* showed the lowest rate (0.4%) **Table (2)**.

Regarding seasonal dynamics of each parasite, it was found that *Trichodina species* recorded the highest infection rate among examined fish (15.3%) with the peak of infection during summer season (27.3%) followed by *Dactylogyrus* sp. with the peak of infection during autumn (14.5%). While the lowest rate was recorded by *Capillaria* (0.4 %) (1/229) with the peak of infection during autumn season (0.8%) **(Table 3).**

Table 1: Total prevalence and seasonal incidence of parasitic infection among clinically abnormal *Carassius auratus.*

Season	CAEFN	IFN	Pre %
Summer	55	30	54.5%
Autumn	124	68	54.8%
Winter	50	10	20%
Total	229	108	47.2%

CAEFN: Clinically abnormal examined fish number, **IFN:** Infected fish number, **Pre %:** Prevalence of infection.

Table 2: The detected parasites and their prevalence in clinically abnormal *Carassius auratus.*

Parasite species	CAEFN	IFN	Pre %
Trichodina spp.	229	35	15.3%
Ichthyophthirus multifillis	229	25	10.9%
Chillodonella sp.	229	2	0.87%
Icthyoboda necator	229	2	0.87%
Protozoa		27.9%	
Dactylogyrus sp.	229	28	12.2%
Gyrodactylus sp.	229	7	3.1%
Monogenea		15.3%	
EMC of Centrocestus formosanus	229	6	2.6%
Lernaea sp.	229	27	11.8%
Argulus sp.	229	2	0.87%
Crustacea		12.7%	
Capillaria sp.	229	1	0.4%

CAEFN: Clinically abnormal examined fish number, **IFN:** Infected fish number, **Pre %:** Prevalence of infection.

Table 3: Seasonal prevalence of different parasites in Clinically abnormal *Carassius auratus.*

Parasite species	Summer			Autumn			Winter			TIN	Pre%
	CAEFN	IFN	Pre %	CAE FN	IFN	Pre %	CAEF N	IFN	Pre %		
Trichodina spp.	55	15	27.3%	124	19	15.4%	50	1	2%	35	15.3%
Ichthyophthirus multifillis	55	10	18.2%	124	12	9.7%	50	3	6%	25	10.9%
Chillodonella sp.	55	-	-	124	2	1.6%	50	-	-	2	0.87%
Icthyoboda necator	55	-	-	124	2	1.6%	50	-	-	2	0.87%
Dactylogyrus sp.	55	5	9.1%	124	18	14.5%	50	5	10%	28	12.2%
Gyrodactylus sp.	55	1	1.8%	124	6	4.8%	50	-	-	7	3.1%
EMC of Centrocestus formosanus	55	-	-	124	6	4.8%	50	-	-	6	2.6%
Lernaea sp.	55	-	-	124	26	21%	50	1	2%	27	11.8%
Argulus sp.	55	-	-	124	2	1.6%	50	-	-	2	0.87%
Capillaria sp.	55	-	-	124	1	0.8%	50	-	-	1	0.4%

CAEFN: Clinically abnormal examined fish number, **IFN:** Infected fish number, **Pre %:** Prevalence of infection, **TIN**: Total infected number

1. Histopathological findings

Histopathological changes of gills infested with protozoan *Trichodina* sp. revealed *Trichodina* spp. were seen as numerous characteristic ring-shaped (disc-shaped) structures with characteristic horseshoe-shaped macronucleus, the parasites were attached to the supporting gill structure and surrounded by a few numbers of inflammatory cells mainly lymphocytes and macrophages with degenerative changes, desquamation and necrosis in both primary and secondary gill lamellae and mucous secretion at the site of attachment of parasite were not uncommon **(Fig. 3A)**. Gills of *Carassius auratus* with trophont stages of *I.multifillis* showed necrotic changes of secondary gill lamellae and congestion of lamellar blood vessels **(Fig. 3B)**. The trophont stage without the characteristic horseshoe shape macronucleus was observed with clubbing and vacuolation of respiratory epithelium **(Fig. 3C).**

Histology of gills infested with *Dactylogyrus* showed sections of *Dactylogyrus* and necrosis of gill filaments at the site of parasite attachment **(Fig. 3D)** with rupture of the lamellar epithelium accompanied by focal hyperplasia and hypertrophy **(Fig. 4A)**.

The histological sections of gills infested with EMC of *Centrocestus formosanus* showed parasitic cysts at the tip of the gill filaments and inside the supporting cartilaginous tissue of the primary gill lamellae. The EMC in such cases were surrounded by several layers of chondrocytes or chondroblasts with hyperplastic gill filaments and inflammatory cells infiltration mainly lymphocytes and macrophages **(Fig. 4B)**. EMC was sometimes surrounded by fibroblast cells **(Fig. 4C and D)**. Extensive cartilaginous proliferation without parasitic cyst was observed, and associated with hemorrhage **(Fig. 4E)**. Some of the examined cases showed mixed infection in the gills of EMC of *Centrocestus formosanus* and the trophont stage of *I.multifiliis* inside the cartilage. In such cases, severe desquamation of lamellar tissue was common **(Fig. 4F).**

Histological section of skin infested with *lernaea* sp. revealed anchor worm penetrating skin in association with degeneration and desquamation of epidermal cells with mononuclear cell infiltration **(Fig. 5A and B)**. Hyperplasia of epidermal cells, hemorrhage and infiltration with lymphocytes and macrophages were also observed **(Fig. 5C and D)**. Histological section of skin infested with *I. multifillis* displaying a horse-shaped nucleus of the parasites, proliferation of mucous cells, hyperplasia and hydropic degeneration of epidermal cells and necrosis with ulceration surrounding the site of parasitic attachment **(Fig. 5E)**.

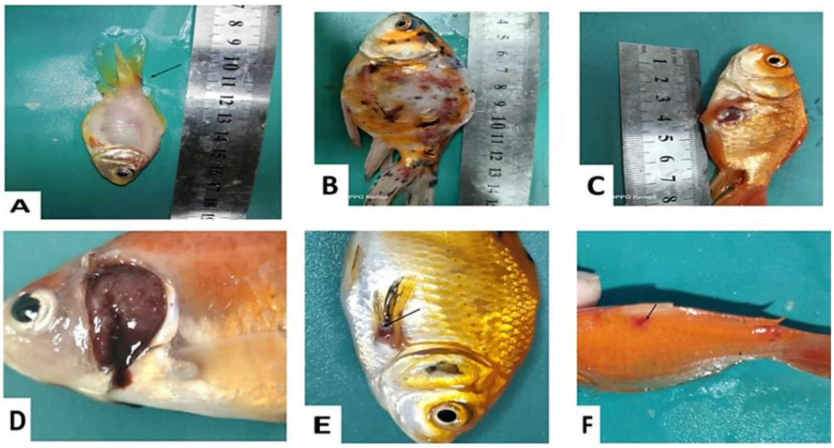

Figure 1: Showing ectoparasitic infestation in goldfish A: Red elevated areas (black arrow) in skin infested with *Trichodina* sp. B: Isolated foci of haemorrhage (petichiation) of skin infested with *Ichthyophthirius multifiliis* particularly near the scale root C: Round ulcer in skin infested with *Ichthyobodo necator* D: Gills infested with *Dactylogyrus* sp. *s*howing congestion with considerable mucus E: White colored worm-like copepods (black arrow) attached to pectoral fin F: Erosions of skin infested with *Argulus* sp. (arrow)

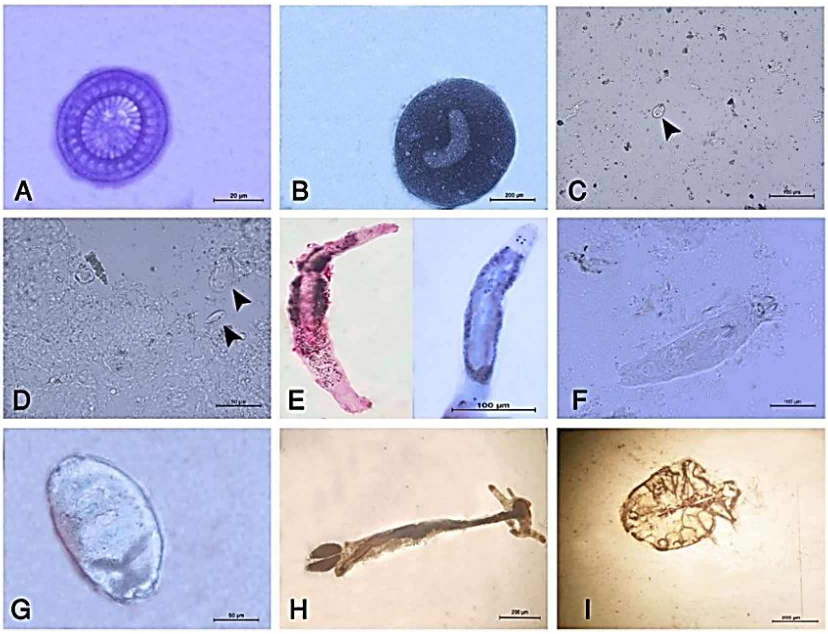

Figure 2: Wet Mount preparation: A: *Trichodina* sp. on the skin and gills stained with Giemsa (a round-shaped parasite provided with denticles) B: Trophont stage of *Ichthyophthirius multifiliis* on skin and gills with huge horseshoe-shaped macronucleus C: *Ichthyobodo necator*. On skin (arrowhead) oval or pyriform in shape and has one flagella D: *Chilodonella* sp. on skin (arrowhead) oval to bear-shaped and dorsoventrally flattened E: *Dactylugyrus* sp. on the gill, eye spots are clearly visible F: *Gyrodactylus* sp. On skin has a V-shaped head and a fusiform body shape G: EMC of *Centrocestus* with an X-shaped excretory bladder, circumoral spines and dark excretory granules H: *Lernaea* sp. on skin I: *Argulus* sp. on skin

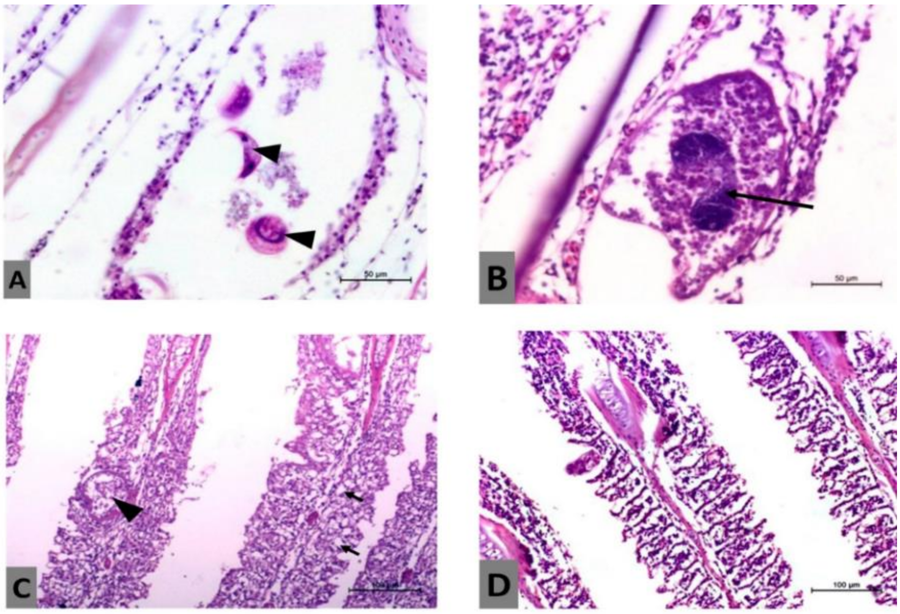

Figure 3: Histological sections of gills infested with ectoparasites and stained with H&E A: *Trichodina* spp. appeared as a multiple ring-shaped structures (arrow head) and surrounded by mild inflammatory reaction B: Mature trophonts of *I. multifiliis* in the gill (C-shaped nucleus; arrow) with necrosis of gill filaments C: vacuolar degeneration (black arrow) and hyperplasia of secondary gill lamellae with trophont stage of *I. multifiliis* (horseshoe shaped macro-nucleus is not apparent) (arrowhead) D: section of *Dactylogyrus* and necrosis of gill filaments at the site of parasite attachment.

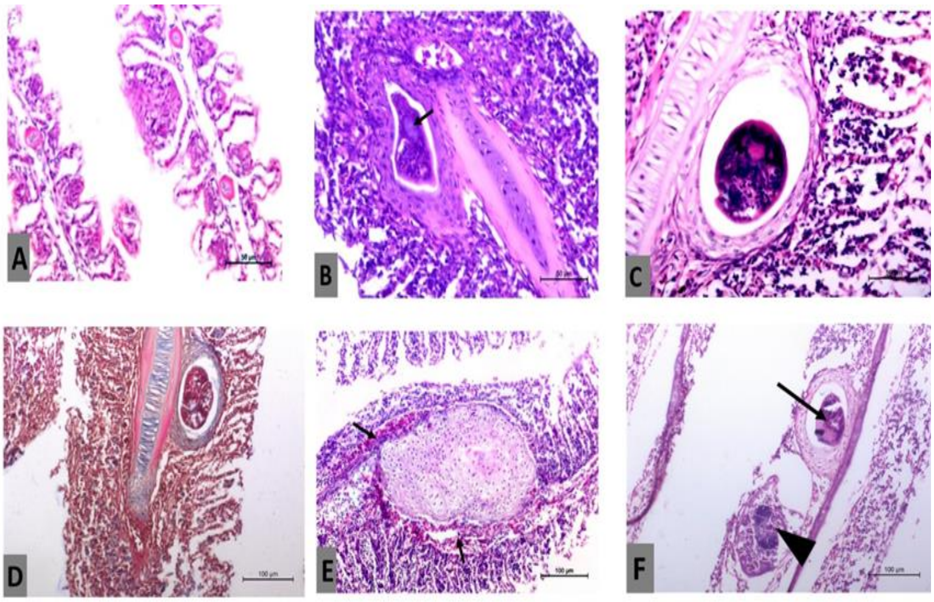

Figure 4: Histological sections of gills infested with ectoparasites and stained with H&E A: Gills showing section of *Dactylogyrus sp.* with focal hyperplasia B: proliferation of chondrocytes around EMC of *Centrocestus* with hyperplasia and fusion of secondary gill filaments C: EMC of *Centrocestus* surrounded by fibroblast-like cells D: EMC of *Centrocestus* stained with Masson trichrome E: Hyperplastic cyst in the lamellar epithelium with hemorrhage (arrow) and the parasite is not apparent F: mixed infection of EMC (arrow) and trophont stage of Ich (arrowhead).

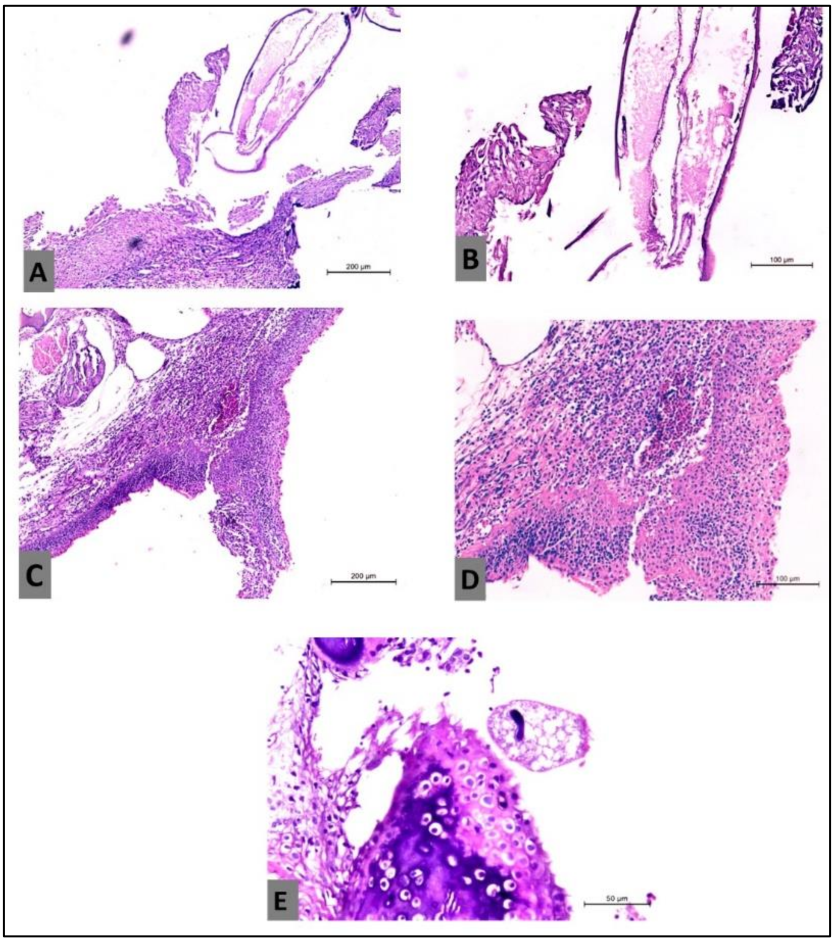

Figure 5: histological section of skin infested with ectoparasites and stained with H&E A: Anchor worm penetrating skin in association with degeneration and desquamation of epidermal cells with mononuclear cells infiltration B: higher magnification C: Hyperplasia of epidermal cells, hemorrhage and infiltration with lymphocytes and macrophages D: Higher magnification E: Skin of *C. auratus* infected with Ich. showing the horse shaped nucleus (arrowhead), hyperplasia and hydropic degeneration of epidermal cells and necrosis with ulceration around the parasite trophont.

DISCUSSION

The cultivation and rearing of ornamental fish has a unique situation in the world and it plays an important part in the exchange revenue of various nations as well as in the occupation. Because of the financial significance of these aquatics, numerous researchers have focused on various factors endangering their health (Meshgi *et al*., 2006).

An estimated $9 billion in economic losses are incurred by the aquaculture sector each year as a result of disease outbreaks which also have an impact on the global aquaculture production (Novriadi, 2014). Parasitism is probably the common problem in tropical and ornamental fishes (Roberts, 2011). Parasitic infection develops as a result of changes in feed, feed quality, fish age, fish size, water condition and aquaculture activities (Novriadi, 2014). Therefore, it is critical to research the most frequent parasite infections affecting freshwater ornamental fishes in markets and pet shops as well as their histopathological consequences.

In the present study, *Carassius auratus* were examined for parasitic infection. Five groups of parasites were identified; protozoans (*Trichodina* sp., *Ichthyophthirius multifiliis*, *Icthyobodo necator* and *chilodonella* sp.), monogenea (*Dactylogyrus* sp. and *Gyrodactylus* sp.), digenea (ECM of

Centrocestus fomanas), copepods (*Lernaea* sp. and *Argulus* sp.) and nematode (*Capillaria* sp.). Because of a small volume of water and high density of fish, parasites of the skin and gills were the most identified parasites.

While a broader range of parasites in ornamental fishes was anticipated, There could be several reasons why specific parasite families were not detected. First, fish keepers treat ornamental fish with chemotherapy when they notice any fish mortality in their aquarium fish which reduces the variety of parasites in the fish population. Second, a full parasitological examination was not performed for every fish.

Protozoans were among the most common parasites found in this investigation. Ciliates are among the most dangerous parasites and can promote secondary bacterial infections in farmed fish (Lom and Dyková, 1992; Bastos Gomes *et al*., 2017).

Trichodinids are harmful ectoparasites of fish in the majority of cases (Thomas and Wellborn, 1967), disrupt the fish respiration and feeding (Ahmed, 1976) and cause total gill epithelial destruction (Eisa *et al*., 1985). Trichodinads are ciliated external parasites that live in the gills and skin of fish and amphibians but can also be found in the U.B. of frogs (Collymore *et al*., 2013).

Ichthyophthiriasis (Ich) (white spot disease) is caused by *I. multifiliis*, a pathogenic parasite of cultured fish (Schaperclaus, 1991). This protozoan is present throughout the world, and almost every freshwater fish is vulnerable to infection. The death rate of affected fish with Ichthyophthiriasis might be around 100% (Meyer, 1974). In certain cases, this parasite causes minimal death, but it nevertheless has a negative influence on the population and individual fish (Lom and Dyková, 1992), attacking by sticking to the mucus layer and depleting red blood cells (Noe, 2006). In the current investigation, some goldfish exhibited lethargy, increased mucus production, and gill filament hyperemia, which is consistent with the findings of (Smith and Roberts (2010). The parasites' lesions can potentially cause secondary microbial infections (Noga, 2010). *I. multifiliis* can induce anemia-dependent pale gill in goldfish (Noga, 2010). This comes in line with our study, but further investigations are required to correlate the infection with the different parasite species in goldfish and secondary bacterial infection.

Our results showed the absence of pathological lesions due to *Chilodonella* sp. as *Chilodonella* species are mostly free-living, however, some parasitize the skin, gills, and fins of freshwater, marine, and estuarine fish (Pdua *et al.,* 2013). According to Bastos Gomes, Miller, *et al*. (2017) certain species as *Chilodonella acuta* and *C. uncinata* may become opportunistic pathogens of aquatic organisms in specific conditions. Only two species have been documented causing injury in fish: *Chilodonella hexasticha* Kiernik, 1909 which is mostly found in tropical fishes, and *C. piscicola* (Shulman *et al*., 1984) (syn. C. cyprini Moroff, 1902) mostly parasitizing fishes from subtropical as well as temperate waters.

Monogenea species are also important parasites affecting freshwater fishes. *Dactylogyrus* and *Gyrodactylus* spp. are the most common genera regarded monogeneans to be among the most important metazoan parasites (Martins *et al*., 2002 and Garcia *et al*., 2009). These parasites have hooks connected to the host's skin and gills generating lesions that allow secondary bacterial infections to occur (Cardoso *et al*., 1990 and Reed *et al*., 2012).

Lesions can vary depending on the monogenetic species and can cause mucus hypersecretion, fusion of gill lamellae and death by asphyxiation. Furthermore, haptor lesions to the integument might promote secondary infection by bacteria and fungi (Cardoso *et al*., 1990).
Gill inflammation, swelling, hyperemia, hemorrhage and excessive mucus production

were observed in infected goldfish gill tissue in the current investigation of monogenea infection. According to (Rodríguez *et al*., 2002), this causes blood vessel occlusion which leads to respiratory and osmoregulatory failure.

Crustacean arthropods' attachment mechanism and feeding preference on the body surface and fins cause lesions such as erosions, ulcerations, haemorrhages, desquamation, and gateways of entrance for secondary invaders (Yanong, 2012a,b).

Lernaea sp. infection constitutes a major issue in cultured and ornamental fish (Iqbal *et al*., 2012a; Iqbal and Haroon, 2014). Because the parasite adheres to the skin of ornamental and aquarium fish, it causes more problems than other types of fish. This may reduce the marketability and look of fish, diminishing their value. Although the parasite may be removed with forceps, the wound creates a breeding ground for viral, bacterial, and fungal diseases, causing the fish to die. *Lernaea cyprinacea*, a natural parasite in goldfish, can act as a vector (Boane *et al*., 2008 and Rohlenová, 2021). *Lernaea* parasites inhabit all parts of the fish's body and attach via anchor mouth and fangs. The parasite will drain blood and mucous from the host. The risk of *Lernaea* parasites is injuring skin tissue besides causing fish mortality (Steckler; Yanong, 2012a,b). Infection of fins is indicated because the fin is one of the organs that gives benefits to the parasite, such as protection from water currents or abrasion; hence, tissues of fin bases are readily penetrated by the parasite (Galindo and Millán, 2005). Infestations of *Argulus foliaceus* produce cutaneous irritation characterized by fin flicking (Bauer, 1991). This is frequently accompanied by increased mucus production on the skin's surface as well as the formation of tiny haemorrhages (Richards, 1977).

The rate of infection among *Carassius auratus* was (47.2%). The overall prevalence of examined fish in fish markets and pet shops was high as the collected fish from different aquariums showed signs of disease (clinically abnormal). This prevalence was lower than that described for 8 species of ornamental fish captured from the Rio Negro river, Amazonas state (64.1%) (Tavares-dias *et al*., 2010) and higher than detected in six species of ornamental fish in northwestern Iran (26.33%) (Rahmati *et al*, 2022). The presence of detectable parasites at any level is important because the majority of parasites recovered from freshwater ornamental fish in Egypt have the potential to cause significant mortality in captive and wild stocks, particularly parasites that do not require an intermediate host, such as protozoans and monogenean trematodes. The high prevalence of protozoan parasites in *C.auratus* (27.9%) may be due to their ability to survive in fish-free environments because of their high tolerance to non-fish hosts (Rahmati-Holasoo *et al*., 2022). The low predominance of crustaceans in *Carassius auratus* (12.7%) may be explained by the fact that their life cycle can be extended by up to three months depending on water temperature (Soulsby, 1982). The bulk of commercial ornamental fish species are only bred for two to three months before being sold. As a result, those fish are less susceptible to crustacean parasites. Because crustaceans like *L. cyprinacea* and *A. japonicus* can be seen with the naked eye, breeders can take more functional control measurements in advance and remove the seriously infested fish from stock while ignoring the less diseased specimens before transportation (Xu *et al*., 2007).

The low prevalence of encysted metacercariae in *Carassius auratus* (2.6%) referred to digenea needs an intermediate host to complete their life cycle. Snail species represent the principal intermediate hosts of these helminths while fish species are the secondary intermediate hosts, and fish-eating birds are the definite hosts (Carvalho *et al*., 2012). As a result, with proper management, infestations can be handled (Citino, 1996), this could justify the very low reported prevalence of *Centrocestus* spp. metacercarial stage as well as the absence of additional metacercarial stages during the investigation.

Nematode infection rates were the lowest (0.4%), which was to be predicted given that most nematodes do not frequently represent a problem in aquaria when compared to terrestrial vertebrates (Anderson, 1996) due to their inability to complete their extensive life cycles that require at least two hosts. Our results are consistent with (Rahmati *et al.*, 2022), who identified one nematode in ornamental fish with an incidence rate of (0.33%). The present findings also indicated that *Trichodina* sp. showed the highest prevalence (15.3%) among *Carassius auratus*. The high percentage of *Trichodina* sp. infection is related to binary fission reproduction, which is favored by the pond's high organic content (Ogut and Palm, 2005). This parasite has no host or location preference (Thilakaratine *et al*., 2003). *Trichodina* sp. infection in ornamental fishes was reported by Thilakaratine *et al*., 2003) and Tavares-Dias *et al*., 2010) in Sri Lanka and Brazil, respectively. Some studies have found trichodinids in ornamental fishes, such as 9.3% of *Trichodina nigra* (Thilakaratine *et al*., 2003), 16.3% (Kayis *et al*., 2013), and 57% (Marques *et al*., 2015) of *Trichodina spp.* on the body surface of *C. auratus*.

Although *lernaea* spp. is not host-specific, this parasite is prevalent in goldfish and other carp [(Thilakaratine *et al*., 2003) with an incidence rate in this study of about 11.8%. *Lernaea cyprinacea* has also been found in major and Chinese carp in captivity (Iqbal *et al*., 2012a).

Majumdar *et al*., 2013 and Hossain *et al*., 2008 found that the prevalence of protozoan parasites is highly dependent on seasonal temperature fluctuations.

This study found that *Ichthyophthirius multifiliis* outbreaks occur during the summer months when temperatures are at their peak. This ciliated protozoan was present and active at temperatures above 14 ℃ (Borji *et al*., 2012). The high infection rate of *Dactylogyrus* sp. in autumn (14.5%) as most species showed high infections at medium temperatures ranging from 20 to 30 ℃ (Chubb, 1977).

Infection with *Gyrodactylus* sp. is highest in the autumn with mild temperatures. (Davioda *et al*., 2005 published a similar study. In warmer months, King *et al*., 2009) discovered that the intensity of *Gyrodactylus* sp. was high in warm aquarium waters. According to Anderson, 1998 the mild temperature is ideal for the growth of *Gyrodactylus* sp.

Lernaea spp. only infests fish during the warmer months. This crab, however, causes one of the most prevalent diseases in warm freshwater fish. Temperature is the most important factor influencing the parasite's life cycle and virulence, as the parasite's life cycle does not complete at temperatures below 15°C (Roberts and Shepherd, 1974 & Durham, 2002). *Lernaea* spp. prevalence often increases when water temperatures are above 25°C while parasitized fish can be observed throughout the year (Durham, 2002).

From the infectivity and pathogenicity point of view, in general, small numbers of parasites on the gills and skin of fish do not cause substantial health concerns but high numbers can cause more severe problems and eventually death (Toksen, 2007).

The pathogenicity of *Trichodina spp.* is determined by their abundance or scarcity. These organisms do not induce lesions in fish infected with a low number of trichodinids, however, they become pathogenic as their numbers increase (Collymore *et al*., 2013) which is consistent with our findings in this investigation.

Trichodina sp. can infect fish notably the gills and skin as *Trichodina sp.* provided with denticles that can adhere to fish organs and infect them. *Trichodina sp.* graze on the epithelial layer of the gill surface and fish skin causes epithelial cell hyperplasia, gill destruction and filament fusion. Ectoparasite infection will impair the ability of the gills to maintain appropriate respiratory and excretory processes as well as the skin's ability to perform optimal osmoregulation activities (Lom, 1995).

The pathogenicity of *I. multifiliis* stems from lesions caused by colonization in surface epithelium primarily the gills and skin resulting in the proliferation of the respiratory epithelium, lamellar fusion, epithelium degeneration and necrosis resulting in the formation of multiple ulcers in the epithelium after the release of mature trophonts (Martins *et al*., 2015).. The presence of many trophonts at the gill lamella could be the result of multiplication when feeding on fish. Although the macronucleus was a constant and clear finding in the case of *I. multifiliis* infection, sometimes the microscopic lesions in some examined cases showed the absence of macronucleus and it might attributed to the level of sectioning or the stage of the trophont maturity.

Monogeneans (Platyhelminthes) are fish external parasites. They securely attach themselves to fish tissues via an opisthaptor, a posterior organ with specialized attachment components (Justine *et al*., 2013). The attachment location generates lamellar clubbing which can destroy the gill epithelium and blood vessels. The effects of monogenean infection on gill lamellae include epithelial tissue proliferation and secondary lamellar fusion. Infection of the fish with *Dactylogyrus* in the current study causes significant destruction to the gill lamellae which may be analogous to lesions described by Abdelhalim, (1990).

Dactylogyrus sp. induces excessive mucus secretion as a result of significant irritations in the gill filaments (Daghigh Roohi *et al*., 2019). It was discovered in this investigation that the parasites nestled between the fish's gill lamellae causing hyperplasia in the gills. This parasite binds to the base of primary gill filaments, which contain osmoregulating chloride cells. Infection with the parasite could thus be risky and detrimental particularly for fry-size fish in hatcheries (Shamsi *et al*., 2009). Jalali and Barzegar (2005) found gill filament fusion, secondary lamellar hyperplasia and aneurism in fish infected with Dactylogyrus sp. which is consistent with the findings of this study.

In the current work, lamellar necrosis was found in gills infected with monogenean parasites *Dactylogyrus* sp. The main cause for this lesion is the mechanism by which the parasite may destroy the branchial vessels where the blood pressure is low and no extensive haemorrhages are caused and the very short clotting time of blood causes rapid occlusions of the vessel then thrombus is formed resulting in ischemia which in turn leads to necrosis, similar to (Eissa, 2002).

The cercarial stage attached to and penetrated the fish host, causing infection with *C. formosanus* (Paller, 2008). When cercaria connects, they create a mucus-like fluid, shed their tail, and engage in a brief period of leech-like creeping behavior before penetrating and encysting on the surface of primary gill lamella (Gjurčević *et al.* 2007)..The site of metacercaria encysted on the fish gills underwent morphological modifications. The modifications were characterized by hyperplasia of the cartilage of the primary gill lamellae that envelop the metacercaria. As a result of the encapsulation of the fish host response, the metacercaria was enclosed by a thick capsule. The host cyst wall was made up of chondrocytes that were generally elongated next to the parasites (Gjurčević *et al.* 2007). The similar histological changes of *C. formosanus* infection in freshwater fish have previously been described by(Gjurčević *et al.* 2007).

Cercariae migration into host tissues might result in modest mechanical damage and haemorrhage [105]. Unlike other trematodes, *C.formosanus* causes substantial gill damage in many freshwater fish (Hoffman, 1999). Histopathological sections of infected gills show that metacercariae lodged next to the cartilage of gill filaments and triggered an intense inflammatory response that included hyperplasia of the cartilage of the primary gill lamellae that encircles the metacercarial cysts, lamellar fusion, and proliferation of fibroblasts and chondroblasts that formed a cartilaginous encapsulation around this parasite, leading to destruction of the normal gill tissue and a decrease in Other authors who observed *C. formosanus* infection in *Carassius auratus* have previously described the same changes (Yildiz, 2005). Previous research found haemorrhages in the gills of fish infected with metacercariae. Similarly, the haemorrhage reported in our study could be caused by the invading parasites that mechanically damage gill capillaries. Oedema and leucocyte infiltration in the gills were most likely caused by the initiation of inflammatory reactions in the host as a consequence of the injury (Hernandez, 1988).

In response to the injury caused by this parasite, the parasite was discovered to be next to the cartilage of primary gill lamellae and surrounded by just a few layers of host cells. The host cell layer that ringed the parasite grew thicker over time, eventually transforming into chondroblasts or chondrocytes. The proliferation of cartilage cells found in this study is most likely a defense mechanism of the host toward the parasite as demonstrated by previous research (Rezaie, 2017).

Infection with *L. cyprinacea* can have major consequences for fish health, particularly for tiny fish. This parasite can induce rupture and necrosis, whereas adult female *L. cyprinacea* attachment produces haemorrhage, necrosis, muscular stiffness, inflammatory responses and occasionally subsequent bacterial infections. This is due to the fact that the portion of the *L. cyprinacea* that attaches to the skin deeply pierces the fish body causing internal organ damage (Lester and Hayward, 2006). Infection of fins is warranted because the fin is one of the organs that provide benefits to the parasite such as protection from water currents or abrasion; consequently, tissues of fin bases are easily pierced by the parasite (Galindo and Millán, 2005).

Pathogenic effects of *Argulus* sp. on their hosts include skin damage which manifests as skin lesions (dermatitis) (Oprean and Vulpe, 2002). The dermatitis is caused by the suckers' and proboscis' harmful action. There are numerous crimson spots and hemorrhagic regions on the skin. *Argulus sp.* inject digestive enzymes into the fish's body using a preoral stinger and the lice suck out liquid body fluids with their proboscis-like mouth. These toxic enzymes cause irritation and inflammatory lesions on the skin of fish culminating in the formation of ulcers owing to microbial infections Purivirojkulm, 2012 and Mayer, 2013). The lesions in the current study were minimal as pathology in fish is determined by the population density of *Argulus* (Kumar *et al*., 2017).

Capillaries can cause chronic wasting when parasite levels are high. They can be very invasive, penetrating the intestinal mucosa and eliciting massive inflammatory responses (Heckmann *et al*., 1987), as well as causing intestinal obstruction and nutritional deficiency (Thatcher, 2006), leading to protracted death (Wildgoose 2001). In the current study, no significant digestive lesions or mortalities were associated with the presence of *Capillaria* spp., which might be attributed to the low parasitism level. These parasites frequently cause fewer problems in fish than in terrestrial vertebrates(Anderson, 1996). This study lacks the results of bacteria and virus detections due to technological limitations of field sampling. However, histological research suggests that the parasites described in this work caused harm to the host's epithelial tissue. They can behave as primary pathogens to the host, causing secondary infection with bacteria or viruses and illness (Basson & Van As, 2006). Improvements in living standards and other lifestyle changes have raised demand for high-quality, low-cost aquaculture products in Egypt and have encouraged agencies to give priority to freshwater fish farming's production, environmental and food safety issues. As a result, a precise list of ectoparasites should be created to help aquaculturists identify these significant parasites accurately and treat them effectively.

CONCLUSION

This study revealed that the prevalence rate of parasites in goldfish is relatively high as well as the pathological findings induced by these parasites negatively affected the proper function of the skin and gills of the host. This could have a negative impact on fish health and result in significant economic losses through higher mortality.

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دراسات باثولوجية على الاصابات الطفيلية الخارجية والداخلية التى تصيب الأسماك الذهبية ذات الذيل المروحى

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يعتبر استزراع أسماك الزينة على مستوى العالم قطاعا رئيسيًا لتربية الأحياء المائية ويمثل جزءًا كبيرًا من صناعة الحيوانات الأليفة. فى الفترة بين يونيو ٢٠٢٢ وفبراير ٢٠٢٣، تم فحص إجمالي ٢٢٩ سمكة ذهبية تم جمعها من سوق السيدة عائشة بمحافظة القاهرة، مصر وبعض محلات بيع الحيوانات الأليفة في محافظتي بني سويف والفيوم لفحص التغيرات الهستوباثولوجية المرتبطة بالطفيليات. وقد بلغ معدل الاصابة الطفيلية ٤٧,٢%.

تشمل الطفيليات المعزولة الطفيليات وحيدة الخلية (التريكودينا و الاكثيوفثيريس ملتيفيلس و الكيلودونيلا و إكثيوبودا نيكاتور) و الطفيليات أحادية المنشأ (الدكتيلوجيرس والجيرودكتيلس) والطفيليات ثنائية المنشأ (سنتروسستس فورموسانوس) و القشريات (القمل والدودة الشوكية) والديدان الخيطية (كابيلاريا) وكانت معدلات الإصابة ٢٧,٩%، ١٥,٣%، ٢,٦%، ١٢,٧% و ٠,٤% على التوالي .تنوعت الفحوصات الشاملة للأسماك المصابة بأنواع مختلفة من الطفيليات وظهرت على شكل مناطق حمراء مرتفعة، ونزيف، مع زيادة إنتاج المخاط ومجدافيات الأرجل بيضاء اللون تشبه الديدان في أجزاء مختلفة من الجسم وتقرحات، وفقرالدم الشاحب أو احتقان الخياشيم الملونة. التغيرات النسيجية المرضية شملت تفريغ ونخر خلايا البشرة، تضخم واندماج الصفائح الخيشومية الثانوية، بالإضافة إلى ارتشاح خلايا الدم البيضاء بشكل رئيسي الخلايا الليمفاوية والبلاعم، تضخم غضروف الخياشيم والكيس الطفيلي المحاط بعدة طبقات من الخلايا الشبيهة بالليفية.

الخلاصة، إن معدل انتشار الإصابة بالطفيليات في الأسماك الذهبية مرتفع نسبيا، بالاضافة الى ان التغيرات الباثولوجية التي تسببها هذه الطفيليات قد تؤثر سلبا على وظيفة الجلد والخياشيم.

الكلمات المفتاحية : طفيليات, نسبة الاصابة, باثولوجى, الاسماك الذهبية