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DIAGNOSTIC AND PATHOLOGICAL STUDY OF *ARGULUS JAPONICAS* IN GOLDFISH (*CARASSIUS AURATUS*)

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ABSTRACT
This study was carried out in one of fish markets located in AL-Najaf province / Iraq during the period from October 1, 2013 to February 28, 2014. A total of 90 common goldfish (*Carassius auratus*) was collected and examined macroscopically and microscopically. The results indicate that all fish were infected with *Argulus japonicas* according to the morphology of this parasite. Gross lesion showed hemorrhage spot on fins and skin. While, histopathological examination showed focal deposition of melanocytes in the epidermal layer, skeletal muscles, at with edema and hyalinization affecting of skeletal bundles and degenerative with necrotic changes in the epithelial cell and hyperplasia of fin filaments.

KEY WORDS: Goldfish (*Carassius auratus*), Ectoparasite, *Argulus*, Pathological lesions.

INTRODUCTION

Infectious diseases are broadly categorized as parasitic, bacterial, viral, or fungal (Duijn, 1973). Like any animals, fish are susceptible to a range of problems such as tumors, heart and other organ disease, as well as metabolic disorders such as diabetes. However, the overwhelming majority of common health problems involve external parasites, fungus, viral and bacterial infections (Notash, 2012). The genus *Argulus* (Crustacea: *Branchiura*), or lice, are common parasites of freshwater fish (Bauer *et al.*, 1991; Yildiz and Kumantas, 2002). *Argulus* species (Family: *Argulidae*), more commonly known as fish lice, are members of a large group of branchiuran parasites infestation that cause disease in fish. The argulids are crustaceans and are related to crabs, lobsters and shrimp (Mohamed and Kenawy, 2013). Approximately 100 different species of *Argulus* are distributed in worldwide depending upon species, can infest freshwater and saltwater fishes. The three most studied species are *Argulus foliaceus*, *A. japonicus*, and *A.coregoni* that are found in freshwater systems. Infections with these are most common in wild and pond-raised freshwater fish, particularly goldfish, koi, and other cyprinids (carps and minnows); centrarchids (sunfishes) and salmonids (salmon and trout). *Argulus foliaceus* has also been found on frogs and toads (Al-Dulaimi, 2010). These parasites are 5-10 mm in size and consist of a head, thorax and abdomen (Yildiz and Kumantas, 2002). The head is covered by a flattened horseshoe-shaped carapace, maxillipeds, peroral sting and basal glands. The thorax has four segments, each bearinga pair of swimming legs. The abdomen is a simple bilobed segment (Soulsby, 1982). *Argulus* can cause considerable damage to trout through their aggressive attachment and feeding behaviour. They feed by inserting a long spine-like structure into the skin, with breaks down tissues through the secretion of enzymes. The repeated puncturing of the skin, combined with activity of the parasites' serrated mouth-parts, can cause substantial damage and irritation (Noga, 2010). *Argulus* infestations tend to peak in the summer and fall, The lice can be attached to the skin, gill chamber, and mouth. Localized inflammation occurs at the contact site because of mechanical damage from hooks and spines on the stylet and appendages, causing irritation from digestive enzymes. In heavy infestations, the fish lice may be seen all over the skin and fins of the fish and in the water column fish without visible lice (non specific signs) of infestation (Hoffman, 1999). The signs include spot or pinpoint hemorrhages, anemia, fin and scale loss, increased mucus production, lethargy, erratic swimming, reduced feeding, hanging at the surface (avoiding swimming into the water column) and poor body condition. Fish may "flash" orrub against surfaces in an attempt to relieve irritation or to remove the parasites. In some cases, there may be no obvious signs of disease other than presence of the parasite (Stoskopf, 1993). *Argulus* is also capable of acting as a mechanical vector or intermediate host for several fish diseases. The parasite can carry and transmit spring viremia of carp a reportable viral disease of koi, common carp, and goldfish among other hosts. *Aeromonas salmonicida*, an important bacterial pathogen, has been isolated from *Argulus coregoni*, and experiments demonstrated higher rates of *Aeromonas* infection when *Argulus* are present, but direct transmission fo louse to fish has notyet been proven. *Argulus* can also serve as the intermediate host for several species of nematodes (round worms) (Hakalahti-Siren *et al*., 2008).

MATERIALS & METHODS

Study design

This study was carried out during October 1, 2013 to February 28, 2014 about fishery market were selected and

in summation 90 goldfish were obtained from their and were suspected of being affected by ectoparasites. The fish were shipped to laboratory of College of Veterinary Medicine, University of Kufa for assayed both macroscopically and microscopically. Macroscopically, we examined the fishes and in times of existence of parasite on body of fishes, they were come outside and were examined microscopically. In outside, the fish were anesthetized by clove powder and the parasite was detached from fish's skin and was examined microscopically to approve.

Clinical examination

Clinical examination of fish species, *Carassius auratus* was adopted using the methods described by (Lucky, 1977) for the determination of any lesions or abnormalities on the external body surface.

Parasitological examination

The external body surface of fish *C. auratus* and were carefully examined macroscopically. The detected parasites, which could be easily seen with the naked eye were collected by using fine brush, washed for several times in warm saline solution and left in refrigerator at 4 C^{o} for complete relaxation (Eissa, 2002). The collected crustaceans were counted, fixed in 70% ethanol, preserved in alcohol glycerol (4:1) for permanent mounts, cleaned and mounted according to the methods described by (Lucky, 1977). The parasites were identified microscopically by using dissecting microscope as described by (Woo, 1995). Also, the samples examined under dissecting microscope and light microscope for identification of genus and species of parasite were samples put on slide and a drop of Lugol's iodine added as staining then add DPX for fixation and put a cover slide and examined under 4x, 10x and 40x for diagnosis of parasite (Levine, 1961).

Identification of infesting species

Identification and morphometric characteristics were conducted according particularly to (Bykhovskaya-Pavlovskaya *et al*., 1964).

Histopathological examination

Sections were taken from the affected skin and gills of diseased fish and fixed in 10% formol saline for twenty four hours. Sections were washed in tap water and passed in serial dilutions of alcohol (methyl, ethyl and absolute ethyl) for dehydration. Specimens were cleared in xylene and embedded in paraffin. Paraffin wax tissue blocks were prepared for sectioning at 5-7microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparafinized, stained by hematoxylin and eosin and then examined by using light microscope **(**Roberts, 2001).

RESULTS & DISCUSSION

The result of laboratory microscopical examination revealed that goldfish (*C. auratus)* lice belong to *Argulus japonicus* and this ectoparasite consist of a head, thorax, and abdomen. The head is covered by a flattened horseshoe-shaped carapace, maxillipeds, peroral sting and basal glands. The thorax has four segments, each bearing a pair of swimming legs.The abdomen is a simple bilobed segment. While the dorsal aspect, two prominent movable compound eyes are visible in the head region (Figure 1). The current results agree with (Bykhovskaya-Pavlovskaya *et al*., 1964). The percentage of infestation with *Argulus* were 100% because all 90 collected samples of goldfish were infested with lice. This high rate of infestation return to import of fish from neighboring countries which are infested with these parasites that consider one of the important ways of transmission of parasitic infestations where a great number of different species of ornamental fish including different varieties of goldfish were imported annually from these countries. In addition to the fish breeders did not use any kind of pesticides as means of therapeutic which led to the aggravation of the problem, our result come compatible with (Mousavi *et al*., 2011). The clinical signs of natural infested *C. auratus* with argulosis were revealed the presence of erratic movements, abnormal swimming, rubbing themselves against the wall of the tank, frayed fins and off food. Development of inflammatory lesions at the site of parasitic existance, characterized by abundant secretion of mucous and haemorrhages causing erosions of the injured areas were observed on the skin and fin (Figure 2). However, some infested fishes showed nervous manifestation with easily detached scales and abraded areas (Figure 3 and 4). Because of this ectoparasite feeds on blood and other bodily fluids, and causes further harm to the fish by injecting digestive enzymes that can lead to systemic illness in addition to the role of parasites as vectors for other diseases such as Aeromoniasis or Pseudomoniasis (Richards, 1977; Mousavi *et al*., 2011). these results are in agreement with the results that revealed by (Eissa, 2002; Noga, 2010). Histopathological alterations of infested fish species with argulosis clarified that the skin demonstrated focal deposition of melanocytes in the epidermal layer (Figure 5). Skeletal muscles showed edema and hyalinization affecting some skeletal bundles (Figure 6). However, degenerative and necrotic changes in the epithelial cell (Figure 7) with hyperplasia of fin filaments associated with chronic inflammatory cells infiltration (Figure 8), these results are in agreement with the results recorded by (Amlacher, 1996). Taylor *et al*. (2005) reported that the parasites cause a reactive hyperplasia of the epithelium and increased mucus production. Hyperplasia appears as a cloundiness to the skin and leads to hypoxia if occur on the gills. The parasites pierce the host tissue with the pre-oral stylet, inject a cytolytic toxin, and feed on the blood released by the resultant wound. The surface of the host at the point of stylet entry can become erythemic and hemorrhagic. A hemorrhagic factor is produced by some species. Several parasites feeding in close proximity may cause edema and localized swelling of tissues.

FIGURE 1: female of *Argulus japonicus*.

FIGURE 2: *Argulus japonicus* on the fin.

FIGURE 3: hemorrhagic spot on the fin (grossly)

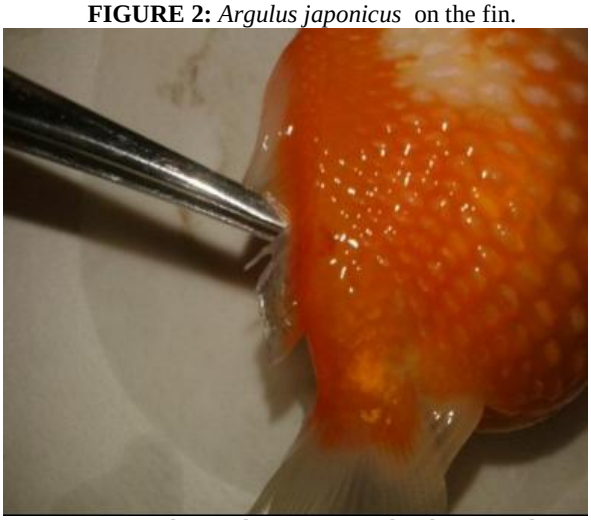

FIGURE 4: hemorrhagic spot on the skin (grossly)

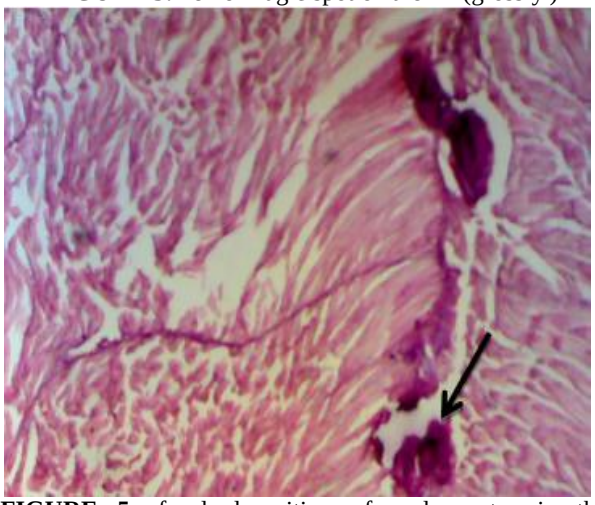

FIGURE 5: focal deposition of melanocytes in the epidermal layer (H and E X10)

FIGURE 6: skeletal muscles showed edema and hyalinization affecting some skeletal bundles. Presence of parasite (H and EX 10)

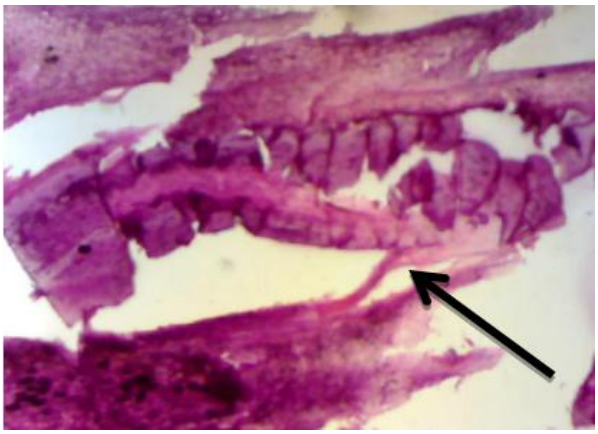

FIGURE 7: degenerative and necrotic changes in the epithelial cell with hyperplasia of fin filaments (H and E X 10)

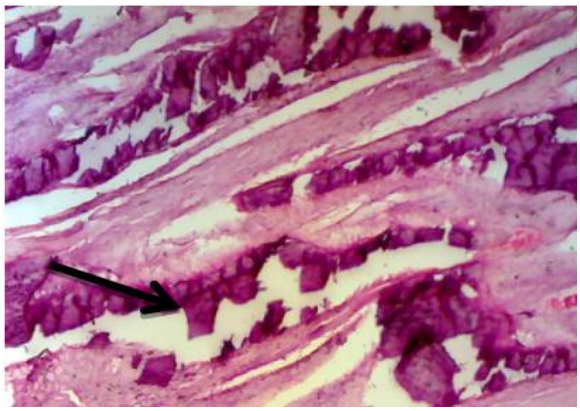

FIGURE 8: degenerative and necrotic changes in the epithelial cell with hyperplasia of fin filaments associated with chronic inflammatory cells infiltration (H and E X10)

REFERENCES

Al-Dulaimi, F. H. A. (2010) Infection with a fish louse *Argulus foliaceus* L. in a goldfish (*Carassius auratus)* at earthen ponds and aquarium fish in Babylon province, Iraq. Journal of Babylon University/Pure and Applied Sciences, 2(18): 468-473.

Amlacher, E. (1996) Textbook of fish diseases Translated by Canroy D.A. and Herman, R.L.T.F.H. Publ. 308pp.

Bauer, R. (1991) Erkrankungen der Aquarienfishe. Verlag Paul parey. Berlin und Hamburg.

Bykhovskaya-Pavlovskaya, I. E.; Gusev, A. V.; Dubinina, M. N.; Izyumova, N. A.; Smirnova, T. S.; Sokoloskaya, I. L.; Shtein, G. A.; Shulman, S. S. and Epshtein, V. M. (1962) Key to parasites of freshwater fish of the U.S.S.R. Akad. Nauk, S.S.S.R., Moscow: 727 pp. (In Russian).

Duijn, van, Jnr. C. (1973) Diseases of fish .lliffe Books, London, 3rd ed., 372 pp.

Eissa, I. A. M. (2002) Parasitic fish diseases in Egypt. El-Nahda El-Arabia Publisher, 32 Abd El-Khalek Tharwat street, Cairo, Egypt.

Hakalahti-Siren, T., Mikheev, V. M. and Valtonen, E. T. (2008) Control of freshwater fish louse *Argulus coregoni*: a step toward an integrated management strategy. Diseases of Aquatic Organisms 82: 67-77.

Hoffman, G. L. (1999) Parasites of North American freshwater fishes. Cornell University Press, Ithaca, NY.

Levine, N. D. (1961) Protozoan parasites of domestic animals and of man. Burgess Publishing Co., Minneapolis, MN.

Lucky, Z. (1977) Methods for the diagnosis of fish diseases Amerial Publication Co., PVT, Ltd, New Delhi and New York.

Mohamed, A. H. and Kenawy, A. m. (2013) Studies on argulosis in some freshwater ornamental fishes with special reference to treatment trials. New York Science Journal, 6(10): 37-41.

Mousavi, H.A. E.; Behtash, F.; Rostami-Bashman, M.; Mirzargar, S. S.; Shayan, P. and Rahmati-holasoo, H. (2011) Study of *Argulus spp.* infestation rate in goldfish, *Carassius auratus* (Linnaeus, 1758) in Iran .HVM Bioflux, 3(3): 198-204.

Noga, E. J. (2010) Fish disease diagnosis and treatment. 2nd ed., Mosby-yearbook, Inc. watsworth publishing Co., USA. pp.366.

Notash, S. (2012) Study on prevalence of *Argulus* in goldfishes (*Carassius auratus*) of East Azerbaijan province of Iran, Annals of Biological Research, 3(7): 3444-3447.

Richards, R. (1977) Diseases of aquarium fish- 2.Skin diseases. Vet. Rec. 101:132-135.

Roberts, R. J. (2001) Fish Pathology 3rd ed.,W.B. Saunders, An imprint of Harcourt Publishers.

Soulsby E. J. L. (1982) Helminths, arthropods and protozoa of domesticated animals, 7th ed., Bailliere Tindall.

Stoskopf, M. K. (1993) Fish medicine. W.B. Saunders company, Philadelphia, PA.

Taylor, N. G. H; Sommerville, C. and Wootten, R. (2005) A review of *Argulus spp*. occurring in UK freshwaters. Journal of Fish Biology 11: 349.

Woo, P.T. (1995) Fish diseases and disorders. Protozoan and metazoan infections V1, 486-494.

Yildiz, K. and Kumantas, A. (2002) *Argulus foliaceus* infection in a goldfish (*Carassius auratus*). Isr J Vet Med., 57(3):118-120.