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Studies on Cultured Silver Carp (*Hypophthalmichthys Molitrix*) Diseases Induced by Some Bacterial, Fungal and Parasitic Pathogens in Sharkia Governorate

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ABSTRACT

Carps are the largest family of freshwater fishes cultured world-wide and introduced into Egypt for food security purpose. The aim of the present investigation was to through light on infectious diseases agents leading to mass mortalities among cultured silver carp (*Hypophthalmichthys molitrix*). Two hundreds of live or recently dead silver carp were collected from fish farms suffered from heavy mortalities. All collected fish were subjected to clinical, postmortem, bacterial, fungal and parasitic examinations. Also, trials for treatment were done. Treatment by Ciprofloxacin was the drug of choice both under in-vitro and in-vivo laboratory conditions and the percentages of bacterial, fungal and parasitic infections among the examined fish were 45, 10 and 60%, respectively. The isolated causative agents were *Aeromonas hydrophila* (30%) and *Pseudomonas fluorescens* (15%), *Achlya* sp. (10%), *Trichodina* sp. (15%), Monogenetic trematodes (5%) and *Lernaea cyprinecea* (60%). The clinical signs and postmortem changes associated with infectious diseases were described. Also, the detected parasites were described briefly.

INTRODUCTION

Among the various fish species cultured worldwide, cyprinids consider the largest group of species cultivated. Asian food fish carps includes: grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), and common carp, *C. carpio.* Silver carp were first imported into Egypt in the early 1980s for food security purpose because of fast growth rate and lower cost of feeding in comparison with other fish species. Also, silver carp control of plankton in aquaculture

ponds, reservoirs, and sewage treatment lagoons. However, such introduction of new species increases the probability of introducing new pathogens (Woo and Bruno, 1999).

Diseases are a crucial factor which hindering the development and sustainability of aquaculture. Bacterial diseases were shown to be a definite problem in fish culture (Toranzo *et al.*, 2005). Many fungi have been associated with disease in fish. Although some are apparently primary pathogens, many are thought to require predisposing factors such as adverse water temperature, poor water quality, handling, or over crowding in order to establish infection (Udomkusonsri, 2003). A wide variety of parasitic diseases have been recorded as major factor in the limiting of fishes production (Woo, 1995).

Chemotherapeutic agents are commonly used for the control of bacterial diseases in cultured fish. Currently in the United States, there are only two available antimicrobial products (Oxytetracycline and combination of ormethoprim and sulfadimethoxine) approved by the U.S. Food and Drug Administration (FDA) for the treatment of bacterial diseases in fish (Plumb, 1999).

The aim of the present investigation was to through the light on infectious diseases affecting cultured silver carp (*H. molitrix*) with special reference to its control.

MATERIALS AND METHODS

Naturally infected fish

Two hundred fish specimens of cultured silver carp (*H. molitrix*) were collected from commercial fish farms suffered from heavy mortalities in Sharkia Governorate. They were transferred to the laboratory alive or recently dead and subjected to clinical and postmortem examinations as described by Lucky (1977).

Experimental fish

An apparently healthy silver carp (*H. molitrix*) weighting 25-30 gm were collected from El-Abbassa fish farm and transferred alive to laboratory, maintained in glass aquaria filled with dechlorinated tap water supplied with continuous aeration and acclimatized to laboratory conditions for 15 days before the start of experiment. The temperature was kept at 24±1^{o}C throughout the experiment. Fish were fed twice daily with standard commercially prepared pellets at 2% of their body mass/day throughout the

experimental period. Uneaten feed and feces were siphoned daily. Dead fish were removed daily.

Bacteriological examination

Samples were taken under complete aseptic condition from gills, liver, spleen, kidneys, gonads and intestine and inoculated into tryptic soya agar and incubated at 22 C. Purified isolates were identified according to standard biochemical tests (Bergey *et al.,* 1984 and Austin and Austin, 1993).

Mycological examination

Mycological examination was performed according to the method described by Robert (1982).

Parasitic examination

Parasitological examinations were performed according to Lucky (1977).

Sensitivity test

Sensitivity of *Aeromonas hydrophila* and *Pseudomonas fluorescens* to different antibiograms, were estimated according to Carter and Cole (1990).

Experimental infection

The pathogencity of the isolated bacteria was confirmed by dividing 30 silver carp into 3 equal groups. The first and second groups were injected with *A. hydrophila* and *P. fluorescens* and at a dose of 0.2 ml of saline containing 10^8cells/ml intraperitoneally (i.p.), respectively. The third group was served as a control group and injected with 0.2 ml sterile saline (i.p.). Fish were observed daily for clinical signs abnormalities and mortalities. The pathogens were reisolated as a pure culture from the freshly dead fish.

Laboratory evaluation of Ciprofloxacin (Cf)

A total number of 40 apparently healthy silver carp were divided into four equal groups. The first group was subjected to a treatment by 1.6 g Cf was dissolved in ounce of 36% acetic acid to accelerate dissolution and then added to the water aquarium to give a final concentration of 10 µg/ml of Cf and injected i.p. with *A. hydrophila* (Guo *et al.,* 2005). The second group was injected with Ciprofloxacin at a dose of 10 mg/kg fish, then challenged by 0.2 ml of pathogenic strain of *A. hydrophila* (Bowser *et al.,* 1994). The third group was challenged with *A. hydrophila* and kept without any treatment. The fourth

Table (1): The percentage of bacterial, mycotic and parasitic agents recovered from the examined silver carp.

Number of examined fish	Bacterial agents		Fungal agents		Parasitic agents	
	Number	%	Number	%	Number	%
200	90	45	20	10	120	60

group was left as a control. All fish were kept under observation for any clinical abnormalities, mortalities and for the presence of *A. hydrophila* for 20 days.

RESULTS

Among 200 cultured silver carp specimens examined, the percentages of pathogenic agents were shown in Table (1).

The Gram-negative bacteria isolated from clinically diseased silver carp were *Aeromonas hydrophila* (30%) and *Pseudomonas fluorescens* (15%). The detected fungus was *Achlya* sp (10%). The identified parasites were *Trichodina* (15%), Monogenetic trematodes (5%) and *Lernaea cyprinecea* (60%) as showing in Table (2).

Table (2): ***The percentage of the identified fish pathogens.***

Fish pathogen	Number of examined fish	Number of infected fish	Percentage of infection
A. hydrophila	200	60	30
Ps. Fluorescens		30	15
***Achlya* spp.**		20	10
Trichodina spp.		30	15
Monogenetic trematodes		10	5
Lernae cyprinecea		120	60

The common clinical signs due to bacterial infections were loss of appetite, swam near the surface of water, dullness, increased mucus secretion and congested gills. Hemorrhages were noticed at the external surface of fish(Fig. 1, 2, 5&6). Abdominal distention and intestinal prolapse were observed. The postmortem findings were congested liver, in other cases congesion at the margins with necrosis and in others pale colour and yellowish white patches were observed. Spleen was dark in colour. Kidneys and gonads were congested and watery ascitic fluid was recorded (Fig. 3&4).

Table (3) shows that both bacterial species were sensitive to Ciprofloxacin, novobiocin, Vancomycin, Trimethoprim and Tetracycline while it was resistant to Ampicllin and Kanamycin.

The results of pathogenicity of *A. hydrophila* and *Ps. fluorescens* among silver carp (Table, 4) showed that the mortality rate was 70 % and 40%, respectively. No mortality was recorded in the control group. The clinical signs and postmortem changes were similar to naturally infected fish.

Regarding to the laboratory trial for the treatment of fish artificially infected with *A. hydrophila* with use of Cf , our result showed that Cf was effective against MAS. The clinical signs were disappeared and the treated fish returned to the normal state of health. *A. hydrophila* was not isolated from the treated fish. Table (5) showing the mortality rate among the treated and non-treated groups.

Naturally infected fish by *Achlya* showed cotton like tuft on external surface (Fig. 7), increased mucus secretion and congestion of gills. Squash preparation of ulcerated skin of infected fish revealed the *Achlya* spp. grow in branched, thin white filaments and terminal hyphal swelling that will differentiate into the primary zoospores. *Achlya* spp. is among oomycete (order Saprolegniales) which produced flat, white colonies or hyphae on solid agar.

Regarding to parasitic infections, naturally infected fish exhibited lethargy, respiratory distress, frayed fins and epidermal erosion. *Trichodina* species was detected in gills and skin. The clinically diseased fish were suffered from sluggish swimming, loss of appetite, swimming near the water surface, gasping air and showing an increase in the breathing frequency. Moreover, pale color, slimy skin and

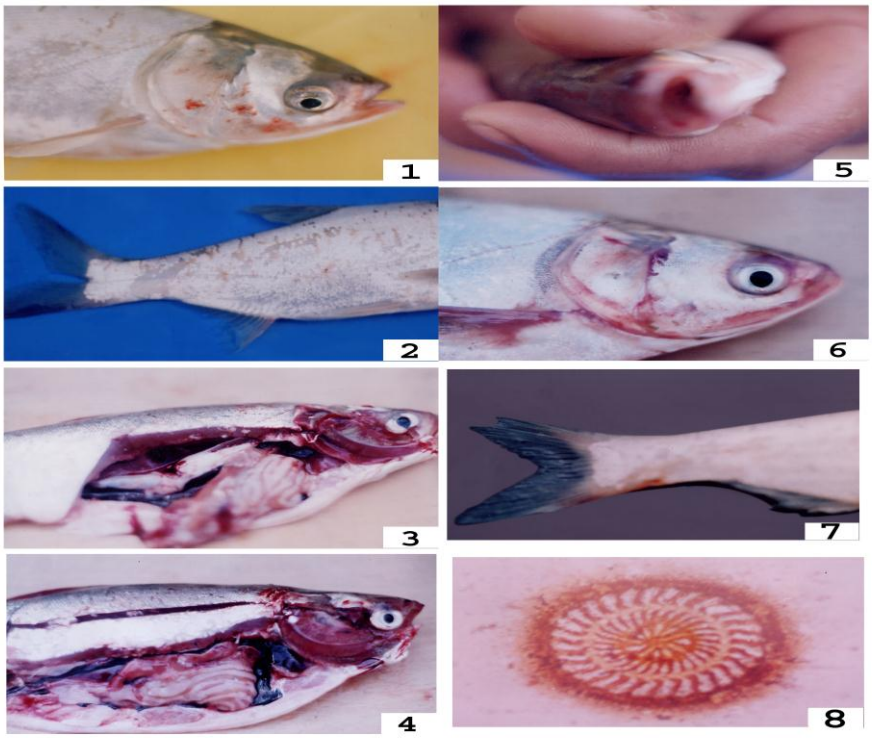

Figure (1): Showing hemorrhage in mouth and gill cover of silver carp.

Figure (2): Pin head hemorrhage and scale less of tail silver carp.

Figure (3): Post mortem(P.M) lesion of silver carp showing congested gills, kidney and herorrhagic of internal organs.

Figure (4): Post mortem(P.M) lesion of silver carp showing congested gills, kidney and hemorrhagic of internal organs.

Figure (5): Red mouth of silver carp.

Figure (6): Hemorrhagic of head,gill cover and pectoral fin.

Figure (7): Showing tail and fin rot, ulcer on tail with cotton like structure.

Figure(8): Trichodina species stanined by silver nitrate.

Table (3): Sensitivity of some bacterial isolates to different antibiograms.

Antibiotic againt	symbol	Concentration (mcg)	*A. hydrophila* Sensitivity reaction	*Ps. Fluorescens* Sensitivity Reaction
Ampicllin	**AM**	**10**	**R**	**R**
Ciprofloxacin	**CIP**	**5**	**S**	**S**
Novobiocin	**E**	**15**	**S**	**S**
Kanamycin	**K**	**30**	**R**	**R**
Trimethoprim + sulphamethoxazol	**Sxt**	**1.25 / 23.75**	**S**	**S**
Tetracycline	**TE**	**30**	**S**	**S**
Vancomycin	**VA**	**30**	**S**	**S**

I: Intermediate. **R**: Resistant. **S**: Sensitive.

detatchment of scales were observed. The gills showed pale appearance at the extremities of the filaments with severe erosion giving the gills a ragged appearance. Microscopic smears from the external body surface of naturally infected silver carp showed large bodies with disc-shaped or wheel- like bodies were isolated. The parasites were provided with 3 rows of cilia with prominent denticular rings (Fig. 8).

Table (4): *Pathogencity test of A. hydrophila and Ps. fluorescens among silver carp.*

Fish group	Bacterial Pathogens	Route of injection	Number of infected fish	Number of dead fish	Mortality rate
I	***A. hydrophila***	**I/P**	**10**	**7**	**70**
II	***Ps. Fluorescens***	**I/P**	**10**	**4**	**40**
III	**Control**	**I/P**	**10**	**0**	**0**

Table (5): ***Laboratory efficacy of Ciprofloxacin for the control of A. hydrophila infection.***

Fish group	Number of fish	Number of dead Fish	Mortality rate
G1	**10**	**2**	**20**
G2	**10**	**1**	**10**
G3	**10**	**7**	**70**
G4	**10**	**0**	**0**

G1: Treated by immersion and infected ***G2: Treated by injection and infected***
G3: Non-treated and infected ***G4: control.***

Microscopic smears from external body surface and gills of the naturally infected silver carp showed flat worm parasites, possessed two pairs of eyes, with two anterior conical projections, disc like ophthaptor which was provided with two selerotenoid anchors and a connecting bar with 14 pair of peripheral hooklets (Fig. 9) Such adult worms are monogenic trematodes belonged to family Dactylogyridae, genus *Dactylogyrus*.

Isolation of large crustacean parasite from external body surface of silver carp was obtained from skin and musculature. The copepod was characterized by the configurations of its head region which comprising a pair of cephalic horns, branching dorsal horns and pair of shortened, more slender and ventrally undivided one. The horns were arranged perpendicular to the long axis of the body. The female was elongated, cylindrical and somewhat enlarged posteriorly. The trunk section following cephalon and further back pregenital protuberance was found. Ovaries were elongated containing rounded yellowish brown egg. Such adult copepod is related to family Lernaeidae, *Lernaea cyprinecea* (Fig.10).

DISCUSSION

The planktivorous filter-feeding silver carp (*Hypophthalmichthys molitrix*) has a great potential in the developing countries for lower cost of feeding in comparison with other fish species. Disease has become the most

critical factor hampering the development of fish culture in Egypt. The nowadays knowledge analysis within this domain points out that nursery pond cyprinids pathology, especially the morph-physiology basis of diseases, was not entirely been studied, mainly within the sphere of systematic arrangements of intensive breeding, in which large fish populations are used as patterns (Lazar, 2009).

Basic knowledge of fish etiology is needed to develop strategies for prophylaxis and control of diseases in tilapia aquaculture (Abd El-Ghany *et al.,* 2009). The current study showed that the parasitic agents were more prevalent pathogens. Similar finding was mentioned by Woo, (1995) and Lazar, (2009).

Dealing with bacteriological agents isolated from the naturally infected fish recorded that the most common isolated bacteria was *A. hydrophila*. Similar observation was noticed by Jeney and Jeney, (1995); Otta *et al.,* (2003); Abd El-Ghany *et al.,* (2009); Lazar, (2009); Awad, (2011) and Sahu *et al.,* (2012) who mentioned that among the bacterial pathogens, the most frequently encountered bacterial agent associated with fish diseases is *A. hydrophila*. It is found to be distributed widely in the aquatic environment and responsible for causing several diseases in cold-blooded animals besides fish like reptiles, amphibians and in warm blooded animals like mammals and birds.

In the present study, the inspection of naturally infected fish showed loss of appetite, dullness, increased mucus secretion and congested gills. Hemorrhages were noticed at the external surface of fish. Abdominal distention and intestinal prolapse were observed. The post mortem examination of the diseased fish showed congestion in the internal organs. Similar pictures were previously described by Austin and Austin (1993); Plumb, (1999); Woo and Bruno, (1999); Abd El-Ghany *et al.,* (2009); Lazar, (2009); Awad, (2011) and Sahu *et al.,* (2012) who mentioned that *Aeromonas* and *Pseudomonas* species had septicemic picture. Stress factors as temperature, overcrowding, availability of iron and poor water quality plays an important role in the incidence of infection (Woo and Bruno, 1999). *Pseudomonas* species has been considered as a secondary invader of damaged fish tissue as well as a primary poor and weak pathogen (Schaperclaus, 1992).

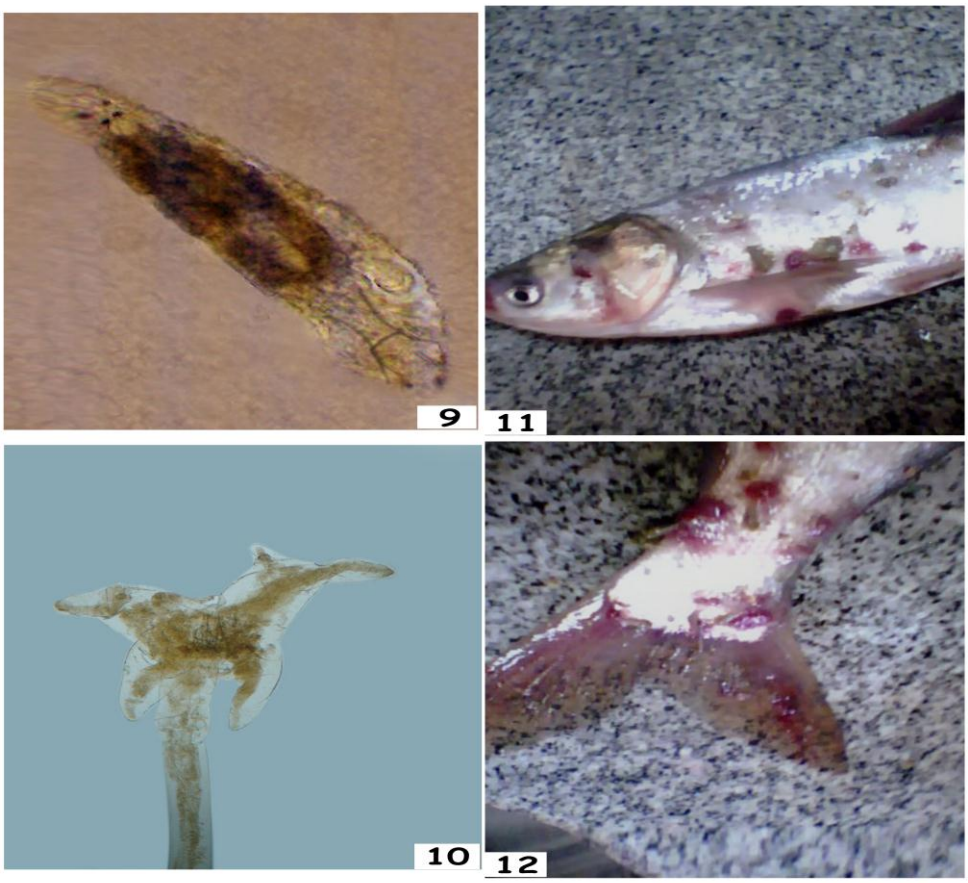

Figure (9): Monogenetic trematode (Dactylogyrus spp.) freshmount.

Figure (10): Adult Lernea cyprincea.

Figures (11&12): Silver carp showing heavy infestation with Lernea cyprincea .

Pathogencity of the isolated bacterial pathogens was confirmed by experimental infections. The experimental infection showed that the isolated bacteria were highly pathogenic to fish via i.p. route. The clinical signs and postmortem changes associated with experimental infection were similar to the naturally infected fish. Similar findings were described by Woo and Bruno, (1999); Abd El-Ghany *et al.,* (2009); Lazar, (2009); Awad, (2011) and Sahu *et al.,* (2012).

Food and Drug Administration (FDA) approved only Oxytetracycline and ormethoprim and sulfadimethoxine as feed additives for the treatment of bacterial disease in fish (Plumb, 1999). Therefore, due to an increase in acquired resistance of bacterial pathogens to oxytetracycline and the combination of ormetoprim and sulfadimethoxine, the lack of other FDA-approved antibiotics for fish, and the need for *in vivo* data on pharmacokinetics and withdrawal periods to support the use of other chemotherapeutics in aquaculture and ornamental fish industry (Somjetlertcharoen, 2001). The present investigation examined the usefulness of the antimicrobial drug, Ciprofloxacin and evaluates the potential of this drug for consideration as an alternative bacterial therapeutic agent for finfish. Ciprofloxacin was the drug of choice under in vivo and in-vitro conditions. The same result had been recorded by Abd El-Rahman and El-Ashram, (2005); El-Refaee *et al.,* (2008) and Awad, (2011).

Achyla species is often accompanied by opportunistic bacteria (Plumb, 1999). *Achyla* species is a serious winter disease that affects all species and ages of freshwater fish, and also affects many estuarine fish (Stueland *et al*. 2005). Fungal infection usually increases in fish as water temperatures drop to a near lethal level (Aly and El-Ashram, 2000). The infected fish are easily recognized by the cotton-like, white to grayish patches on the external surface, visible to the naked eye. Similar findings were observed by Plumb, (1999); Woo and Bruno, (1999); Aly and El-Ashram, (2000); Udomkusonsri, (2003) and Abd El-Ghany *et al.,* (2009).

The parasitic diseases of fish may be the main cause of low body weight gain, high mortality, immarketability and some of these diseases may have a zoonotic importance (Eissa, 2002). The parasitic agents the ectoparasites or endoparasites are taking a superior

position as infectious agents. Heavy infestation with ectoparasites (*Trichodina* and monogenea) usually resulted in loss of appetite, sluggish movements and the fish swam near the surface of water. The infected fishes showed increased breathing frequency, and the gill covers were stretched open widely. The gills were pale in some fish, hyperemic in others and covered with mucus secretions forming cloudy film of slime. Infected fishes were anemic and the fins were ragged with partial detachment of scales. Such obtained results are nearly similar those of (Post ,1987; El Gawady *et al*.., 1992; Hool *et al*.., 2001; Eissa ,2002; Schatch and Moraes, 2005 and Osman 2005). These clinical signs and postmortem changes attributed to disruption and irritation of skin by the parasitic denticles of trichodina and/or its rotatory movement and the organs of attachment and mode of feeding of monogenea. Fry and fingerlings were more sensitive to infection (monogenea and protozoa) and their sensitivity are increased with the higher density of fishes usually found in pond (Jalali and Barzegar ,2005). *Trichodina, Dactylogyrus* and *Gyrodactlus sp*. occurred mainly at low water quality, bad zoo hygienic conditions in ponds and an increase in water temperature (Tomee *et al*, 1995).The parasitic gill disease found according mixed infection of(monogenea and protozoa) *Dactylogyrus spp and Trichodina spp* (Kim and Sim ,1993 and Osman,2005) .

In Egypt, the dangerous copepod *Lernaea cyprinecea* was introduced with the imported brood stocks of carps and their progeny in different hatcheries (Faisal et al,1988 and Azza Abd El Rahman, 2000). Clinically infested carp by *L. cyprinecea* infestation revealed, sluggish movement, poor appetite and appeared emaciated, and they scraped those selves on the side of aquaria with extruding the rest of their bodies with the egg sacs out of the fish body, which was easily observed by naked eyes, the attachment point was often accompanied with distinct lesion occasionally inflamed and hemorrhagic with swollen margins, focal distribution along body surface of fish and fins with scale loss and ulcers. The clinical signs and postmortem findings of lernaeosis were nearly similar as those recorded by (Faisal *et al* 1988; Guoxianzhen 1989; Schaperclause 1992; Aly and El-Ashram, 2000; Azza Abd Elrahman ,2000; Hanna, 2001; Awad, 2007; Lazar, 2009 and Saleh et al., 2010). These results may be attributed

to strong hooks of lernaea, which make destruction of musculature causing ulcer. Such ulcers are considered the gate for secondary infection by bacteria, fungus and protozoa.

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دراسات عن الأمراض البكتيرية, الفطرية والطفيلية التي تصيب أسماك المبروك الفضي المستزرع فى محافظة الشرقية

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تم إجراء هذا البحث علي عدد200 سمكة مبروك فضى حية أو حديثة النفوق من المزارع السمكية التى تعاني من نسبة وفيات عالية. وكان الهدف من الدراسة الحالية إلقاء الضوء على الأمراض المعدية التى تصيب المبروك الفضى وتؤدى إلى نفوقه. كل الأسماك المجمعة تم فحصها ورصد العلامات المرضية الظاهرة والصفة التشريحية من الناحية البكتيرية، الفطرية والطفيلية. أيضا، تم إجراءالتجارب لتلقي العلاج. وكانت النسب المئوية للإصابات البكتيرية، الفطرية والطفيلية بين الأسماك التى تم فحصها هى 45٪، 10 ٪ و 60٪، على التوالي. تم عزل مسببات الأمراض وهى إيروموناس هيدروفيلا 30٪،سيدوموناس فلوروسينس 15٪ ، أكليا 10٪ ، تريكودينا 15٪ ، تريماتودا وحيدة العائل (داكتيلوجيرس)5٪ ، ليرنيا سبرناسى 60٪ . أثبتت الدراسة أن السيبروفلوكساسين له تأثير قوى فى علاج الأسماك المصابة ولذلك إستخدم كدواء مختار في إطار معملى وتحت ظروف المجراة فيه.