Some studies on fish deformity in freshwater fish in Egypt

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Abstract: Fish anomalies are defined as presence of defects in particular parts of the body like vertebral column, mouth and caudal peduncle regions. This study was carried out on 400 fishes showed signs of anomalies (250 cultured, and 150 wild) collected from Alexandria, Kafr El-Sheikh and El-Behera Governorate in the period from June 2006 to May 2008. The clinical signs were in the form of, deformity of vertebral column, mouth and caudal peduncle. Also most fish were emaciated with dark discoloration of the external body. Internally, congestion of some internal organs (spleen, kidney and gills) with enlargement and paleness of liver, watery fluid in abdominal cavity were the main observed signs. Ration analysis from affected farms was carried out to detect calcium deficiency effect on fish deformity which revealed 17 samples had calcium deficiency from total examined 250 by a ratio of 6.8%. Deformed fish were examined for cytogenetic effect which revealed 6 samples have cytogenetic anomaly. Infection with ***Ichthyophonus hoferi*** was 68 samples from total number of 250 cultured fish by a ratio of 27.2% and 30 samples from total number of 150 wild fish by a ratio of 20% . Infestation with ***Myxosoma cerebralis*** was 68 from total examined 250 cultured fish by aratio of 27.2% and 14 samples from total examined 150 wild fish by a ratio of 9.3%. The prevalence of infection with ***Ichthyophonus hoferi*** and ***Myxosoma cerebralis*** were higher in Kafr El-Sheikh governorate followed by El- Behera and Alexandria. The prevalence of infection site with ***Ichthyophonus hoferi*** and ***Myxosoma cerebralis*** were higher in liver followed by kidneys, spleen and intestine respectively Histopathological changes of natural infected fish revealed changes of most affected organs as will as presence of cyst of ***Myxosoma cerebralis*** and spores of ***Ichthyophonus hoferi*** in many organs. Through this study we found that fish anomaliesproved to be affect fish economically either by low production or marketabilityAlso infectious causes of anomalies were of high percentage, so more studies and researches are of important in this situation to make planning for control.
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Keyword: fish deformity freshwater .

Introduction

The aquaculture industry has been considered as one of the fastest growing agribusinesses over the past two decades (***USDA2000)***.

Fish anomalies occur in both freshwater and marine fish. They have bad economical effect, as they affect marketability and during processing the fillets might be very soft slimy and strong with some times off odors ***(Reichenbach-klinke, 1965 and Amany 2010) .***

Infectious fish diseases considered as the main cause of reduction of fish farms production and its profitability ***Woo (2004) and Ramaiah (2006) .***

Fish anomalies can be attributed to genetic, pathogenic, environmental and / or nutritional may be involved ***(Noga, 1996 and Easa,1997).***

These anomalies may be genetic, resulting from mutation or recombination either epigenetic, acquired during embryonic development or post embryonic acquired during larval or post larval development***.(Noga, 1996).***

Skeletal anomalies ranging from modification in gill arch structures, fin rays to extreme vertebral deformation have been noted in fish farms polluted habitats ***(Sloof, 1982).***

The type of skeletal deformities differed according to the species of fish and causes ***(Easa, 1997).***

In Egyption study, the prevalence of infection with ***Ichthyophoniosis*** and ***mycobacteriosis*** was 32%. Prevalence was higher in cultured (40%) and female fish (44.7%) than for wild (24%) and males (22.6%). ***(Nadia Abdelghany et al. 2008).***

This study was aimed to throw the light on the causes of fish deformity among wild and cultured fish in Egypt.

2. Materials and Methods

Naturally deformed fish :

A total number of 400 fish (250 cultured *Oreochromis niloticus* and 150 wild fish including 2 *Mugil capito,* 2 *Mugil cephalus,*1 *Bighead carp,* 1 *Gold fish and* 144 *Oreochromis niloticus*) were

collected from 30 farms from different localities at Alexandria, Kafr El-Sheik and El-Behera Governorates (7 farms from Alexandria , 13 farms from Kafr El-Sheik and 10 farms from El-Behera) Ration samples were obtained from each farm for analysis .

The fish samples were collected during the period from June 2006 to May 2008. The body weight of the obtained fish was ranged from 40-150 g.

A total number of 12 fish were obtained a live from farms in El-Behera Governorate for studying the cytogenetic effect.

Clinical examination:

Clinical and postmortem examination of the collected fish were done according to the methods described by *Amlacher (1970) and McVicar*, *(1982)* to detect any clinical abnormalities like (Scoliosis, lordiosis , mouth deformity and loss of tail or fins) and any internal lesions.

Bacteriological and mycological examination:

Samples from affected organs (spleen, liver, kidneys) were used for cultivation of Mycobacterium species on Trypticase soya agar at 32 C for 48 hrs. The suspected colonies was transported to Dorset egg media then incubated at 25C for 2 weeks

Mycological examination was done according to *McVicar (1982)* and Amany (2010) for the fish showing any deformity . Samples were taken by using sterile dissecting needle from the internal organs (liver, kidney, spleen and intestine) and inoculated onto the MEM- 10 and on Sabouraud's dextrose agar with l% bovine serum. The inoculated plates and tubes were incubated at room temperature for 15 day.

Identification of the isolates was done according to the morphological characters including the hyphal growth and multinucleated spores through the microscopical examination of wet mount and stained preparation *McVicar (1982)* . From the nodules appeared in affected organs of naturally infected cases, squash preparations were prepared.

Calcium analysis:

Ration samples were obtained from every farm where fish samples were collected for calcium analysis. The calcium was analyzed according to the method described by *Khoof (1991)* by analytical chemical method and the obtained results were judged according to *N.R.C (1987)*

Diagnosis of *Myxosoma cerebralis*

A fresh fish sample was put between two sterile slides and compressed then examined under light microscope (high power) for refractile bodies (Myxobolus cyst) according to *(Wolf & Markiw 1984)* .

Cytogenetic analysis in deformed fish:

The effect of deformity on the somatic chromosomes of *Oreochromus niloticus* was investigated using micronucleus test (MN) as described by *(Hayashi et al., 1998)*.

X-rays examination X-ray technique was carried out for 10 samples of deformed fish.

Histopathological studies

Fresh specimens were collected from liver, spleen, gills, muscles and vertebral column for histopathological examinations Sections were stained by hematoxyline and eosin (H,E) according to the method described by *Culling (1983)*.

3. Results

Isolation and identification of *Ichthyophonus hoferi*:

The young culture of *Ichthyophonus hoferi* on SDA + 1% bovine serum showed rupture of multinucleated bodies and release of spores through extra material discharge after 9 days while the, culture of *Ichthyophonus hoferi* on MEM-10 PH 7.0 showed hyphae with different sizes and formation of multinucleated bodies after 8 days of incubation. Localization and fixation of multinucleated bodies (ameaboblast) at the end of each hyphae with rupture of some ameoboblasts were noticed .

At pH 3.5 showed starting of hyphal growth after 24 hours post incubation. The hyphae produced many branches, extending of the hyphae to grow and increased in length, migration of cytoplasm to the apex of hypae after 3 days was noticed .

Rounding up of the apices of the hyphae after 7 days was also observed , finally all the hyphae rounding up to form spherical hyphae terminal bodies after 10 days .

In old culture chlamydo-spores formation around the multinucleated bodies extend to the test of stacked hyphae at 3 weeks were seen.

Culture of *Ichthyophonus hoferi* showing foamy white color of hyphal growth on M E M- 10 (Fig 4)

Isolation of *Ichthyophonus hoferi* were from 98 fishes (1 gold fish , 1 big head carp and 96 from *Oreochromus niloticus*)

Calcium analysis :

Analysis of rations obtained from farms in which the fish showed deformities for calcium examination revealed calcium deficiency in 17 samples. The ratio was less than the reported ratio by FAO . According to *N. R . C (1987)* , the ratio was less than 3.8 mg calcium/kg feed considered to be Calcium deficiency

Identification *of Myxosoma cerebralis* :

The examination of gills and vertebral column of affected fish revealed the presence of refractile bodies indicates *Myxosoma cerebralis* spores at different stages in 68 samples . The spores were ovoid in shape contain two polar capsules with sporoplsm of different sizes (Fig5, 6, 7, 8, 9).

Cytogenetic analysis:

The genotoxic examination of collected fish revealed that , six samples monosex *Oreochromus niloticus* gave genotoxic effect for deformity (Fig. 10, 11)

Clinical signs and postmortem lesions of naturally deformed fish due to:-

Ichthyophonus hoferi* infection*:

Clinical signs of the deformed fish were in the form of excessive mucous on the skin, deformity of the vertebral column and congestion of some internal organs with paleness and enlargement of liver in some cases (Fig.2,3,4). There were 68 fish of *Oreochromus niloticus* from the total 250 by ratio of 27.2 % in cultured fish, and 30 fishes from the total 150 with a ratio of 20% in wild fish.

Calcium deficiency:

Emaciation and dwarfism, head size was comparatively larger than head region. The rays spin of fins were soft and easily turned down. Internally paleness of most viscera and watery fluid in the abdominal cavity were the main observed signs (Fig. 5,6) in case of calcium deficiency samples. There were 17 samples of calcium deficiency from the total 250 with a ratio of 6.8 % .

Myxosoma cerebralis* infestation*:

Emaciation, dark discoloration of external body, deformed mouth and body and internally congested liver, spleen, kidney and gills (Fig7, 8,9) were observed in case of deformed fish associated with *Myxosoma cerebralis* infestation . There were 68 samples from the total 250 with artio of 27.2 % in cultured fish and 14 samples from the total 150 with a ratio of 9.3 % in wild fish (Table 4) .

Cytogenetic deformity*:*

The results of signs which recognized in case of fish with genetic deformity were absence of tail , parrot and bull dog mouth , deformed body and internally congestion of internal organs in some cases . (Fig.10, 11)

Fish had mixed infection by both of *Myxosoma cerebralis* and *Ichthyophonus hoferi* found to be deformed in body , mouth or tail .

There were 15 wild fish samples found to be deformed in a ratio of 10 % from wild examined fish and 3.75 % from the total samples .

X-ray examinations

The X-ray of naturally infested fish by *Myxosoma cerebralis* and by genetically cause showed deformity of vertebral column (Fig 12).

Prevalence of deformed fish in different localities:

Mycological, parasitological examination, nutritional analysis and cytogenetic study of the collected fish revealed the prevalence of infection with *Myxosoma cerebralis*, *Ichthyophonus hoferi*, calcium deficiency and genetic defects among the examined fish. The data revealed that prevalence of infection in cultured fish was higher than in wild fish. With respect to the localities, the fish collected from Kafr- El-Sheikh showed higher infection rate than that obtained from Alexandria and El- Behera Governorates.

Seasonal prevalence of deformed fish:

Regarding to the seasonal prevalence of *Ichthyophonus hoferi*, *Myxosoma cerebralis* and calcium deficiency in the examined fish, in the three Governorates the infection recorded in a higher prevalence during Autumn followed by winter and in a lower prevalence during summer in the examined fish.

Twenty random samples were tested for Cytogenetic effect as a cause of deformity and the results revealed six samples were positive for cytogenetic effect as shown in .

Bacteriological examination:

The result of bacteriological examination revealed no bacterial growth on the dorset egg media .

Histopathological altration :

Result of naturally collected samples, proved the presence of resting degenerated spore of *Ichthyphonus hoferi*, in hepatic tissue with severe eosinophilic granular cells (EGCs) infiltration as a tissue reaction against infection. Perivascular severe lymphocytic infiltration with severe hepatic hydropic vacuolation, and diffuse filamentous necrosis were seen. Degenerated spores between muscle bundles with minimal tissue reaction appeared as infiltration of few melanophores adjacent to spores, enlargement and hyperactivation of melanomacrophage centers., multinucleated resting spores surrounded with fibrosis layers of chronic inflammatory cells as chronic tissue reaction were recorded.

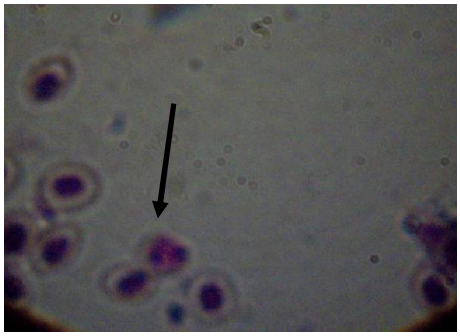

Fig 1 : Peripheral blood erythrocytes of ***Oreochromus. niloticus*** Arrow : Clear cytoplasm without micronucleus.Microscopic magnification X 1000

Fig 2 : ***Oreochromus niloticus*** , showing anomalies due to natural infection with ***Ichthyophonus hoferi***

Fig 3 : ***Oreochromus niloticus*** , showing anomalies due to natural infection with ***Ichthyophonus hoferi***

Fig 4 : ***Big head carp*** showing anomalies due to natural infection with ***Ichthyophonus hoferi***

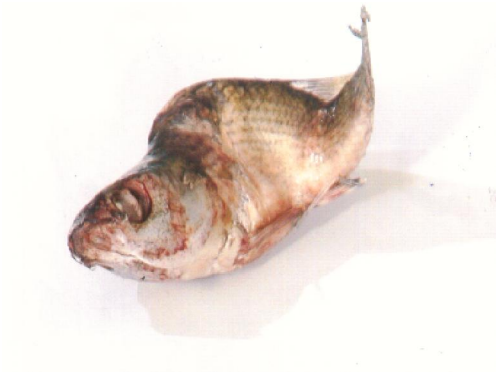

Fig 5 :***Mmugil capito*** showing anomalies due to calcium deficiency

Fig 6: ***Oreochromus niloticus***, showing anomalies due to calcium deficiency

Fig 7 : ***Oreochromus niloticus*** showing anomalies due to natural infection with ***Myxosoma cerebralis***

Fig 8 : ***Oreochromus niloticus*** showing anomalies due to natural infection with ***Myxosoma cerebralis***

Fig 9 : ***Oreochromus niloticus*** showing anomalies due to natural infection with ***Myxosoma cerebralis***

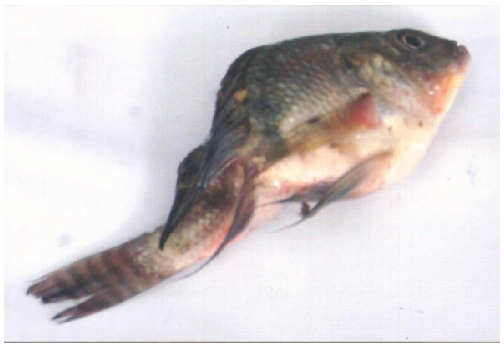

Fig 10 : ***Oreochromus niloticus*** showing anomalies due to cytogenetic effect in the form of mouth deformity .

Fig 11 : ***Oreochromus niloticus*** showing anomalies due to cytogenetic effect in the form of tail loss .

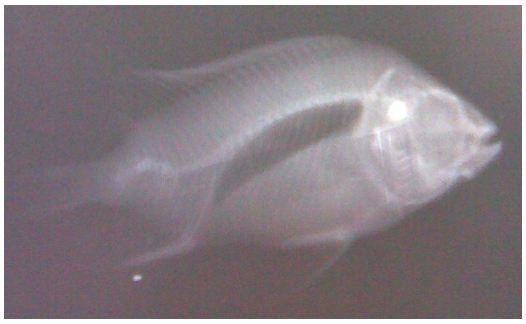

Fig 12 : X-ray film showing deformity of vertebral column of ***Oreochromis niloticus*** fish due to cytogenetic effect

4. Discussion

Lordiosis is one of the most severe deformities developing in reared fish and affect body shape, mostly the posterior abdominal region framed by the anterior and middle base of the pelvic fin. *Shimasaki et al. (2006)*.

The present study was carried out on cultured and wild fish from different species morphologically showed signs of anomalies to investigate the main causes of anomalies via cytogenitical, mycological, bacteriological and parasitological examinations in addition to ration analysis.

The results revealed that the fish anomalies due to either *Myxosoma cerebralis* or *Icthyophonus hoferi* were 136 cases from the total number of 250 cultured fish and 44 from the total number of 150 wild fish . These results proved that fish anomalies may be due to infectious or non infectious causes.

Noga (1996) and *Easa (1997)* mentioned that many factors, genetic, pathogenic, environmental and / or nutritional may be causes of fish anomalies.

The clinical signs of naturally infected fish revealed that the shape of anomalies among fish were varied from deformity of vertebral column to dwarfism, emaciation, deformed mouth or absence of tail. These signs were reported by *McCann and*

Jasper (1972). The differences in the shapes of anomalies may be related to fish species and causes of anomalies as well as severity of infection with the pathogenic agent *(Easa ,1997)* .

Ichthyophoniosis is considered as an important newly recorded disease among cultured tilapia species at different localities in Egypt *(Manal Easa 2002)*.

Spanggaard et al. (1996) and *Mellergaard and Spanggaard (1997)* have reported *Icthyophonus hoferi* as a cause of Ichthyophoniosis and deformity among fish .

During this study, *Icthyophonus hoferi* was isolated from different species of fish. The isolated fungus were submitted to complete morphological and cultural examinations

The young culture of *Icthyophonus hoferi* on SDA+1 %bovine serum showed rupture of multinucleated bodies and release of spores through extra material discharge after 9 days .

Culture on MEM at pH 7 showed hyphae with different sizes and formation of multinucleated bodies after 8 days .

At pH 3.5 showed starting of hyphal growth after 24 hrs post culturing which extend and produce many branches and form spherical hyphae terminal bodies after 10 days .

The growth characters of *Icthyophonus hoferi* were reported by *Ziedan (1999)*

Regarding to the clinical signs and post mortem lesions of *Icthyophonus hoferi* infection were mainly in form of hemorrhage, congestion of body surface with dark discoloration and emaciation. Internally enlargement of liver, congested kidney and gall bladder, heart with abdominal fluid

These may be attributed to the effect of quiescent cyst after settled in the different organs and made tissue damage (*Mcvicar and Mclay*, *1985).*

Deformity is the most important lesion occur due to *Icthyophonus hoferi* these may be due to migration of quiescent cyst (infective stage) to skeletal muscle around vertebral column. The fish try to localize the cyst which usually occurred by surrounding the cyst by connective tissue which replace the myofibers. The end result will be permanent extension of the muscles which lead to moving the vertebrae from its place and finally the deformity occur *(Chauvier and Mortier- Gabet ,1984 and* Amany 2010)

These signs mentioned by *Mcvicar* and *Mclay (1985)* who revealed that the most obvious lesions due to *Icthyophonus hoferi* infection occurred in the white muscle, heart, liver and kidney in herring . In cases of heavy infections normal organ tissue may be replaced by the cyst and C.T. Intern lead to impairing the organ function.

Also *Kocan et al . (2004)* reported that 20% of Yukon river purchased fish were discarded because of muscle tissue damage caused by *Icthyophonus hoferi* .

Myxosoma cerebralis is an important chronic parasitic disease of fish responsible for anomalies especially skeletal deformities *(Wolf et al . 1986)* .

In this study *Myxosoma cerebralis* spores revealed high incidence (21%) from examined (82) samples which found harboring *Myxosoma cerebralis* spores in organs.

This result can confirm the role played by *Myxosoma cerebralis* in fish deformity.

In the present study the signs of deformity resulting from *Myxosoma cerebralis* was mainly in vertebral column and mouth. That comes in accordance with the tropism of myxozoan spores to vertebral column multiplication between vertebrae causing deformity and to the upper and lower jaw resulting in deformity of mouth. In addition the emaciation and ascitis may be related to the chronicity of disease which make depletion of many elements of fish body especially protein which come in contact with that described by *(Wolf et al . 1986)* .

The deformity which occurred in case of *Myxosoma cerebralis* infestation may be attributed to myxozoan spores multiplication make destruction of the cartilaginous elements of the skeleton leading to the chronic phase of the disease characterized by skeletal deformities especially when the infestation occurred in young fish since the calcium precipitation not completed yet *EL Matbouli et al. (1995)*

Also Stoskoph(1993) and *Noga (1996)* reported that trout infected with *Myxosoma cerebralis* developed misshape of body that mainly in the form of deformed caudal area or curvature of the spine with permanently bent and opened mouth

In the present study the prevalence of *Myxosoma cerebralis* infestation was 68 samples from the total number of 250 by ratio of 27.2% in cultured deformed fish and 30 samples from the total number of 150 by a ratio of 9.6% in wild deformed fish. This may refer to the role of over-crowded and other aspects of culture systems which make the infestation more easy than wild fish infestation. Moreover, the chance for infestation is quite high.

Calcium deficiency is considered one of the main causes of fish deformity

During this study the ratio of deformed fish due to calcium deficiency was 6.8% which come in accordance with role of calcium deficiency in fish deformity. The main lesions were softness of fin rays and spins.

The results of ration analysis revealed that Calcium levels were less than 3.8 mg/kg diet . The *N. R. C (1987)* considered Calcium level less than 3.8 mg/kg as Calcium deficiency The negative results of bacteriological, parasitological and mycological examination of deformed fish plus low

level of calcium in ration (less than 3.8 mg / kg diet) gave us support to refer these deformities due to calcium deficiency .

Fish deformity due to calcium deficiency was more prevalent in young ages as This may be concerned to the nature of skeletal apparatus of young fish that mainly is gummy and weak so effect will be more and easy for deformity occurrence *Amlacher (1970).*

Also, in the young fish, the ossification center not closed yet and the precepitation of calcium is week.

Heupel et al . (1999) mentioned that skeletal anomalies are quite common in young fish and may be due to inadequate levels of vitamins, calcium or tryptophan.

Six cases from collected samples were due to genetic causes. These were supported by the genotoxic examination which gave positive results. In the same time the parasitological, bacteriological and mycological examinations were negative which highly supported the causes of genotoxic effect.

This conclusion was supported by *Heupel et al. (1999)* who found that genetic deformities in fish has a minor rank in the risk of aquaculture processes

Kumar and Thakar (2004) concluded that deformity caused alterations in the chromatin conformation of AR promoter and reduction in its accessibility to DN asel in the brain cortex of adult male mice.

Concerning to localities Kafr-elSheikh Governorate showed higher infection rate with *Myxosoma cerebralis*, *Icthyophonus hoferi* and cases of calcium deficiency than that collected from Alexandria and El-Behera. The possible explanation of higher prevalence in Kafr-elsheikh is that the high number of fish farms which present beside each other using the same water drained from the neighbor farms. Moreover they mainly use fish meal for rations and poultry manure which may contain infective stage of *Icthyophonus hoferi* (quiescent cyst) and infected fish used in feed manufacture *(Lauckner 1984)* .

Regarding to the seasonal prevalence of *Myxosoma cerebralis* and *Icthyophonus hoferi* infections in examined fish, the higher prevalence of infections was recorded during the autumn followed by winter, summer and spring seasons respectively.

The possible explanation may be due to the stress effects which caused by low temperature during the winter which interne facilitate the infection with *Icthyophonus hoferi* and infestation with *Myxosoma cerebralis*.

Amany Abdelwahab et al. (2003) reported that *Icthyophonus hoferi* proved to be higher during winter (68.1%) in *Oreochromis niloticus* .

In concerning to distribution of *Icthyophonus hoferi* and *Myxosoma cerebralis* in different organs of infected fish showed higher prevalence of infection in liver , kidney, spleen and intestine respectively

These can be explained that the infection occur mainly in the highly vascular organs with high blood supply. Also, these organs may be the right tropism for these causes .

Faisal et al. (1985) recorded a higher prevalence of infection in liver followed by kidney, spleen and intestine of examined *Claris lazera* infested with *Icthyophonus hoferi* in a rate of 42%, 36%, 14% and 4% respectively

Also *Noga (1996)* and *Ziedan (1999)* stated that the principle infected organs with *Icthyophonus hoferi* are that rich with blood (parenchymatous organs)

Histopathological changes were mainly in the form of viable multinucleated spores beside degenerated one in hepatic and pancreatic tissues with atrophy and necrotic focci , advanced acute cellular swelling of hepatic cells, perivascular severe lymphocytic infiltration with severe hepatic hydropic vacuolation this may be attributed to the effect of *Icthyophonus hoferi* infection .

Also severe eosinophilic granular cells infiltration of hepatopancreas , degenerated spores between muscle bundles with infiltration of melanophores adjacent to spores of *Myxosoma cerebralis* , degeneration of perichondrial tissue of cartilage these changes can be occurred due to *Myxosoma cerebralis* infestation.

These results refers mainly to the tissue reaction against both *Icthyophonus hoferi* and *Myxosoma cerebralis* spores beside the nature of multiplication through target organs lead to signs of inflammation. At the same time presence of the spores through infected tissues insure the infection

Rand (1991) detected the resting and germinating spores surrounded by chronic granulomatous reaction with pleocellular infiltration in the infected organs with *Icthyophonus hoferi* . Moreover the positive results of the roles played by *Icthyophonus hoferi* in deformity of vertebral column were proved by X- ray which done for the infected fish.

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