SOME STUDIES ON ICHTHYOPHONOSIS AMONG MARINE FISHES WITH REFERENCE TO MYCOLOGICAL SIGNIFICANCE OF CAUSATIVE AGENT

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Abstract

A total of 300 fishes examined related to 10 marine fish species, out of which 193 samples proved to harbour *Ichthyophonus hoferi* (64.3%) from Lake El-Temsah and Suez Canal. The percentage of isolation was higher in winter (74%) than in summer (51.6%). The results of clinical investigation revealed that, some naturally infected fishes did not show any clinical abnormalities, while, other suffered from emaciation, exophthalmia, abdominal swelling and erection, easily detached scales. P.M. lesions were manifested as white nodules of *Ichthyophonus hoferi* in liver, kidney, heart, spleen and intestine. *Ichthyophonus hoferi* was isolated in Sabouraud's dextrose agar plate +1% calf serum and in Minimum Essential Medium + 10% fetal calf serum at pH 3, 5, 7 and 9 within 10-15 days. Morbidity and mortality rates, clinical signs and post-mortem changes in experimentally infected fish were discussed in details with reference to mycological significance of the causative agents.

INTRODUCTION

Ichthyophonosis is a chronic, systemic, infectious and granulomatous fungal disease of most organs and tissues of many marine and freshwater fishes causing high mortalities (McVicar, 1982). Ichthyophonosis is primarily a disease of marine origin reported as pathogen of elasmobranch fishes, freshwater crustaceans, amphibians, reptiles and piscivorous birds (Rand *et al.*, 2000). Ichthyophonosis is of worldwide distribution as reported by many authors from USA, Europe, Australia, Switzerland and Japan (Galuppi *et al.*, 1994, Rahimian 1998 and Jones & Dawe 2002). This fungus is an obligate parasite with a wide host spectrum (Spanggaard *et al.*,1994). The pathogenicity of *I. hoferi* varies accorrding to the fish species.

In Mediterranean region, Ichthyophonosis was reported from cultured marine fish in Spain and Greece (Franco-Sierra *et al.*,1997). In Egypt, Ichthyophonosis was recorded for the first time from the wild Nile catfish (*Clarias lazera*) at Alexandria Governorate (Faisal *et al.*, 1985), as well as from both Nile catfish and Tilapia (Manal *et al.*, 1996 and Shaheen & Easa, 1996). Recently, Ichthyophonosis was reported in freshwater fish *O. niloticus* (Manal, 2002). This research was planned to study

Ichthyophonus disease among marine fishes and significance of mycological criteria of the causative agents.

MATERIALS AND METHODS

1-Fish

a-Naturally infected fish

A total of 300 naturally apparently healthy and diseased marine fishes were collected from different localities of Suez Canal and El-Temsah lake to be used for studying the prevalence and seasonal prevalence of ichthyophonosis (Tables 1).

b- Experimental fish

A total of 180 apparently healthy normal Nile catfish (*Clarias gariepinus*) with an average body weight 130±20 g were caught from River Nile at Giza Governorate during summer, and transported alive to Microbiological lab. of Fish Diseases Department, Animal Health Research Institute, Dokki, Giza. Ten full glass aquaria 120 ×60× 40 cm were used for holding the experimentally infected fish (including the maine items of management through O_2, pH and dechlorinated water) throughout the experimental period at 20±2 ºC for 2 weeks for acclimatization. Random samples of 20 fish were sacrificed and examination for determining the incidence of *I. hoferi* during the period of experimental infection (Table 3).

2- Clinical and Post mortem examination

The collected fishes were examined clinically according to the methods described by McVicar (1982).

3- Mycological examination

A-Squash preparation: It was carried out from organs of moribund and apparently healthy fishes according to method described by Faisal (1985).

B-Field diagnosis: Samples were taken from different organs and kept at room temperature for 4-5 hours to detect post-mortem germination of *I. hoferi* described by McVicar (1982).

C- Fungus isolation: Samples taken from the internal organs (liver, kidney, spleen, heart, ovary, testes and eyes) of natural and experimental fishes and embedded in Minimum Essential Medium +10% fetal calf serum (MEM10) at pH 3.0, 5.0, 7.0 and 9.0 were incubated at room temperature for 15 days for isolation of the fungus as described by McVicar (1982).

D-Staining

Staining of the fungus was carried out by Lactophenol Cotton Blue Stain (L.P.C.B.) according to Spanggaard *et al.* (1994).

4- Experimental infection

A total of 180 *Clarias gariepinus* (130±20) g were used. Twenty of them were used to determine the prevalence of natural infection, the remained fish were divided into 8 groups each of 20 fish per aquarium, having the principal items of management through O_2, pH and dechlorinated water receiving the maintenance feeding during the experimental and experimentally infected as shown in Table 3. All fish were observed over eighteen weeks period for clinical abnormalities, mortalities and morbidity. At the end of the experiment, all survivors were sacrificed. Squashed preparations from organs were used for mycological examinations in dead and sacrificed fish.

RESULTS AND DISCUSSION

Prevalence of Ichthyophonosis among the examined marine fishes was recorded in Table 1. The result revealed that 193 out of 300 fish (64.3%) were positive for Ichthyophonosis. This result was higher than that recorded by Sitja-bobadilla & Alvarez Pellitero (1990) who found that 24.4% of examined cultured sea bass was positive, while, Rand *et al.* (2000) found that 0.6 to 10% of yellow-tail flounder were diseased with Ichthyophonosis. Moreover, Marty *et al.* (1998) found that 29% of Pacific herring were infected with Ichthyophonosis, and Jones & Dawe (2002) found that the prevalence of infection in Pacific herring from coastal British Columbia ranged from 10.5 to 52.5% in 2000 and 2001, and lower than that found by Sindermann (1990) who found that 85% of haddock were diseased with Ichthyophonosis.

Clinical examination of naturally diseased marine fishes revealed the presence of emaciation, exophthalmia, abdominal swelling and erection, easily detached scales with fin rot. In addition, slight to severe darking of skin all over the fish body surface was recorded. Some fishes revealed presence of infection without any clinical abnormalities. The present observations agreed with those recorded by Okamoto *et al.* (1985). McVicar (1982) noticed ulcers and emaciation in herring which suffered from Ichthyophonosis. He reported also that clinical signs are not specifically diagnostic of the disease. On the other hand, skin roughness (sand paper-like) or ulceration was the main external lesion reported by Galuppi *et al.* (1994) in rainbow. In the present study, the absence of skin roughness in naturally infected fishes agreed with that reported by Rahimian (1998) and Manal (2002), and this may be due to absence of

red muscle systems in the lateral muscle of many fishes which are highly vascularized and showed rapid fungal multiplication (Rahimian 1998).

Post-mortem examination of naturally diseased marine fishes revealed the presence of paleness and enlargement of the liver with white to grey nodules of variable sizes (Fig. 1) and enlarged gall bladder. The kidneys were hemorrhagic, enlarged and contained minute white to grey spots distributed along the kidneys surface. The spleen was enlarged, congested and contained greyish white nodules. The intestine was corrugated with white spots in its wall, and the intestinal lumen was filled with hemorrhagic viscous materials. Skeletal muscles were soft and flabby. Moreover, the heart was congested and contained nodules of variable sizes (Fig: 2). These resultes were in agreement with those obtained by Faisal *et al.* (1985) and Sindermann (1990). Oslon (1986) revealed that the P.M. lesions of examined Pacific staghorn scolpin were appeared in heart and liver.

The result of microscopic examination of squash preparation taken from different organs of naturally infected fishes revealed the presence of resting spores with different developmental stages of the end spores (uni, bi and multinucleated spores) and hyphae. These cysts had variable sizes scattered between the tissues of the infected organs. The fungal elements may replace the tissues of the infected organs forming granulomatous lesions which were characteristic to Ichthyophonosis. These results are similar to those obtained by Mcvicar (1982), Shaheen & Easa (1996), Manal *et al.* (1996) and Manal (2002). *I. hoferi* occurred in the tissues of infected fish as spherical, thick-walled and multinucleated cell. This cell has been named variously by many authors, multinucleated cyst, spherical multinucleated cyst (Sindermann, 1990), resting spore, large multinucleated bodies and spherical hyphal bodies in MEM (McVicar, 1982).

Mycological examination of the infected fish with *I. hoferi* reaveled the isolation of the fungus on MEM-10 as abundant characteristic hyphal growth into the substrate of the medium within 10-15 days at pH 5.0 (Fig. 3). Regarding the fungal growth on MEM-10 at low pH (3 and 5 pH), it was found that, the characteristic hyphal growth into the substrate is accompanied by moving of the cytoplasmic contents to each of the hyphal tips which subsequently were rounded up to produce a thick spherical multinucleated hyphal terminal body and became separated from the hyphae, then, ruptured and released the endospores. These resultes were similar to those reported by Faisal *et al.* (1985), Shaheen & Easa, (1996) and Manal (2002).

Microscopic examination of the wet preparation at pH 5.0 from *I. hoferi* culture isolated from marine fishes showed development of thin hyphae which divided into many branches (Fig. 4A). After the cytoplasm had moved to each of the hyphal tips,

the cytoplasm rounded up to produce a spherical multinucleated hyphal terminal body and became separated from the hyphae, then, ruptured and released the endospores (Fig. 4B). The development of hyphae at pH 5.0 was similar to that at pH 3.0. Microscopic examination of the wet preparation at pH 7.0 from *I. hoferi* culture isolated from marine fishes showed that the hyphae were developed, branched out once or two lanes and rarely twice (Fig. 5A). The cytoplasm of hyphae migrated to the hyphal tips. Spherical uni-or binucleated bodies were not separated from the hyphal tips and the cytoplasm endogenously cleaved into spherical uni-or binucleated bodies in the upper part of hyphae. Spherical uni-or binucleated bodies formed at pH 7.0 and pH 9.0 were attached together by mucous-like material (Fig. 5B). These results came in agreement with those described by Millero & Sohn (1992), while, Spanggaard *et al.* (1994) and Manal (2002) found that germination and growth of fungus require low pH, and that can only be induced in the stomach. Ichthyophonus can be considered a dimorphic fungi having a mold from at pH 3 and 5 and spheric form at pH 7 as multinucleated spherical thick walled cysts. These findings are similar to those reported by Spanggaard *et al.* (1994).

The seasonal prevalence of *I. hoferi* infection was recorded to be high in winter (74%) followed by autumn (68.75%) and low in summer (51.6%) (Table 2). These results are in agreement with those reported by Sindermann (1990) who recorded that the growth of fungus occurs over a wide range of temperatures (3-20°C) with an optimum at 10°C, and also, he added that the prevalence of infection in herring was found in shore waters, particularly, during late winter and spring. Also, to the same result, came Sitja-bobadilla & Alvarez Pellitero (1990) who reported a seasonal pattern of Ichthyophonus infection among wild and cultured seabass.

The distribution of the organism among different organs of the examined fishes revealed that, the fungus was isolated in a higher prevalence from liver followed by spleen, heart, kidney, intestine, ovary and eyes at a rate of 93.2%, 85.4%, 77.7%, 75%, 64.7%, 20.7% and 10.4%, respectively. Also, the results supported those obtained by Mellerggaard, & Spanggaard (1997) who stated that *I. hoferi* causes systemic infection in fish spread via the circulatory system. The principal infection sites are the organs rich with blood.

The results of examination of fish used for experimental infection are shown in Table 3. The prevalence of natural infection among fish used for experimental infection was (50%). Ten fish out of 20 were positive to *I. hoferi* isolation and 10 were free. All experimentally infected groups with *I. hoferi* showed emaciation, pale colouring, abdominal swelling, cloudy eyes, and exophthalmia and skin ulceration. Occasionally, a dark colour with pigmentation on skin, depressions in the bone of the

head above or under eyes with exophthalmia was obvious. Post-mortem examination of apparently healthy, moribund and dead fish (*Clarias gariepinus*) in the early stage of disease showed slight enlargement of liver with pale colour, congested spleen and hemorrhage with corrugated intestine. At the end of experiment, there appeared white, well defined, macroscopic nodules of variable sizes in liver, kidney and testes. Spleen was congested and enlarged. *I. hoferi* was re-isolated from the internal organs of most experimentally infected fish (Fig. 6). The mortality rate among the experimentally infected fish is to be seen in Table 3. The results were supported by those of Manal (2002) who found that the mortality rate reached 20 and 30% in *O. niloticus* which was experimentally infected with *I. hoferi* by heavily infected minced organs and bath challenged with pure culture of *I. hoferi,* respectively. Also, Manal *et al.* (1996) found that the mortality rate ranged from 90-100% in Tilapia and Nile catfish (infected via forced feeding on infected minced organs, oral inoculation with broth culture and by contact infected fish with uninfected ones). Also, Okamoto *et al.* (1985) inf ected rainbow trout orally by feeding them with thick-walled spherical multinucleated bodies and recorded that the mortality rate was 100%.

Table 1. Prevalence of Ichthyophonosis among the examined marine fishes.

Location	Fish	No. of exam.	No. of infect	% of infect
lake El.Temsah	Sigans Fish (*Siganus spp.*)	24	20	83.3%
	Snapper nei (*Lutjanus spp.*)	40	30	75%
	Jake and Horse Mackerel (*Trachurus spp.*)	24	16	66.6%
Suez Canal	Thin lipped Grey Mullet (*Liza ramada*)	40	19	47.5%
lake El.Temsah	Narrow-Barred Spanish Mackeret (*Scomberomorus commerson*)	24	15	62.5%
	Saddled seabream (*Oblada melanura*	24	17	70.8%
	Marine Tilapia spp.(*Oreochromis spp.*)	40	29	72.5%
	Flat heads fish (*Playcephaloidei spp.*)	40	20	50%
	Tigger fishes (*Balistidae*)	24	15	62.5%
	Brushtooth lizasrd fish (*Saurida undosqumis*)	20	12	60%
	Total	300	193	64.3%

Table 2. Seasonal prevalence of Ichthyophonosis among the examined marine fishes.

Season	No. of examined	No. of infected	of% infection
Winter	100	74	74
Spring	60	33	55
Summer	60	31	51.6
Autumn	80	55	68.75
Total	300	193	64.3

Table 3. Experimental desigen and prevalence of ichthyophonosis among the experimented fishes

Group No.	No. of Fish	No. of Naturally Diseased fish	Mode of infection	Dose	Morbidity %	Mortailty %
1	20	10/20 50%	By feeding on heavily infected minced organs	3% of fish body weight	13/20 65%	6/20 30%
2	20	10/20 50%	By bath with pure culture of *I. hoferi* on MEM-10 at pH 5.0	0.5 ml/L of the aquarium water	14/20 70%	7/20 35%
3	20	10/20 50%	By force-feeding of *I. hoferi* on MEM-10 culture	1 ml/fish for one time	17/20 85%	8/20 40%
4	20	10/20 50%	Intraperitioneal with *I. hoferi* on MEM-10 culture	1 ml/fish for one time	19/20 95%	18/20 90%
5	20	10/20 50%	By feeding on uninfected minced organs	3% of fish body weight	---	---
6	20	10/20 50%	By bath with sterile MEM-10 media at pH 5.0	0.5 ml/L of the aquarium water	---	---
7	20	10/20 50%	By force-feeding of sterile MEM-10 media at pH 5.0	1 ml/fish for one time	---	---
8	20	10/20 50%	Intraperitioneal with sterile on MEM-10 culture	1 ml/fish for one time	---	---

Fig . 1. Brushtooth lizard fish (saurida undosqumis) infected with *I. hoferi* showing greyish white nodules of variable size in liver.

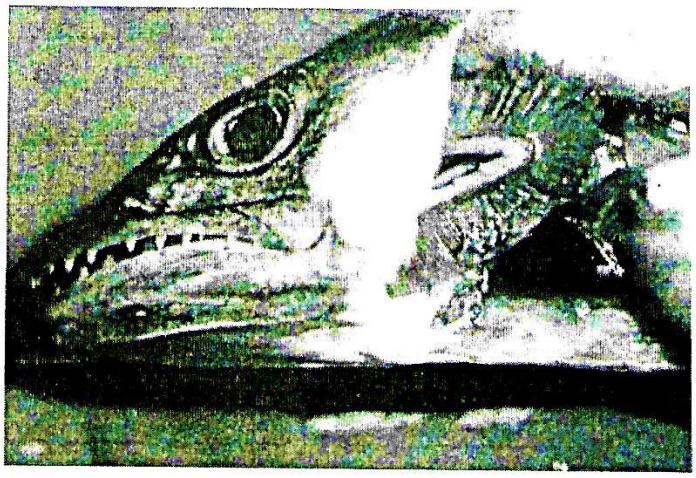

Fig.2. Narrow- Barred Spanish mackerel, (scomberomorus commerson) infected with *I. hoferi* showing congested heart with white nodules.

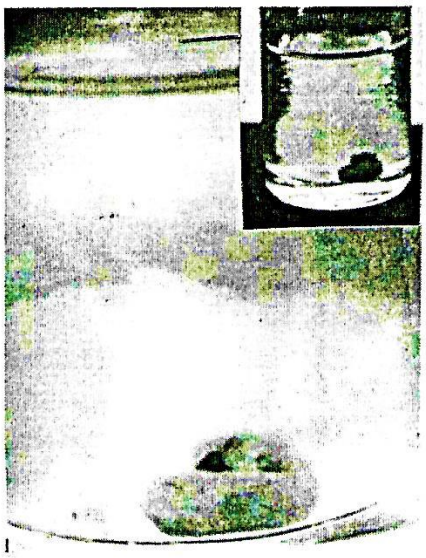

Fig.3. MEM- 10 culture of *I.* hoferi showing hyphal at pH 5 of infected marine fish.

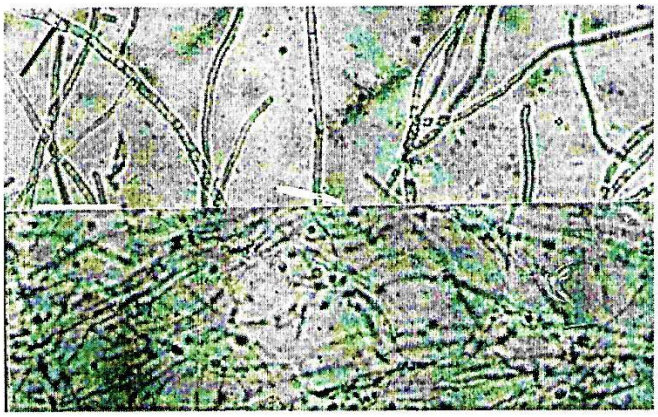

Fig.4. culture of *I.* hoferi on MEM- 10 at pH 5 showing may branches of hyphae with budding end (A) (x 125) , spherical multinucleated hyphal terminal bodies produced in each of the hyphal tips (B) (x 125).

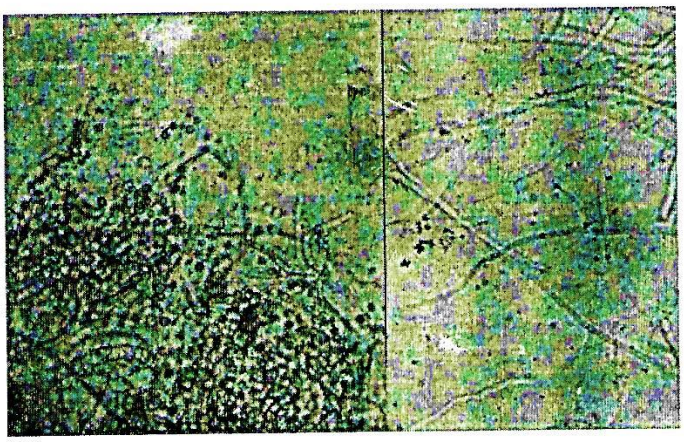

Fig. 5. culture of *I. hoferi* on MEM- 10 at pH 7 from marine fish showing the hyphae developed with one or two lanes (A) (X200), endogenous cytoplasmic cleavage, spherical uni or binucleate bodies in the upper part of hyphae (B) (200) L. C.P.B. stain.

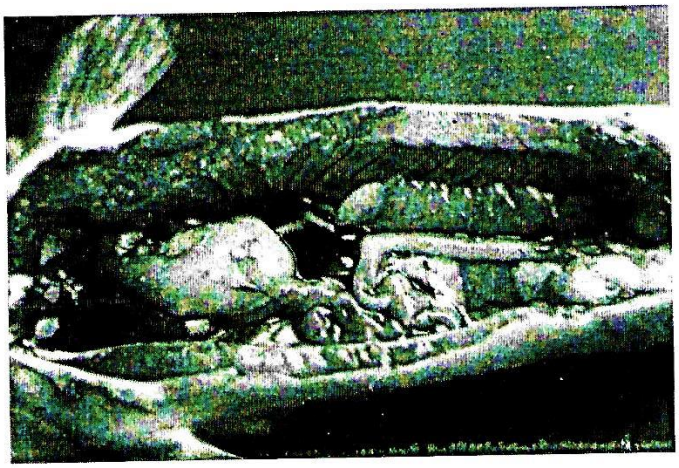

Fig.6. clarias gariepinus experimentally infected with *I. hoferi* showing enlarged, congested spleen and enlarged liver and greyish white nodules varying in sizes in liver, intestine wall, gronads and spleen.

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بعض الدراسات على مرض الأكثيوفونوزس فى أسماك المياه المالحة بالإشارة إلى الخصائص الفطرية للمسبب المرضى

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تبين من فحص ٣٠٠ سمكة من الأسماك البحرية من قناة السويس و بحيرة التمساح تنتمى الى ١٠ أنواع من الأسماك البحرية ان ١٩٣ سمكة كانت موجبة لعزل فطر الاكثيوفونس هوفرى بنسبة ٦٤,٣% وكانت أعلى نسبة إصابة فى فصل الشتاء(٧٤%) وأقل نسبة إصابة فى الصيف(٥١,٦%).

وقد وجد من الفحص الظاهرى أن بعض الأسماك المصابة طبيعيا لم يظهر عليها أيه علامات خارجية بينما البعض الأخر كانت الأعراض فى صورة وجود اللون الاسود فى جلد الاسماك مع جحوظ وعتامة فى العينين ونتشوهات فى العظام والرأس. وأوضحت الصفة التشريحية للاسماك المصابة وجود حويصلات بيضاء مختلفة الحجم فى الكبد والكلى والقلب والطحال والامعاء. تم عزل فطر الاكثيوفونس هوفرى المسبب لهذا المرض على بيئةالسبارود ديكستروز+١% مصل العجل أوعلى بيئة الميم +١٠% مصل جنين العجل عند الأس الهيدروجينى (٣،٥،٧،٩)من الأعضاء المصابة. وكانت نتيجة العدوى الصناعية للاسماك المعرضة للاصابة سواء عن طريق الفم أو الحقن البريتونى أو إذابة مزرعة الفطر المستزرع فى ماء الحوض أو باستخدام أعضاء الأسماك المصابة بنسب تتراوح من ٣٠-٩٠%.