

**STUDIES ON ICHTHYOPHONOSIS IN NILE TILAPIA,
"OREOCHROMIS NILOTICUS"**

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SUMMARY

Ichthyophonosis was recorded from naturally infected cultured and wild *O. niloticus* in a rate of 66% and 30%, respectively. The disease proved to be higher during winter (68.1%). Culture characters of the isolated fungus were studied. With respect to pH, *I. hoferi* culture on MEM of pH 3.5 revealed abundant hyphal growth (mold form), while at pH 7 the culture showed thick walled spherical multinucleated bodies (spherule form). Identification of the isolated fungus was done. Post mortem germination of *I. hoferi* cyst was also noticed. Signs and lesions observed on the natural and experimental fish were recorded. Histopathological examination of tissues sections from liver, spleen and kidneys revealed presence of multiple characteristic granuloma of *I. hoferi* which formed from central resting spores enclosed by fibrous connective tissue capsule with inflammatory cells and melanin carrying cells and gave positive PAS reaction. Degenerative changes in both hepatocytes and renal epithelia with splenic, ~~also~~ congestion were seen. Catarrhal enteritis with numerous PAS positive fungal spores in lamina propria and submucosa of intestine were recorded .

INTRODUCTION

Ichthyophonosis is a systemic granulomatus disease of fish and was firstly recorded in cultured brown trout by **Hofer (1893)**. It has a wide distribution in both fresh and saltwater fishes, and has been reported from close to 100 species (**Rand, 1990**). The causative agent was described as a fungus but new investigations have shown that *I. hoferi* is not a classic fungus but it belongs to the protistan parasites clade (DRIPs) (**Spanggaard**

et al., 1995; Ragan et al., 1996 and Fredricks et al., 2000). The pathogenicity of *I. hoferi* varies between fish species (Spanggaard et al., 1994) and the lesions induced are commonly observed in blood-rich organs such as, heart, red musculature and viscera (Mellerggaard and Spanggaard, 1997). Many researchers have studied the gross and internal manifestation of *I. hoferi* infection in fish (Egusa, 1992; Armstrong et al., 1993; Galuppi et al., 1994; Spanggaard et al., 1995; Noga, 1996; Sierra et al., 1997 and Rahimian, 1998). Several studies have been dealt with the culture characters and the morphology of the fungus (McVicar, 1982; Okamoto et al., 1985; Spanggaard et al., 1994; Spanggaard et al., 1995 and Rahimian, 1998). Tissue responses to experimental infection with the parasite like fungus in rainbow trout were described by Okamoto et al., (1985) and Okamoto et al., (1987). Moreover, histopathological lesions in naturally infected fish were observed in the examined seabream (Athanasopoulou, 1992), in northern pike (Armstrong et al., 1993) and herring (Sindermann and Chenoweth, 1993 and Rahimian, 1998).

In Egypt, the disease was recorded in wild *Clarias lazera* (Faisal et al., 1985), in ornamental fish species (Nahla et al., 1997) and in Nile tilapia (Manal et al., 1996; Shaheen and Easa, 1996 and Ziedan, 1999). The economic importance of the disease is not only due to high losses of fish stocks but also due to the extensive postmortem germination which occur in fish fillets during processing industry even, after chilling, storage or smoking making the product soft, slimy and with strong off odour (McVicar, 1982 and Athanasopoulou, 1992). The present work was planned to study the prevalence of Ichthyophoniasis, isolation and identification of *I. hoferi* with histopathological lesions in naturally and experimentally infected *O. niloticus*.

MATERIALS AND METHODS

Naturally infected fish:

A total number of 500 *O. niloticus* (400 cultured and 100 wild) were collected from their natural sources at Kaluobia and Sharkia Governorates.

Private fish farms, El-Riah El-Tawfiki, Bahr-moiess and their trabeculae during the period from June 2000 to May 2001. The body weight of the obtained fish ranged from 30-100g. The fish were transported alive or freshly dead under all accurate methods of transportation and sent to the Wet lab, Faculty of Veterinary Medicine, Moshtohor. They were examined grossly as described by Amlacker (1970) and McVicar (1982).

Clinical examination

Mycological examination:

Under complete aseptic precautions, samples from liver, kidnes, spleen, heart, intestines, ovaries and eyes were taken by using sterile dissecting needle. The obtained samples were inoculated onto minimum essential medium contained 10% fetal calf serum (MEM-10) at pH 7 and 3.5 and on sabouraud's dextrose agar supplemented with 1% bovine serum. The cultured media were incubated at room temperature for 15 days as described by McVicar (1982). The isolated *I. hoferi* strains were identified according to Aguis (1978) and McVicar (1982).

Experimental infection:

Aquaria

Twelve aquaria were used, each measured 1 x 0.5 x 0.5m. They were supplied with dechlorinated tap water and sufficient aeration using electric air pumping compressors. The water temperature was adjusted at $16 \pm 1^\circ\text{C}$.

Experimental fish:

A total number of 125 apparently healthy *O. niloticus* fish, of an average body weight $45 \pm 5\text{g}$ were taken from a private fish hatchery and brought alive in big tanks to the Wetlab. Random samples of 5 fish were sacrificed and examined mycologically to ensure their freedom of *I. hoferi*. The remaining fish were divided into 12 groups each of 10 fish. The fish were fed on pelleted commercial fish ration in a rate of 3% of their body weight according to Eurell et al., (1978).

Preparation of inoculum:

- 1- Seven day MEM-10 pure culture of *I. hoferi* at pH 7 and 3.5 were used according to Spanggaard et al., (1995).
- 2- Heavily infected minced organs.

A synthetic corticosteroid, triamcinolone acetonide suspension (Kenacort-A)^R (Bristol-Myers Squibb Egypt Company) used as immunosuppressants as described by Anderson et al., (1982).

Experimental design:

The design was summarized and presented in Table (1).

The fish in the groups 2, 4, 6, 8, 10 and 12 were injected with cortizon at a dose of 20 mg/kg body weight of fish. Both experimentally infected and control groups were put under observation for 2 months. Signs and lesions that developed in experimentally infected fish were observed and mortality rates were also recorded.

Histopathological examination:

Samples for histopathological studies were taken from liver, spleen, kidneys and intestines of naturally and experimentally infected fish were fixed in 10% neutral buffered formalin. Paraffin sections of 3-5 micron in thickness were prepared and stained with hematoxylin and eosin stain (H & E) as described by Roberts (1978). Periodic acid schief staining technique (PAS) for demonstration of mycotic spores within the tissues were performed according to Emmons et al., (1977).

Table (1): Group number, number of fish, type of infective materials, dose and route of infection.

Group No.	No. of Fish	Infective materials	Dose	Route of infection
1	10	Heavily infected	3% of fish body	Oral
2 (*)	10	minced organs	weight	
3	10	Pure culture of <i>L. hoferi</i> on MEM-10 at pH 7.0	0.5 ml/L of aquarium water	Bath challenge
4 (*)	10			
5	10	Pure culture of <i>L. hoferi</i> on MEM-10 at pH 3.5	0.5 ml/L of aquarium water	Bath challenge
6 (*)	10			
Control groups				
7	10	Sterile minced	3% of fish body	Oral
8 (*)	10	organs	weight	
9	10	Sterile MEM-10	0.5 ml/L of	Bath challenge
10 (*)	10	media at pH 7.0	aquarium water	
11	10	Sterile MEM-10	0.5 ml/L of	Bath challenge
12 (*)	10	media at pH 3.5	aquarium water	

(*) Fish of these groups injected with cortizon at dose of 20 mg / kg body weight.

RESULTS AND DISCUSSION

The present study revealed that 294 out of 500 examined *O. niloticus* were positive to *Ichthyophonus hoferi* isolation (58.8%). The infected fish showing signs of excessive mucus on skin, darkness of skin, (Fig. 1a), emaciation, abdominal swelling, exophthalmia (Fig. 1a), erected easily detached scales and fin rot. While, postmortem examination revealed paleness and enlarged liver, spleen and kidneys with white to gray nodules of variable size (Fig. 1a) in many cases; enlarged gall bladder. Meanwhile, the intestine, was corrugated with white to gray nodules of variable size (Fig. 1a) and ascetic fluid in the abdominal cavities. In some cases, soft and

flabby muscles with congested heart containing nodules of variable size. These findings are agreed with that recorded by **Shaheen and Easa (1996)**, **Manal et al., (1996)** and **Ziedan (1999)** and partially agreed with that recorded by **Schaperclaus (1992)**.

Squash preparations from nodular lesions showed different developmental stages of the endospores (uni, bi and multinucleated spores) scattered between the tissues and characteristic granuloma of ichthyophonosis (Fig, 1b). These results were similar to that obtained by **McVicar (1982)**, **Shaheen and Easa (1996)**, **Manal et al., (1996)** and **McVicar (1999)**. Wet tissue smears (rapid field test) from the infected sacrificed fish showed visible nodules in their organs were taken and kept at room temperature. The examined smears revealed early germination of spores, budding of the cyst, (Fig, 1c) after 4-5 hours, the hyphae were observed in the tissues and in some cases the fungal elements replaced the tissues which appeared as disorganized mass. These observations came in accordance with that reported by **Aguis (1978)**, **McVicar (1982)** and **Lauckner (1984)**. Such results may be attributed to the rise in carbon dioxide levels as the host dies that stimulate *Ichthyophonus* spores to germinate (**Spanggaard et al., 1995** and **Spanggaard and Huss, 1996**).

In the present work, macroscopical examination of the inoculated plates of sabouraud's dextrose agar with 1% bovine serum white revealed hyphal growth of different levels on and into media and grow to fullfill the plate within 10-14 days post inoculation (PI) (Fig, 1d). Wet preparations from the culture showed branched non septated macro and micro hyphae. The hyphal cytoplasm migrating to the apex, leading to formation of pseudo hyphae. These results were closely similar to those previously described by **McVicar (1982)** and **Shaheen and Easa (1996)**. On MEM-10 the inoculated tubes showed abundant hyphal growths in the media either at pH 3.5 (Fig, 1e) or 7.0 (Fig, 1f). Keel formation which considered as a characteristic feature of *I. hoferi* occasionally appeared in MEM-10 at pH 3.5 one day PI (Fig, 1g). Wet preparations from culture at pH 3.5 showed beginning of hyphal growths and second branching of the hyphae formed 5

hrs PI (Fig. 2a). Extensive hyphal growths with many branches were noticed 12 hrs PI (Fig. 2b). Hyphae may extended to grow and increase in length and number 24 hrs PI (Fig. 2c). The characteristic evacuated non septated hyphal branches with their cytoplasm migrating to the apex appeared 3 days PI (Fig. 2d), the hyphal tips started to round up 7 days PI (Fig. 2e) and finally almost all hyphal tips had rounded up to form spherical hyphal terminal bodies 10 days PI (Fig. 2f). In contrast, the hyphae of the fungus at pH 7.0 appeared with different sizes and began to form multinucleated bodies 5 hrs PI (Fig. 3a), many multinucleated bodies were formed 12 hrs PI (Fig. 3b) and they start to separate from the hyphae 24 hrs PI (Fig. 3c), complete separation of these bodies occurred 3 days PI (Fig. 3d), releasing of multinucleated bodies of variable size with large number of nuclei were noticed 7-10 days PI (Fig. 3e) and rupture of the thick walled spherical multinucleated bodies with releasing of their endospores (Fig. 3f). The alternated pH showed great effect on

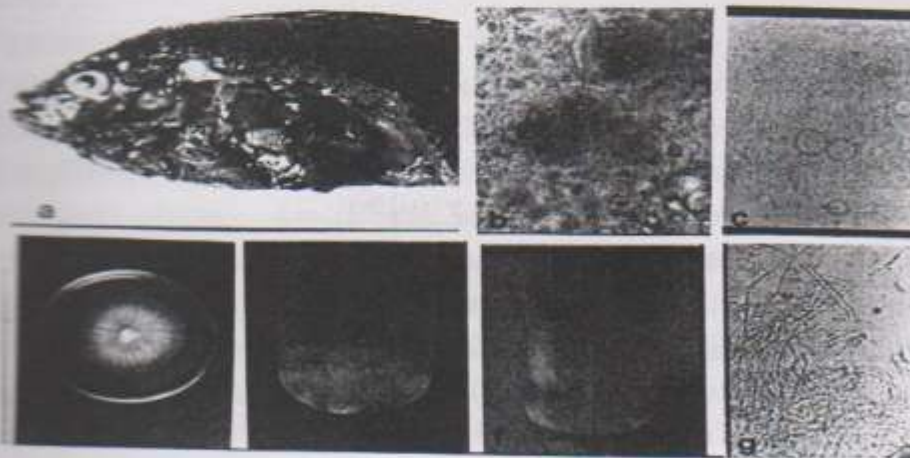


Fig. (1): Showing naturally infected fish with dark skin, enlarged liver and presence of grayish white nodules in the internal organs (a); thickwalled multinucleated bodies in liver (wet preparation) (b); postmortem germination showing budding of cyst (wet smear from intestinal serosa) (c); *I. hoferi* culture on sabourand, dextrose agar (d); on MEM-10 at pH 3.5 (e); on MEM-10 at pH 7.5 (f) and keel formation (g).

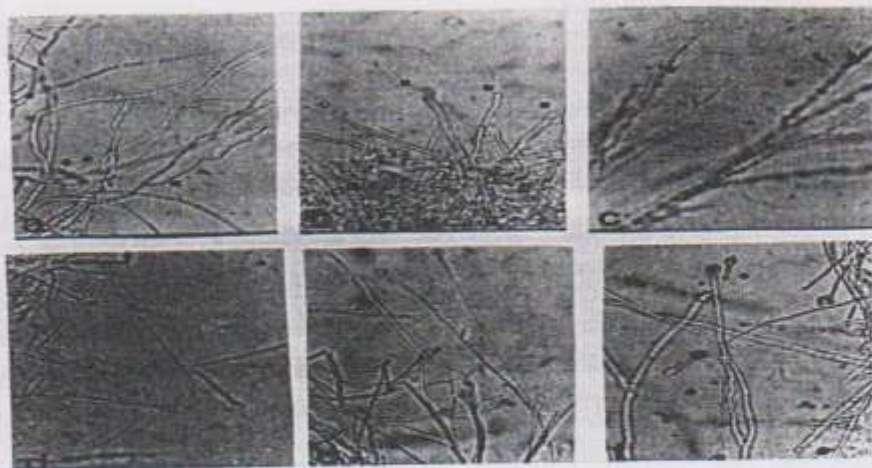


Fig. (2): Culture of *I. hoferi* on MEM-10 at pH 3.5 showing: hyphal growth 5 hrs PI (a), many branches formed 12 hrs PI (b), elongated hyphae 24 hrs PI (c), migration of their cytoplasm to the apex 3 days PI (d), rounding up of their tips 7 days PI (e) and formation of spherical hyphal terminal bodies 10 days PI (f).

both growth and germination of *I. hoferi*. Transferring the culture from pH 7.0 to pH 3.5, revealed abundant hyphal growth. But transferring the culture from pH 3.5 to pH 7.0 showed scanty hyphal growth. Meanwhile, subculturing from pH 7.0 to pH 7.0 caused cessation of the growth of the fungus. These findings are closely similar to that described by **Millero and Sohn (1992)** and **Spanggaard et al., (1994)** and partially in agreement with those of **Manal et al., (1996)** and **Ziedan (1999)**.

The present results indicated that the total prevalence of *I. hoferi* infection in *O. niloticus* was 58.8% (Table, 2). These findings were higher than that recorded by **Rand (1991)** in yellow-tail flounder, **Shaheen and Easa (1996)** in Tilapia, **Hodneland et al., (1997)** in Norwegian herring and **Marty et al., (1998)** in Pacific herring and lower than that observed by **Nahla et al., (1997)** in ornamental fishes (Veil tail) and **Ziedan (1999)** in male Tilapia. Such variation may be due to the difference in the fish species. Moreover, the infection rate in cultured fish was higher (66%) than that in

the wild fish (30%). These results were agreed with that recorded by Shaheen and Easa (1996). In this respect, Sitja-Bobadilla and Alvarez Pellitro (1990) found a higher infection rate in cultured seabass (24.4%) than the wild one (14%), and Athanassopoulou (1992) who observed a higher prevalence of infection in cultured *Sparus aurata* (19.8%) than in the wild one (9.7%).

Regarding to the season, the maximum prevalence among the examined fish was recorded during winter (68.1%), followed by autumn (63.33%), spring (51.25% and summer (37.78%) (Table, 3). In Sharkia Governorate, the disease was reported in a higher incidence during autumn (72%) in cultured fish and during winter (33.3%) in wild fish. In Kalubia Governorate, the highest prevalence was observed during winter in both cultured and wild fish. The fungus was isolated for a higher incidence from liver followed by kidneys, spleen, intestines, ovarus, heart and eyes in a rate of 100% followed by 88.09%, 78.23%, 32.31%, 12.24%, 11.22% and 4.08%, respectively. These results nearly agreed with that reported by Ziedan (1999) who found that the prevalence of infection in the organs of male *O. niloticus* was higher in liver(81%), followed by kidneys(79%), spleen (54%), intestines(60%, and eyes(47%).

Regarding the of experimental infection^{it} was revealed that all infected groups showed signs of dark skin (Fig. 4), emaciation, abdominal swelling, exophthalmia and turbidity of eyes. Internally, dead and sacrificed fish showed severely enlarged liver which appeared pale and had white nodules of variable size. Gall bladder was distended. Spleen and kidneys were congested, enlarged and contained white nodules. The intestines were congested and had minute white nodules. These observations were nearly similar with those recorded by Manal et.al ., (1996).

On the other hand, skin roughness (sand paper-like) or ulceration which are considered the main external lesions were not observed in the present study, these findings may be attributed to absence of red muscle systems in the lateral muscle of *O. niloticus*, as the red muscles are highly vascularized and showed rapid fungal multiplication (Rahimian, 1998).

The mortality rate among the experimentally infected fish groups treated with *Cod liver oil* ranged from 80-100% while in non treated group reached 20-30%.

Histopathological examinations of internal organs of both naturally and experimentally infected fish revealed nearly similar lesion. Liver of diseased fish showed characteristic granulomas which formed central resting spores enclosed by fibrous connective tissue capsule with mononuclear inflammatory cells and melanin carrying cells. These spores gave positive reaction with PAS stain (Fig. 4.1). Moreover, hyperplasia of bile ductal epithelium with lymphocellular infiltration of the portal area were also seen (Fig. 4.2). Spleen sections revealed multiple characteristic granulomas of *L. hoferi* with different sizes (Fig. 4.3) and their spores gave positive PAS reaction (Fig. 4.4). Moreover, severe congestion and lymphoid depletion in spleen were also noticed. Kidneys showed characteristic granulomata scattered throughout the renal interstitial tissues (Fig. 5.1), congestion of renal blood vessels, multiple area of hemorrhages (Fig. 5.2) and degenerative changes of the lining epithelia of the renal tubules. While, the intestinal sections revealed catarrhal enteritis evidenced by hyperplasia of the mucosal epithelium, numerous strong PAS positive fungal spores (Fig. 5.3) and mononuclear inflammatory cellular infiltration in the lamina propria and submucosa were observed. Moreover, eosinophilic granular cellular infiltration of the muscular layer of the intestine was also detected (Fig. 5.4). These observations were partially in agreement with those reported by Manal et al., (1996); Shaheen and Easa (1996) and Ziedan (1999) in Tilapia fish and nearly similar with those reported by Faisal et al., (1985); Mclay (1987); Egusa (1992); Sindermann and Chenoweth (1993); Nahla et al., (1997) and Rahimian (1998) in other fishes.

Moreover, PAS positive spores were detected in the intestinal mucosa, submucosa and lamina propria. These spores within mucosa appeared rounded and some of them had short hyphae representing the beginning of germination. While, within submucosa the spores were spherical in shape. These changes could be explained the pathogenesis of *I. hoferi*, where the hyphae of germinating spores were responsible for invasion of the intestinal mucosa. Such opinion was supported by Spanggaard et al., (1995). In contrast Post (1987) and Egusa (1992) mentioned that uni_ and binucleated spores are responsible for invasion and infection.

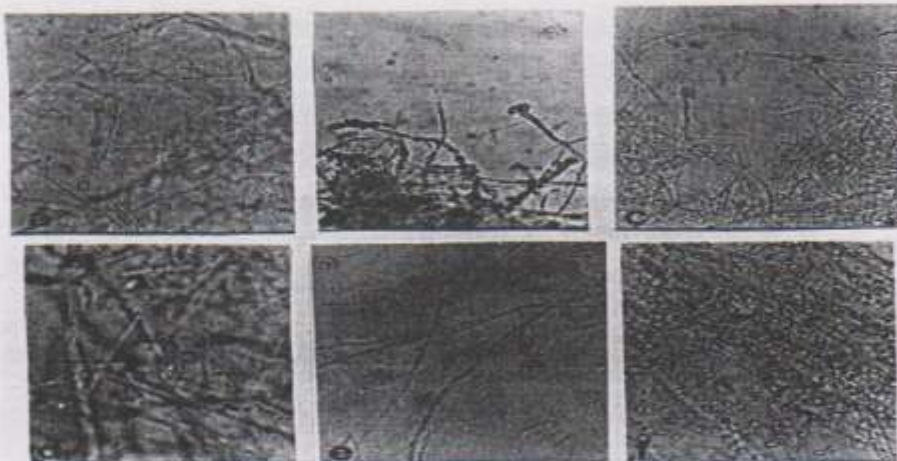


Fig. (3): Culture of *I. hoferi* on MEM-10 at pH 7 showing hyphae and beginning of multinucleated bodies formation 5 hrs PI (a); well developed thick wall multinucleated bodies 12 hrs PI (b); start of separation of these bodies 24 hrs PI (c); complete separation of these bodies 3 days PI (d) release of multinucleated bodies in media 7-10 days PI (e) and rupture of these bodies and release of endospores 14 days PI (f).

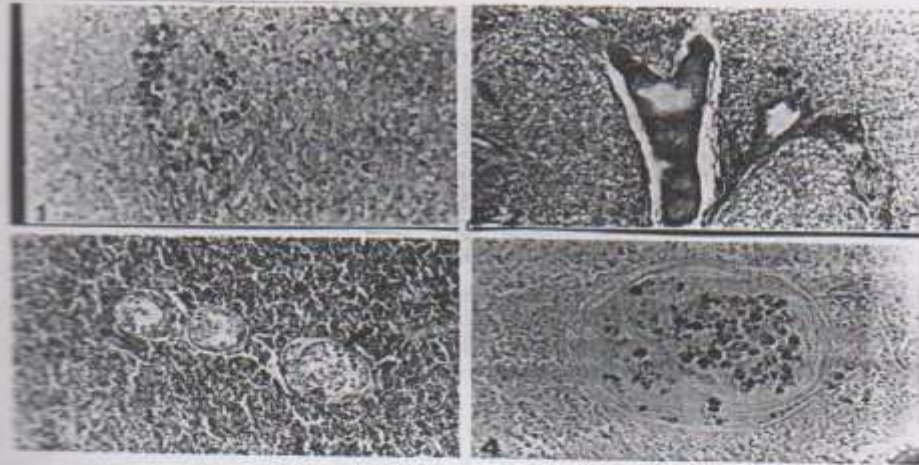


Fig. (4): Showing histological section in liver notes, PAS positive spores within the mycotic granuloma (1); PAS stain and hyperplasia of bile duct epithelium with lymphocytic cellular infiltration of the portal area (2) H & E stain; spleen section showing multiple granulomas surrounded by melanin carrying cells (3) H & E stain and granuloma with PAS positive spore (4) PAS stain.

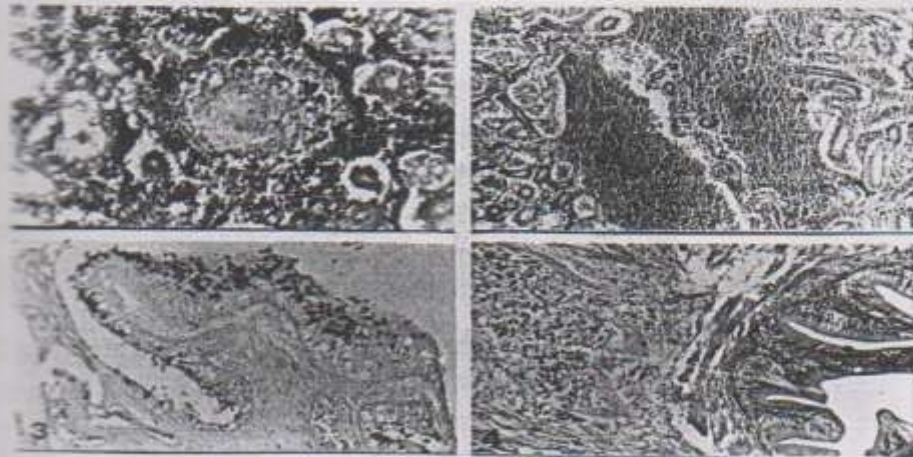


Fig. (5): Showing histological section of kidney notes characteristic granuloma of *I. hoferi* surrounded by inflammatory cells (1) H & E stain and focal area of hemorrhage replaced the renal tissues (2) H & E stain; intestinal section showing PAS positive spores inside lamina propria (3) H & E stain and eosinophilic granular cellular infiltration of muscular layer (4) H & E stain.

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دراسات على مرض الأكتيوفونيموز في أسماك البلطى النيلية
لمتى عباس ، عادل شاهين ، أحلام عبد اللطيف* ، منال حقنى*
قسم أمراض ورعاية الأسماك - كلية الطب البيطرى - جامعة الزقازيق - فرع بنها * معهد بحوث
صحة الحيوان بالدقى

أجريت هذه الدراسة على مرض الأكتيوفونيموز وتم عزل الفطر المسبب للمرض (الأكتيوفونيموز هوقرى) من أسماك البلطى النيلية المستزرعة والتي تعيش طبيعياً فى المياه بنسبة 66% ، 30% على التوالي. أثبتت الدراسة أن نسبة الإصابة كانت أعلى فى فصل الشتاء (68.1%) وقد درست الصفات البيئية للفطر ووجد أنها تختلف باختلاف الأس الهيدروجينى (3.5 ، 7) حيث أنه عند الاستراخ على بيئة ميم عند (PH) 3.5 وجد ظهور نمو كثيف من الفطر على البيئة بينما عند 7 فقط ظهرت على البيئة حويصلات جرثومية مستديرة ذات غشاء خلوى سميك. وقد تم التعرف على الفطر المعزول أيضاً من خلال الإنبات الذى يحدث للحويصلة بعد التحلل الرمى للسمكة.

وقد تم وصف العلامات الظاهرية والصفة التشريحية للمرض. ولقد أظهر الفحص الميكروسكوبى للأشجة المصابة وجود حويصلات جرثومية عديدة مختلفة الأحجام محاطة بنسيج ليفى مع خلايا التهابية صبغية فى الكبد والطحال والكلى ، وقد أظهرت هذه الحويصلات إيجابية شديدة مع التبيور صبغة PAS.

كما أظهر الفحص وجود تغيرات فى خلايا الكبد والكلى مع احتقان فى الطحال وكذلك التهابات فى الأمعاء مع وجود العديد من الجرثيم أحادية وثنائية الخلايا الموجبة لصبغة PAS فى طبقات الأمعاء.