

Treatment of Monogenic Parasites in Imported Hybrid Mono Sex Red Tilapia Fries (*Oreochromis SPP*) in the Sultanate of Oman

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معالجة الإصابة بالطفيليات وحيدة العائل في الأسماك المستزرعة من نوع البلطي الأحمر وحيد الجنس بسلاطنة عمان

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الخلاصة: في فبراير/شباط، ٢٠٠٨، تم استيراد ١٢٠٠٠ زريعة أسماك من نوع البلطي الأحمر وحيد الجنس بمتوسط وزن ٠,٢ جرام / سمكة كما تم استيراد ١٢٠٠٠ زريعة من نوع البلطي النيل وحيد الجنس بمتوسط وزن ٠,٥ جرام / سمكة. وقد تم استيراد الأسماك من مفرخ في جنوب شرق آسيا. ووضعت في حوض خرساني مستطيل الشكل داخل مزرعة بمنطقة بركاء والتي تبعد حوالي ٨٠ كيلومتر من محافظة مسقط. بعد ثلاثة أيام من وضع الأسماك بالحوض لوحظ نفوق الأسماك بمعدل متزايد في الزريعة من نوع البلطي الأحمر وحيد الجنس داخل أحواض التربية. ولقد أوضح الفحص البدئي الظاهري للأسماك عن وجود حركة لامعة شاذة في الأسماك أثناء العوم وكذلك ميل الأسماك للاحتكاك بجدران حوض التربية. كما لوحظ وجود لون أبيض رمادي خارجي على الجلد والزعانف بالمناطق النزفية البؤرية. الفحص الجهري لعينة من الخياشيم والجلد حدد وجود أعداد كبيرة من الديدان الطفيلية وحيدة العائل تهاجم هذه المناطق من الجسم. وعند الفحص النسيجي كان رد فعل الأنسجة المصابة ضد الطفيليات واضحاً جداً على هيئة التهاب حاد في المناطق المصابة من الجلد بمنطقة الأدمة. وجمع للسوائل في النسيج الرقائق للخياشيم مع حدوث تضخم كمي من النسيج الخيشومي. في هذه الدراسة تم وضع خطة معالجة سريعة قصيرة الأمد أدت إلى تقليل عدد الأسماك النافقة في المزرعة. ولقد استخدم في نظام المعالجة مواد الميندازول والملح والفورمالين لمدة ثلاثة أيام متعاقبة وكرر العلاج ثلاث مرات أسبوعياً. وتم عمل إجراءات متوازنة لتحسين نوعية الماء أثناء المعالجة. بعد المعالجة لوحظ نقص أعداد الأسماك النافقة خلال ثلاثة أيام وتوقف النفوق تماماً بعد أسبوع واحد من النظام العلاجي المستخدم. وعند فحص عينات الأسماك بعد العملية العلاجية لوحظ اختفاء الديدان الطفيلية تماماً من على جسم وخياشيم الأسماك. وأوضحت الدراسة عدم إصابة الأسماك من نوع البلطي النيل بأية إصابات طفيلية على الرغم من تربيتها داخل نفس المزرعة و تحت نفس ظروف التربية.

ABSTRACT: In February, 2008, 12000 fries of monosex red tilapia hybrid (*Oreochromis SPP*) of 0.2 g and 12000 fries of monosex Nile tilapia (*Oreochromis niloticus*) of 0.5 g were imported from a hatchery in South East Asia and stocked in concrete raceways at an agriculture farm in the Barka Region about 80 km from Muscat. Three days after stocking, mortality problems were observed. The preliminary inspection revealed abnormal flashing movements of the fish with body scratching against the walls. Faint grayish white discoloration was observed externally on the skin and fins with focal hemorrhagic areas. Examination of skin and gill biopsies demonstrated the presence of a large number of small-sized monogenea parasitic worms attacking the tissue of these body regions. Histopathological observations revealed tissue reactions against the parasites and demonstrated a severe dermatitis of the skin, lamellar oedema and hyperplasia of the branchial tissue. A concomitant treatment regime using mebendazole, salt and formalin was practiced for three successive days and repeated three times every week together with parallel management procedures to enhance water quality during treatment. After treatment, a reduction of mortality was noticed within three days and completely ceased after one week at which time biopsy examinations revealed the absence of any parasitic agents. There were no deaths reported among the Nile tilapia, which were raised on the same farm.

Keywords: Red tilapia, monogenic parasites, treatment, histopathology, Oman.

Introduction

As the world population has increased parallel to the increase in demand for fish, and with stable production from the sea, aquaculture has become one of the main sources for aquatic organisms. Aquaculture continues to grow more rapidly than

other animal-production sectors. Recent data showed that it has grown by 8.8 % since 1970 compared to 1.2 % for capture fisheries and 2.8 % for the terrestrial-farmed meat-production system over the same period (FAO, 2007).

The total aquaculture production in 2006 was 51.7 million tons (without aquatic plants) with a value of 78.8 billion US\$ (FAO, 2008). The share of aquaculture of the total aquatic

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production increased from 3.9 % by weight in 1970 to 36 % in 2006.

Tilapia production increased from 830,000 tons in 1990 to 1.6 million tons in 2005. Tilapia is among the top ten produced species in 2004 with productions of 1.7 million tons. The value of this production was 1.8 billion US\$. China is the main tilapia producing country with a production of 1 million tons. Egypt has a production of 200,000 tons. Currently, Asian countries represent about 63 % of the total tilapia production. The main tilapia species produced is Nile tilapia (*Oreochromis niloticus*). It is expected that the supply of tilapia will continue to increase in the future.

Currently, many new tilapia species (hybrids) have been introduced to the field of aquaculture. Tilapia hybrids have been geared towards the improvement of some characteristics, such as: color, salinity resistance and weight gain (Romana and Eguia, 1999). Thus, red tilapia is not a species of tilapia, instead it is a name used for several different manmade tilapia variants that sport attractive red coloration. These variants are the result of continuous selective breeding. Many farmers prefer to cultivate red tilapia since it is very sought after in certain markets.

A few examples of red tilapia variants commonly encountered in fish markets and grocery stores are Florida Red, Jamaican Red, Taiwan Red and ND56. Unlike wild tilapia species – which tend to be black or grayish – these fish have showy red and pink colors. In this concern, Lutz (2001) mentioned that, Florida Red tilapia is the result of Blue tilapia (*Oreochromis aureus*) mating with

Mozambique Tilapia (*Oreochromis mossambicus*). On the other hand, hybridization could increase fish susceptibility to some diseases such as monogenea infestation (Robinson *et al.*, 2008).

The freshwater culture of Nile tilapia (*Oreochromis niloticus*) is practiced in some private farms in Oman, particular in the northern part of the country. This type of aquaculture is carried out on small farms where high levels of saline groundwater prevent or reduce normal agriculture activities. The first introduction of tilapia species to Oman was originally done by the Ministry of Health to control mosquitoes in the natural water bodies (FAO, 2008). As a result of the adaptability of this species to the environment, their numbers increased and the local people began to rear tilapia on their farms. There are very few agricultural farms, located in the north of Oman that produces Nile tilapia on a small commercial scale. Irrigation water is used in the production process. The Fishermen Training Institute (Ministry of Manpower) started an aquaculture training program in one of the tilapia farms in Barka, where the fry of tilapia was imported from a hatchery in South East Asia.

One of the most significant problems that hinders fish farming is abnormal mortality increases especially with recently introduced fries.

In this study, the aim was to investigate the cause of early mortality in the imported tilapia spp., and perform treatment regimes to minimize and control mortality rate with the object of coming up with recommendations for local authorities to take the necessary measures against importing diseases into the country.

Table 1. Demonstrate mortality problem of red tilapia fingerlings before and after treatment given at day 4 in the farm during first 11 days of stocking.

Number of Raceway and Fish Species	Total No. of Fish/ Raceway	Mortality	Date Mortality Rate After Stocking										
			Day										
			1	2	3	4	5	6	7	8	10	11	
(1) Monosex Nile tilapia	5000	Number of dead fish	-	6	-	5	7	10	5	6	3	6	
		%	0	0.1	0	0.1	0.1	0.2	0.1	0.1	0.0	0.1	
(2) Monosex Red tilapia	5000	Number of dead fish	-	30	-	*198	600	260	162	**6	10	10	
		%	0	0.6	0	3.9	12	5.2	3.2	0.1	0.2	0.2	
(3) Monosex Red tilapia	5000	Number of dead fish	-	18	-	*37	185	177	12	**5	1	3	
		%	0	0.3	0	0.7	3.7	3.5	0.2	0.1	0.0	0.0	
(4) Nile tilapia	5000	Number of dead fish	-	3	-	3	3	5	3	-	2	3	
		%	0	0.0	0	0.0	0.0	0.0	0.0	0	0.0	0.0	

*Increased mortality and treatment application.

**Decreased mortality after treatment.

Materials and Methods

Fish Source and Stocking

In February, 2008, 12000 fries of monosex red tilapia hybrid (*Oreochromis SPP*) of average weight 0.2 g and 12000 fries of monosex Nile tilapia (*Oreochromis niloticus*) of 0.5 g were imported from a hatchery in South East Asia and stocked in concrete raceways (4×6×1 m) on a farm in the Barka Region, about 80 km from the Muscat.

The imported fish were acclimatized well prior to being released to raceways in FRB tanks, which are facilitated with air and good water exchange. In raceways, fries were stocked at a density of 500 fry per cubic meter, the fish were fed 32 % crude protein commercial feed imported from Saudi Arabia at 7 % BW/D.

Mortality and Clinical Signs

Three days after stocking, deaths were observed (Table 1). On primary inspection, abnormal flashing movements were observed and the fish scratched their bodies against the walls. Faint grayish white discoloration was observed externally on the skin and fins with focal hemorrhagic areas. The clinical signs were suspected to be the manifestation of external parasitic infestations, so further confirmatory procedures were performed as follows:

Sampling: Fifty fish samples in a moribund state were collected randomly from all the raceways of the farm and transported in aerated plastic bags to the Fishermen Training Institute, El Khaboura for further examination.

Examination: The euthanized fish samples (by cervical dislocation) were subjected to the following:

Skin and gill biopsies. Careful external examination and biopsies were performed. Skin and gill scrapings were performed by gently scraping a scalpel along the side of the body, fins and gills while the fish was restrained. The scrapings were spread onto glass slides, and evaluated carefully using an ordinary light microscope.

The procedures were performed according to Noga (1996).

Histopathological examination. Tissue specimens from skin and gills were fixed in 10% neutral buffered formalin. The samples were transported in tightly closed sampling bottles for routine histopathological examinations.

The fixed samples were washed in tap water overnight and exposed to ascending concentrations of ethanol (70,80,90 and 100%), cleared in xylene and embedded in paraffin. Tissue slides of 5 µm thick sections were prepared and stained by hematoxylin and eosin (H&E). The histopathological preparation was performed according to Bancroft *et al.* (1996).

Histopathological examination was performed in the Pathology Department, Faculty of Veterinary Medicine, Cairo University, Egypt. Samples were examined and photographed using an OLYMPUS BX 50 microscope.

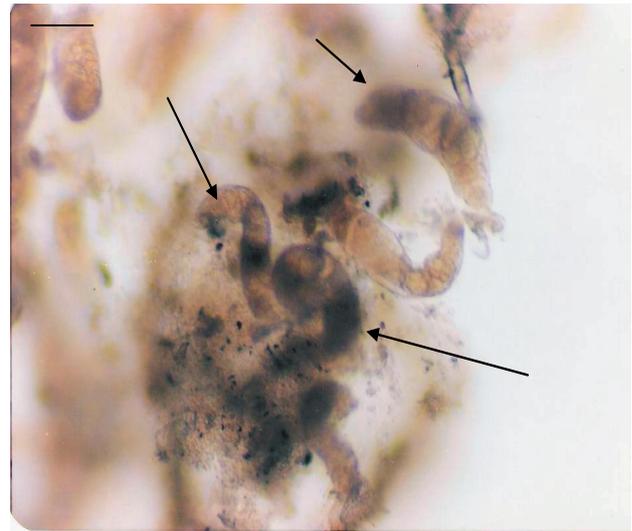


Figure 1. Wet mount preparation from gills of red tilapia fish showed multiple parasites (monogenea Sp.) attached to branchial tissue (arrows) bar, 20 µm.

Treatment Procedures

Chemicals used for treatment: Concomitant mass treatment of the fish in raceways was applied using the following medications:

Anthelmintic. Mebendazole - a bath of mebendazole was added at a dose of 100 mg mebendazole/liter for 10 minutes (Szekely and Molnar, 1987).

Salt. Pure sodium chloride was used in the form of a bath (10 ppt), and the fish were treated for 30 minutes (Scott, 1993).

Formalin. Prolonged immersion in aquaria was performed by the addition of 15 ppm formalin/l (Hoffman and Meyer, 1974). The treatment was applied for three successive days.

Prior to the application of the used chemicals, water levels in the all raceways were reduced to 30 cm to avoid losses of chemicals. Application of the anthelmintic drug (mebendazole) was firstly added for 10 minutes followed by elevation of the water level to wash out the drug and then a salt bath was carried out for 30 minutes followed by prolonged formalin immersion.

Recording of Mortality

Mortality rate before and after treatment were recorded daily (Table 1).

Results

Skin and Gill Biopsy Examination

The skin and gill biopsies revealed the presence of many small-sized worms firmly attached to the skin and gill tissue in the red tilapia fish. The parasites were identified as monogeneans (Fig. 1) because of their jerking or

Table 2. Severity of parasite infestation of the examined red tilapia.

Total Number of Examined Fish	Number of Fish Showing Infestation	Number of Parasites/ Microscopic Field x 100	Percent
50	25	12	50
	9	8	18
	13	5	26
	3	4	6

caterpillar-like motion, in which the parasite repetitively stretched and recoiled and also due to presence of attaching hooks (Noga, 1996).

The gill and skin tissue were severely infested with these parasites. The number of the parasites on x 100 magnification revealed 4-12 parasites per microscopic field. All examined fish samples were infested with the parasites (Table 2). The examined samples of Nile tilapia showed absence of external parasites either on their skin or gills.

Histopathological Examination

On the skin, multiple parasitic agents were noticed on different body regions either in the anterior part attached

to the eyes or in the posterior part in the tail region (Figs. 2 a&b). The parasite caused spongiosis in the epidermal layer of the skin together with dermatitis manifested by mononuclear cell infiltration and dilated blood vessels.

On the gills, multiple parasites were observed either free between secondary gill lamellae or attached to gill filaments.

The infested branchial tissue showed lamellar oedema and diffuse hyperplasia together with mononuclear cell infiltration (Fig. 2c). Desquamated epithelium of the gills was prominent indicating the destructive effect of the parasite on the branchial tissue (Fig. 2d).

Effect of Treatment

The treatment regime induced a dramatic positive effect on the parasites.

Mortalities were reduced 2 days after application of the treatment (day 6 of stocking) and completely ceased after 4 days (day 8 of stocking). At this time, examination of the skin and gill biopsies revealed absence of any parasitic agents. The percent of mortality before and after treatment is demonstrated in Table 1.

Discussion and Recommendations

Our study shed light on the effective concomitant treatment plans in case of parasitic infestation of red tilapia fish

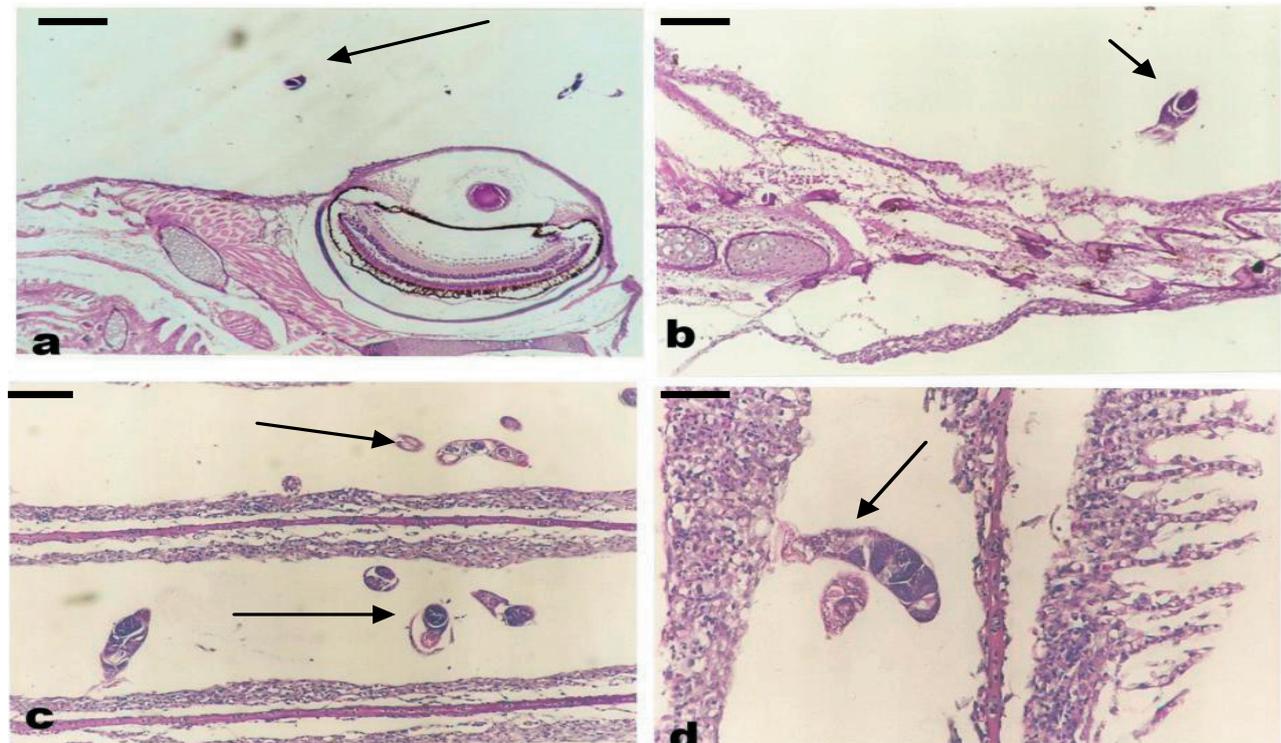


Figure 2. a. Histopathological section of anterior part of red tilapia SPP showing monogenean parasites near ocular region (H&E stain bar, 300 µm). b. Histopathological section of posterior part of red tilapia SPP frays showing monogenean parasites attacking tail region with dermatitis(H&E stain bar, 150 µm). c. Histopathological section of gills (branchial tissue) of red tilapia SPP frays showing multiple monogenean parasites with diffuse lamellar hyperplasia and mononuclear cells infiltration (H&E stain bar, 75 µm). d. Histopathological section of gills (branchial tissue) of red tilapia SPP frays showing multiple monogenean parasites and desquamated lamellar epithelium (H&E stain bar, 38 µm).

fries. The study also stresses problems associated with trans-boundary (introduced and transferred) fish diseases, with emphasis on parasitic diseases of tilapia fries.

It has become increasingly clear that many of the human-assisted movements of fish into new areas have been responsible for the introduction, establishment and spread of pathogens and parasites into new geographic areas. Hoffman (1970) and Bauer and Hoffman (1976) summarized the state of knowledge on the transfers of fish parasites along with host movements through human activities. Hoffman (1970) documented movement and establishment on new continents of at least 48 species of parasites (5 Protozoa, 31 Monogenea, 3 Nematoda, 5 Digenea, 1 Acanthocephala and 3 Copepoda).

Because Nile tilapia is considered one of the most cultured species, the possibilities of trans-boundary disease transmission are very high. In this concern, Arthur (1996) noted that 50% (9 of 18) of the parasites known to infest Nile tilapia in the Philippines were probably introduced into the country along with the introduction of this fish for aquaculture and stocking in natural waters.

The number of diseases has increased significantly with the increased ease of air travel and the recent explosive growth of the aquaculture industry. In general, fishery managers must be faulted for not giving pathogens adequate consideration when contemplating the introduction and transfer of aquatic animals. In many cases this has led to serious pathogens becoming established in new areas and hosts. Once established in natural waters, such pathogens are usually impossible to eradicate. With proper planning, the introduction of many of these pathogens could have been avoided.

Monogenea are one of the most important parasites affecting fresh water fish causing flash movement and other characteristic signs on the fish. The detected signs in our study were in agreement with the findings of Thoney (1990). The parasite can be treated using various types of anthelmintic drugs as mebendazole (Szekely and Molnar, 1987; Thoney, 1989; Thoney and Hargis, 1991); also long-term exposure to suboptimal salinity may be highly effective against productivity of some monogenea as *Neobenedenia melleni* (Mueller *et al.*, 1992) or *Polylabroides multispinosus* (Diggles *et al.*, 1993). The use of formalin as a chemical treatment for monogenea has been recommended by Langdon (1992), who stated that, freshwater or saltwater baths usually work only on small monogenean species unless followed 48 hours later by formalin or organophosphate treatment. The control requires weekly treatments for at least 3 weeks.

From a pathological point of view, monogenea parasites cause severe pathological lesions either on skin and/or gills in many fish species (Whittington, 1998). In our study, the observed lesions were severe on skin and gills. It appeared as dermatitis; spongiosis (intracellular oedema) and some of the parasite fragments were noticed and attached on the outer epithelial part of the skin. On gills, the lesions were prominent and characterized by

destruction of the branchial epithelium and hyperplastic proliferation of the cells. Our findings are in accordance with those of Kear (1998) who studied the lesions caused by monogenean parasites in several fish species.

From our study, it was clear that, although red and Nile tilapia were imported from the same place and reared under the same conditions, only red tilapia were affected by the parasites. In this concern, Robinson *et al.* (2008) mentioned that, red tilapia is a hybrid of *O. aureus* × *O. Mossambicus* and added that hybrid species are more sensitive to monogenean parasites than other tilapia species.

The concomitant treatment regime applied in our study was a very effective means of eradicating the external parasites.

Recommendations

To ensure absence of any parasitic agents and to avoid entrance of new pathogens to native fish, a preferable approach would be to impose a mandatory quarantine on imported fish.

It is recommended that fish-health laboratories (pathology, bacteriology and parasitology laboratories) should be established and considered as a reference laboratory for Oman to specify and survey different types of fish parasites and diseases.

Further studies should be implemented to specify the types of monogenea.

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