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BIOCONTROL OF ENCYSTED METACERCARIA AND VIBRIOSIS INFECTION AFFECTING THIN-LIP GREY MULLET (*LIZA RAMADA*)

By

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ABSTRACT

Co-infections are very common in nature and occur when hosts are infected by two or more different pathogens either by simultaneous or secondary infections so that two or more infectious agents are active together in the same host. *Vibrio parahaemolyticus* and the encysted metacercaria of *Heterophes sp.* were obtained from *Liza ramada* with prevalence rate 50% and 80% respectively at marine fish farm in Damietta Governorate (Egypt). *Vibrio parahaemolyticus* were retrieved and presumptively identified using morpho-chemical characterization and API*20E system. Antibiogram has revealed that isolates were sensitive to ciprofloxacin, enrofloxacin, nalidixic acid and oxolinic acid while resistant to ampicillin, amoxicillin, and lincomyci. The naturally infected *Liza ramada* could be treated successfully in the farm by Biofarm in a single dose (3ppm for 30 minutes) as a short duration bath and then fed a ration containing Ciprofloxacin (150 mg / Kg body wt.) for ten days. The recovery rate increased, the clinical signs disappeared and the fish returned to a normal state of health. The aim of this study is assessment of applying antibiotic after sensitivity test in comparing with using biofarm in treatment of co- infection by encysted Metacercaria and Vibriosis.

Keywords:

Vibrio parahaemolyticus, *Liza ramada*, encysted metacercaria, Biofarm, antibiogram,

INTRODUCTION

Egypt has large areas of fresh, brackish, and marine water bodies suitable for fishing. Moreover, the climatic conditions are optimum for fish farming, with high growth rates for the fish that are raised, which constitute a cheap source of animal protein for Egyptians **(Oczkowski *et al.*, 2009)**. In aquaculture, up to 50% of production loss is caused by diseases

(Assefa and Abunna 2018). Grey mullet (*Mugil cephalus*) and Nile tilapia (*Oreochromis niloticus*) are the most consumed fish spp. in the Egyptian market, and the fish productions are about 1.5 million tons per year **(FAO, 2003-2020**). Various diseases including parasitic infections pose a threat to fish cultivation, which is a valuable source of food and employment in developing countries **(Yooyen *et al.*, 2006)**.

Bacterial diseases mount the most influential sector of disease problems that has direct colossal impacts on Egyptian Mari culture **(Grisez and Ollevier 1995).**Vibrios come on the top list of pathogens with direct jeopardy to Mari culture development due to high mortalities among fishes (**Austin and Austin 2012)**. It is important to know that, Vibrios are ubiquitous to marine environment, while clinical disease outbreaks occur only when a stressed fish get exposed to the flaring up infectious agent **Austin and Austin 2012**. *Vibrio* spp. is Gram-negative, facultative anaerobic bacteria that are widely distributed in seawater, marine life, offshore sediments and seafood products **(Hackbusch *et al.*, 2020)**. The most prevalent pathogenic Vibrio species in human infections include *Vibrio parahaemolyticus*, *Vibrio cholera, Vibrio vulnificus,* and among which *V. vulnificus* is more life threatening and *V. parahaemolyticus* exhibits a higher prevalence in sea food **(Bonnin-Jusserand *et al.*, 2019)**. Furthermore, the vibriosis caused by Vibrio species (e.g., *V. parahaemolyticus, Vibrio alginolyticus, Vibrio ordalii*, and *Vibrio anguillarum*) has been found to cause diseases in more than 50 economic fish species that were considered severe economic losses to the marine aquaculture industry **(Yu *et al.*, 2016)**.

Digenetic trematodes and their metacercariae were be considered as one of the most prevalent parasites infecting fish giving rise to low weight gain, high mortality, unmarketability and some of these parasites may possess zoonotic importance **(Taher 2009).** Digenetic trematodes and its larval stages (encysted metacercariae) take a great interest in most countries especially for the human care against the transmissible diseases **(Taher 2009; Hassan *et al.*, 2012)**. Eating raw or improperly cooked or processed fish is the main source of these infections for humans **(Park *et al.*, 2009)**. Fish-borne trematodes, particularly those have zoonotic nature (fish-borne zoonotic trematodes ‘‘FZT’’) are threats to human and domesticated animals health, food safety, as well as aquaculture industry **(Hung *et al.*, 2013 and Soliman and Yacout 2016)**, It is estimated that about 40 million people are affected by fish-borne trematode infections worldwide **(WHO, 1995)** and more than 18 million people are reported to be affected with fish borne heterophyids **(Sohn *et al.*, 2009)**. Eating raw mullet is the main

source of heterophyiasis infection **(Chai and Jung 2017).** Many members of the trematode family Heterophyidae Odhner, 1914, use fishes as intermediate hosts and humans as definitive hosts **(Cribb *et al.*,2010)**, Heterophyidiasis caused by these and related species has now become a major public health threat **(Yousif *et al.*,2016)**. The existence of heterophyid metacercariae in wild and farmed fishes has been recorded in Egypt **(Salem *et al.*, 2010)**.Family Heterophyidae includes a set of over 50 genera having similar morphological features **(Waikagul and Thaenkham 2014).** Heterophyiasis is highly endemic disease in Egypt where the habit of consuming raw or inadequately cooked mullet or pickling mullets is a common habit **(Youssef and Uga 2014; Mousa *et al.*, 2018).**

Risks associated with many of traditional chemical therapeutants to fish, to the environment and to consumers have led to bans for their use in the aquaculture industry of many countries. The search for alternative environmentally friendly approaches for diseases has increased during the last decade **(Thora *et al.*, 2020)**. In Egypt, **WHO (2006)** aquatic farmers and paramedics rottenly use large quantities of antibiotics during disease without veterinary supervision, also the insufficient regulations for the antimicrobial registration facilitating its illegal use. The use of antibiotic in aquaculture and food animal production settings contributes to the development of antibiotic-resistant bacteria (ARBs). ARBs and ARGs have been isolated from aquatic environments **(Pham *et al*., 2018)** and aquatic animals **(Naik *et al.*, 2018)** in the last decade.

Biofarm is considered a potential natural compound used as parasiticide in fish **(Hussien *et al.*, 2010 and Noor El-Deen *et al.*,2010).** Humic substances (HS) have been used as antimicrobial agents **(Arif *et al.*, 2019).** The main substances that make up the HS are the humic acids (HA), fulvic acids (FA) and humins **(Arif *et al.*, 2019**). Humic substances are another promising, natural immunostimulants which will be considered in depth. **Lieke *et al.*, 2020**, reported an overview of risks and benefits of current treatment options and new **a**pproaches to replace harmful therapeutants and minimize the number of toxic residues discharged into the environment. **Gryndler *et al.* (2005)**, has been described that, dissolved HS have a general bacteriostatic effect toward pathogenic bacteria.

In different studies conducted on fish, it was reported that hAs increased the survival rate against fish pathogens while the effect changed depending on fish type, dose or time **(Rousdy and Wijayanti, 2016). Liltved and Landfald (2000)** found that HS has a disinfecting effect

toward fish pathogen bacteria *Aeromonas salmonicida* and *Vibrio anguillarum* and **Yılmaz *et al.* (2018)** reported that, humic acid might replace antibiotics in diets for rainbow trout to control yersiniosis.

Therefore, in this study, the effects of Biofarm on treatment, control and survival rate against of co- infection of encysted Metacercaria and Vibriosis infection affecting thin-lip grey mullet *(Liza ramada*) in a private marine fish farm in Triangle of Deeba-Damietta Governorate (Egypt).

MATERIAL AND METHODS

Fish samples and site of fish farms:

A total of 200 alive and moribund *Liza ramada* were randomly collected from a private marine fish farm in Triangle of Deeba- Damietta Governorate (Egypt), rearing thin-lip grey mullet (*Liza ramada*) in earthen ponds. Fish were transported alive in aerated water tanks. At the arrival time, fish were euthanized by immersion in MS222 solution (250 mg/L; 25-30°C) that assumed to cause rapid unconsciousness according to **AVMA (2007).** Alive adult *Liza ramada* (80 $\pm$ 10 gm) transported to the Fish Disease Researchs Department lab. In Animal Health Research Institute, Dokki, Giza **(Langdon and Jones, 2002)**.

Clinical picture and Post-mortem examination of fish:

Fish samples were examined carefully, externally and internally for detection of any abnormalities. The specimens were examined externally according to **Noga (2010)** and also, were dissected for detection of any abnormality internal lesions.

Water sampling:

Water samples were collected from marine fish farm, according to **Canadian Council on Animal Care(2005).**Waterparameters pH, temperature,dissolved oxygen,salinity,Un- ionized ammonia,Nitrate (NO3), Nitrite (NO2) and sulphate were measured.

Isolation and identification of bacteria:

Primary isolation of bacteria:

Fish samples were washed with sterile 3% NaCl solution to remove surface microorganisms, debris,sand,and slime. The collected fish were bacteriologically examined. Briefly, bacteriological swabs were obtained from the spleen, liver, gills, ascetic fluid, gall bladder and kidneys of the fish according to **Woo and Bruno (2014).** Samples were streaked onto trypticase soy agar supplemented with 3% (w/v) sodium chloride, Rimler - Shotts medium (RS) and thiosulphate citrate bile salt agar (TCBS) plates then incubated at 28°C at 24-48 hr. according to **(Austin**

and Austin, 2012). All media used in the bacterial analyses were produced by Oxoid, Canada.

Identification of the bacteria:

The similar and dominant bacterial colonies are examined microscopically and biochemically according to Bergey's Manual of Systematic Bacteriology (**Holt, *et al.*, 1994) and (Whitman 2004).** Bacteria spp. were identified using API*20E (BIO-Merieux) for identification. Pure colonies were transferred to glycerol broth 20% at -80°C **(Pujalte *et al.*, 2003).**

Antibiogram sensitivity discs:

Antibiotic susceptibility of the retrieved bacterial isolates was determined using the Kirby Bauer disk diffusion method according to **(Bauer *et al.*, 1966)**. The following antimicrobial discs (Oxoid) were used: Ampicillin 10 lg (AML 10), Amoxicillin 25 lg (AX 25), Ciprofloxacin 5 lg (CIP 5), O/129 150 lg (129/150), Erythromycin 15 lg (E 15), Gentamicin 10 lg (CN 10), Nalidixic acid 30 lg (NA 30), Nitrofurantoin 300 lg (F 300), Novobiocin 30 lg (NV 30), Oxolinic acid 2 lg (OA 2), Oxytetracycline 30 lg (TE 30), Streptomycin 10 lg (S10), Tetracycline 30 lg (T 30), Trimethoprim/Sulfamethazole 25 lg (SXT 25) and Enrofloxacin 5 lg (ENR 5). In vitro antimicrobial susceptibility was screened on Mueller-Hinton agar (MHA) (Oxoid, Hampshire, UK) supplemented with 1.5% (w/v) sodium chloride. At the end of incubation period, antibiotic inhibition zones were measured in mm using a measuring caliber. Susceptibility testing was conducted according to the recommendations of **(ClSI, 2017**).

Parasitological examination:

Tissue samples were examined by compression method. The muscles, gills and internal organs were pressed between two glass plates and searched for digenetic encysted metacercariae under a dissecting microscope then stereomicroscope. Encysted Metaceraciare were removed and fixed in warm 10% formalin and stained with acid carmine **Noga, (1996).**

Chemicals used for treatment:

Biofarm® dry: (Totally natural Humate) (Teba agri-Vet. Tanta, Egypt): It is a synergistic combination of humic acid, ulmic acid and folvic acid with the trace minerals (Na, Pot., Iron, Ca, Mg, Ph, Zn, Co, Si). The drug is a product of Farmavet International Istanbul-Turkey. Biofarm was used for treatment of infected *Liza ramada* at the dose of 3ppm /30 min.

Experimental design: (Experimental fish).

The experiment was performed in the private marine fish farm in The Diba triangle- Damietta

Governorate (Egypt), a number of 160 *Liza ramada* which naturally mixed infected with *V. parahaemolyticus* and EMC with an average body of 80 ± 10 gm. We're holding its in 4 small earth ponds, it contains almost 200 liter of water from same source as fish farm with 40 fish in each group (A-D).Fishes were subjected to different treatments, Group (A),was kept as a control group and fed on non-treated ration. While group (B),was exposed to a single dose of Biofarm® (humates) in the concentration 3PPM as a short duration bath for 30 min. Group(C),was fed on ration containing Ciprofloxacin (150 mg / Kg Body wt.) for ten days. Group(D),was exposed to a single dose of Biofarm® (humates) in the concentration 3PPMfor 30 min.then followed by feeding on ration containing fed on ration containing Ciprofloxacin (150 mg / Kg Body wt.) for ten days (Table 1). The incidence of encysted metacercaria were recorded post exposure to Biofarm®. The morbidity and mortalities were recorded and at end of experiment, *Liza ramada* was examined for the presence of pathogens to evaluate the efficiency of treatment methods.

Biosafety measures:

This study applied biosafety measures according to Pathogen safety data sheets: Infectious substances- *V. parahaemolyticus*, Pathogen Regulation Directorate, **Public Health Agency of Canada (2019).**

RESULTS

Clinical and post-mortem changes:

Clinically, the fish is very lean compared to the age of the fish with thinning of the head. Diseased fish showed emaciated dark coloration of skin, detached scales, hemorrhages around the mouth opening and operculum, abdominal distension and superficial ulcerations Fig. (1). during the dissection of the infected *Liza ramada*, a decaying odor was found with the presence of fluid in the abdomen. The main post mortem lesions were, marked adhesion of the internal organs and sever congested kidney, congested spleen and liver, pale gills with excessive mucous (in some cases the gills were congested), enlarged gall bladder, ascetic fluid in the abdominal cavity and in some cases inflammation of swim bladder with thickening of its wall Fig. (2).

Physicochemical parameters of the farm water:

The recorded water quality results throughout \ e study were recorded in (Table 2) revealed that all parameters within permissible limit and within the normal ranges for *Liza ramada* culture as reported by **(Tookwinas *et al*., 1986)**.

Bacteriological assay:

Morphologically, the retrieved colonies exhibited round, green and 2-3 mm colonies with dark blue center on TCBS. Such colonies were able to grow on TSA with different salt concentrations (0%, 3% and 6%). Morpho-chemically, the bacterial isolates were Gram-negative, straight or curved rods, facultative anaerobic, motile, cytochrome oxidase positive, and catalase positive with high sensitivity to vibriostatic agent (novobiocin, 30μg).

The biochemical characterization of the retrieved isolates coincided with the standard biochemical criteria of *V. parahaemolyticus* (Table3). ID of *V. Parahaemolyticus* in API*20E was 4146107 & 4146105.

Antibiogram sensitivity:

Antibiogram of retrieved has revealed *V. parahemolyticus* isolates were sensitive to ciprofloxacin, oxytetracycline, tetracycline, enrofloxacin, trimethoprim-sulfamethoxazole, novobiocin, nalidixic acid, oxolinic and vibriostat (O/129), while resistant to ampicillin, amoxycillin, gentamycin, lincomycin and streptomycin. Intermediate sensitive was found to nitrofurantoin and erythromycin.

Parasitological examination:

The examined samples revealed that *Liza ramada* were infected with metacercaria of *Heterophes sp.* in muscle with infection rate 80%. Examination revealed the presence of two morphologically distinct group's heterophyids' EMC.

These groups were categorized according to their shape, size and thickness of cyst wall. One group was large, lie between the muscle fibers in the centre of a spindle shaped mass of fat globules. Every cyst is isolated from the surrounding fatty tissue by a homogeneous membrane originated from host tissue. The second group of cysts was medium with a few pigment particles. Identification of the parasites was carried out according to according to **Sohn (2009)** Fig. (3).

Treatment Experiment:

In the current study, the trial to treat the infected *Liza ramada* by used one or combined drugs were met with success recorded in (Table 1). The effective treatment considered when it reduces parasitic and bacterial infection with decrease the mortality rate. After treatment, the treated groups (C, D) recorded less level of re-isolation of *V. Parahaemolyticus* than control group (A) and increase of recovery rate%. Encysted metacercaria recorded complete

eradication in group (B, D) with increase the recovery rate%. In the treated fish, the clinical signs recorded were disappeared and the fish returned to normal state of health.

Table (1): Showing application of chemotherapeutic treatment for control the infected *Liza ramada* with *V. parahaemolyticus* and EMC.

No. of Groups	No. of fish	Treatment	concentration	Mode of treatment	Time of exposure	recovery rate%
group (A)	40	non-treated	–	–	–	
group (B)	40	Biofarm	3PPM	bath	30 min	80%
group (C)	40	Ciprofloxacin	fed on ration containing Ciprofloxacin (150 mg / Kg Body wt.) for ten days	feeding	10 days	70%
group (D)	40	Biofarm	3PPM	bath	30 min	90%
	40	Ciprofloxacin	fed on ration containing Ciprofloxacin (150 mg / Kg Body wt.) for ten days	feeding	10days	

Table (2): Water quality of the marine farm containing infected fish.

Water parameters	Results	Permissible limits
pH	8.1	7.5 - 8.5
Tempreature 0°C	25°C±3°C	---
Dissolved oxgyen	5.4	5 – 6 mg/L
Salinity PPT	30	28 – 35 ppt for marine farm
Un ionized ammonia	0.01	0.0 – 0.0125 mg/L
Nitrate(No3)	8	10 mg/L
Nitrite (NO2)	0.3	0.0 - 0.3 mg/L
Sulphate	180	<3000 mg/L

Table (3): Phenotypic characters and API20E profile of *V. parahaemolyticus* isolated from naturally infected *Liza ramada.*

Biochemical tests		***V. parahaemolyticus***	**Biochemical tests**		***V. parahaemolyticus***
NO3	**Potassium nitrate**	**+**	**MAN**	**Mannitol assimilation**	**+**
TRP	**Tryptophan production**	**+**	**MNE**	**Mannose assimilation**	**+**
GLU	**Glucose fermentation**	**+**		**Growth on of 0% Nacl**	**-**
ADH	**Arginine Dihydrolase**	**-**		**Growth on of 3% Nacl**	**+**
URE	**Urease**	**-**		**Growth on of 6% Nacl**	**+**
ESC	**Esculin**	**+**	**OX**	**Oxidase**	**+**
GEL	**Gelatin**	**+**		**H2S**	**-**
PNG	**Para Nitrophenyl D Galactopyranosidase B Glucosidase**	**-**		**Temp. tolerance:** **Growth at 28 ^{o}C** **Growth at 37 ^{O}C**	 **+** **+**
GLU	**Glucose assimilation**	**+**		**Catalase**	**+**
ARA	**Arabinose assimilation**	**+**		**Citrate utilisation**	**-**

Fig.(1): Clinical signs finding of the examined *Liza ramada. (*A) Naturally infected *Liza ramada* showing its very lean compared to the age of the fish and hemorrhages around the mouth opening and operculum (B) Diseased fish showed emaciated with thinning of the head (C) Naturally infected Liza ramada showing dark coloration of skin, detached scales and superficial ulcerations. (D) Naturally infected Liza ramada showing abdominal distension.

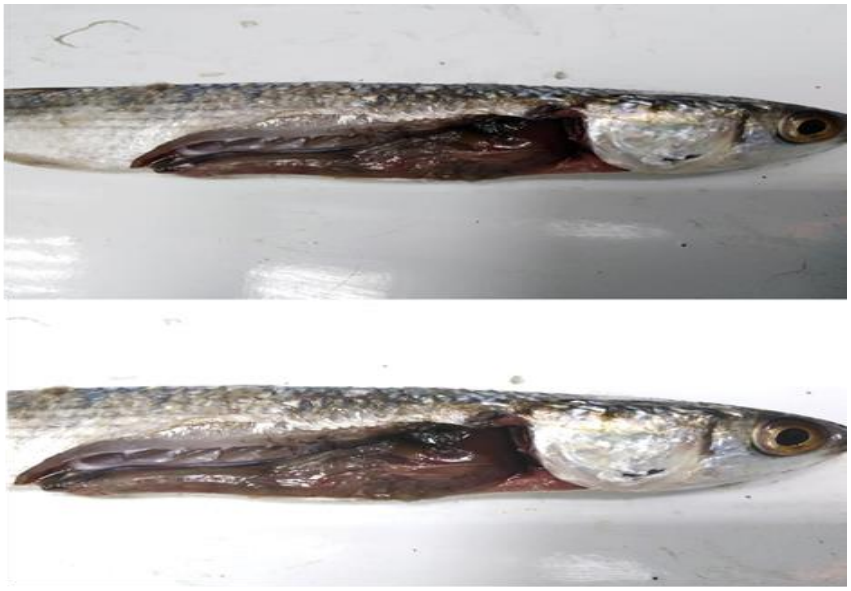

Fig. (2): Post mortem finding of the examined *Liza ramada,* naturally infected fish showing marked adhesion of the internal organs and sever congested kidney, congested spleen and liver, enlarged gall bladder, ascitic fluid in the abdominal cavity.

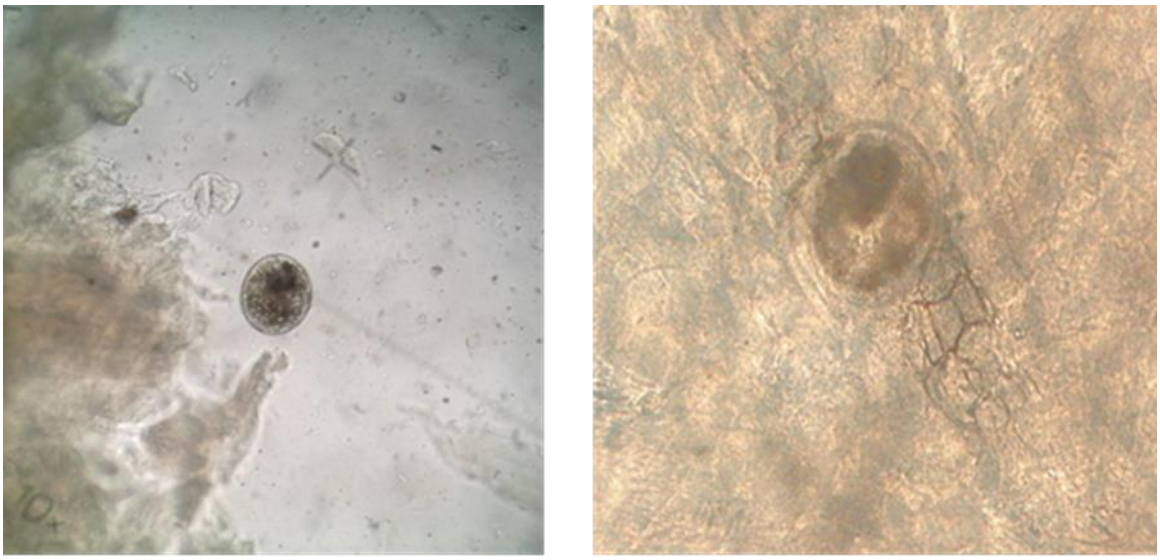

Plate (3): Heterophes sp. encysted metacercariae isolated from *Liza ramada* (X40).

DISCUSSION

During the examination of naturally infected *Liza ramada* and experimental period, the water parameter of the farm used showed: Temperature: 25°C±3°C, pH 8.1, salinity 30ppt, and dissolved oxygen ≥ 5.4mg/I. The level of biofarm, in the experiment was 3PPM in groups B and D and the level of Ciprofloxacin on diet was 150 mg / Kg Body wt. for ten days in groups C and D. *V. parahaemolyticus* is a pathogen of public health concern, the Vibrio species gain global interest of the microbiology community and zoonotic diseases experts **(Austin and Austin, 2016).** It is associated with sewage pollution which is one of the leading causes of bacterial food poisoning pathogens **(Austin *et al.*, 2012).** It is commonly found on all varieties of finfish and shellfishes that are traditionally taken from marine and shore areas. In the current study, clinical signs and post mortem lesions recorded among naturally infected *Liza ramada* similar to clinical signs and post mortem lesions reported by **Moustafa *et al.* (2010), Abdel-Aziz *et al.*, 2013 and Winfield, 2018).** Definitively, the insight analysis of the morpho-chemical characteristics (API 20 NE and conventional biochemical tests) for the majority of retrieved isolates, our findings were in close agreement with those reported for the *V. parahaemolyticus* profiles **(Buller, 2004, Abdel-Aziz *et al.*, 2013, Aly *et al.*, 2020).** Our findings revealed the highest prevalence of *V. parahaemolyticus* was recorded at spring and summer (temp. 25°C±3°C) when temperature fluctuations are more frequent, this diseases was related to a spring syndrome and a fall syndrome(sudden temperature fluctuations), this

finding were agreement with **Abdel-Aziz *et al.*, 2013,Winfield, 2018, Aly *et al.*, 2020**). In respect to intensity of infection, our results showed the highest intensities were in kidney followed by spleen and the lowest level recorded in liver. This result was similarly in agreement with **(Zorrilla *et al.*, 2003 and Abdel-Aziz *et al.*, 2013)** who reported the liver and kidney as the main target organs for isolation of *V. parahaemolyticus* in Gilthead Seabream. Antibiotic resistance poses important risks to human health and can affect the course of infectious diseases. In our study, Ciprofloxacin, enrofloxacin, oxytetracycline, tetracycline, nalidixic acid and oxolinic acid are more effective to treatment of *V. parahaemolyticus* infection. These results were concordant with **Labella *et al.* (2011) and Abdel-Aziz *et al.*, 2013**). These results were nearly similar to those obtained by **(Aly *et al.*, 2020)** who found most isolates of *V. parahaemolyticus* were resistance to ampicillin and gentamycin with intermittent resistance to ciprofloxacin and high sensitivity to sulphamethoxazole/ trimethoprim and oxytetracycline. Similarly, noticed previous studies documented that most isolates of *V. parahaemolyticus* isolates were resistant to ampicillin **(Abdel-Aziz *et al.*, 2013, Eissa *et al.*, 2017 and Aly *et al.*,2020)**. Vibrio spp. were able to develop resistance mechanism to resist antibiotics (**Abdel-Aziz *et al.*, 2013)** which could be related to highly use of these antibiotics reaching to aquaculture and increase its concentration in water agriculture /municipal wastes. The infection of parasites particularly trematodes including *Heterophes sp*. should be managed for successful aquaculture both from economic and zoonotic point of view. The infection affects the feeding habit, acquisition of body weight and reduced fecundity and even death of the host fish **(Silva *et al.*, 2005**). On the other hand, many reports show that they have zoonotic potential as well. WHO has identified fish-borne trematodes as a serious zoonotic and health concern in one of its reports **(WHO, 1995 and Chai *et al.*,2005)** reviewed the same aspect in detail. Many cases of human infection of this parasite have been reported from various regions of the world, therefore it is important to control this infection to check the possibility of zoonoses. Heterophyiasis is prevalent in humans in many countries in the Middle East and Far East. Fish act as the first intermediate host and the habit of consuming raw or inadequately cooked or salted fish is an important determinant of human infection **(Macpherson 2005 and Chai *et al.*, 2005).**

In the present study, the encysted metacercaria of *Heterophes sp*. were obtained from muscle of *Liza ramada* with prevalence rate 80%, These findings agree with that recorded by **Olfat *et al.*,(2020)** who recorded prevalence rates of heterophyid infection in mullets was

80% to 100%. And considered higher than recorded by **Ghobashy *et al.,* (2010)** who reported that the rate of heterophyid infection in Mullet was 37.9%, and **Mona *et al.,*(2014)**who recorded rates of infection with heterophyid metacercariae ranged from 11 to 23% in mullet. Also, agree with **Hany *et al.,*(2008)** who detected *Heterophyes spp*. from *Mugils pp* from brackish water in Dakahlia and with **Hegazi *et al.,*(2014)** who detected *Hetrophyes spp*., from fresh and brackish water fish and with **Simonetta *et al.,*(2016)**who recorded Heterophyes sp. metacercariae in the muscle of mugilids (*Liza ramada*). These findings of EMC in mullet pose alarming signs for a serious zoonotic risk.This uprising in prevalence might be attributed to the sewage pollution **(Mahmoud *et al.,* 2016)** or due to spreading of wild and migratory birds in these geographical localities **(Mona *et al.,* 2014 and Hanan *et al.,* 2020)**.

The morphological features of obtained *Heterophes sp*. are similar to that were described by **(Witenberg 1929**) and very similar with records by **Ebtsam *et al.,* (1998).** Also, agreed with **Hany *et al.,* (2008**) who recorded EMC of Heterophyes spp. singly scattered in the striated muscles of Mugil spp. They lie between the muscle fibers in the centre of a spindle shaped mass of fat globules and cysts was medium with a few pigment particles and is surrounded with small fatty spindles.

Finally, there is an increasing interest in consuming organic and environmentally friendly food. Thereafter, the limitation of chemotherapy in aquaculture and the use of natural treatments could enhance the consumption of aquaculture products. Moreover, their use could reduce costs of treatment and be more environmentally sound as they tend to be more biodegradable than artificial molecules and they are less likely to produce drug resistance in parasites due to the high diversity of plant extract molecules **(Olusola *et al.,* 2013)**. Humate have a positive influence on fish health and constitution. **Heidrich (2005).** Several positive effects have been observed when treating fishes with HS in water as bath treatment or via food **(Schreckenbach *et al.* 1994**). The supplementation of HS in form (water or feed) **Alejandra *et al.,* 2019).**

Concerning, the treatment trials for investigated encysted metacercaria the result proved that, the use of Biofarm® (Humate) at the dose of 3ppm /30 min. was efficient to kill EMC with recovery rate 60%. This result agreed with the result met by **Noor El- Deen *et al.,* (2012)** who found using humic acid at the dose of 3ppm/ 24h. as natural treatment for eradication parasitic diseases is safer than chemical treatment as formalin which has side effects on fish and water as it is reducing agent lowers oxygen level in water, toxic to fish and of public health

importance in food fish when there is a residue in fish musculature **Brown (2000).** master Also, agree with **Yousef (2008)**, who recorded that Biofarm® (Humate) was proved to be the most effective product at concentration 3ppm/ 30 min. to eradicated EMC infection. The modes of action of HS are various. Two papers appeared hypothesizing direct effects of HS. **Münster (1985)** postulated the ability of HS to interact with biomembranes, which was also concluded by analogy by **Visser (1985).** Visser described how low HS concentrations could strongly stimulate, and high concentrations inhibit microorganism development-a so-called hormetic effect **(Calabrese 2005)**. Meanwhile, it is accepted that HS are taken up and induce a variety of response reactions in the organisms **(Kulikova *et al.*, 2006).**

Once internalized, HS can exert specific as well as non-specific effects. So far, specific effects comprise reduction of the photosynthetic oxygen production, estrogenicity, or chemical attraction. Non-specific effects are physical and chemical membrane irritation, induction and modulation of biotransformation enzymes, induction of stress defense proteins (chaperons), or oxidative stress defense. Also programmed cell death reactions may be induced **(Cheng *et al.*, 2003 and Yang *et al.* 2004).**

CONCLUSION

Vibriosis is a worldwide aquatic animal disease posing an actual threat for both human consumers and aquatic species. *V. parahaemolyticus* infection comes on the top list of Vibrios responsible for fatal diseases in both human and fish. Knowledge about the epidemiology, etiology, diagnosis and treatment of the main bacterial diseases of marine farmed fish are crucial to prevent severe economic losses. Thereafter, the limitation of chemotherapy in aquaculture and the use of natural treatments could enhance the consumption of aquaculture products. The present study showed the occurrence of heterophyid metacercariae in commonly consumed fish species (*Liza ramada*) which can transmit *Heterophyes* parasites with vibriosis. The potential risk of human infection is considered to be high. Our results underscore the need to raise the awareness of public health agencies, consumers, and aquaculture operators and managers on the transmission of this trematode. And highlights of Biofarm® (humates) as anti-parasitic effect, control and treatment of vibrio infection and its potential to be used as an alternative to chemical treatments.

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المقاومة الحيوية للعدوى بالميتاسركاريا والفيبريوسيس فى اسماك الطوبار

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الملخص العربى

تعد العدوى المشتركة شائعة جدًا في الطبيعة وذلك عندما يصاب العائل باثنين او اكثرمن مسببات الامراض المختلفة إما عن طريق العدوى المتزامنة أو الثانوية بحيث ينشط عاملان معديان أو أكثر معًا في نفس العائل .وقد تم عزل الفيبريو باراهيموليتكس و الميتاسيركاريا المتحوصله للهيتروفس من سمك الطوبار بمعدل انتشار 50٪ و 80٪ على التوالي في مزرعة أسماك بحرية بمحافظة دمياط (مصر). وقد تم تصنيف الفيبريو باراهيموليتكس وتحديدها باستخدام التوصيف المورفوكيميائي ونظام API * 20E. وأظهرالأنتيبيوجرام أن العزلات كانت حساسة للسيبروفلوكساسين والإنروفلوكساسين وحمض الناليديكسيك وحمض الأكسولين بينما كانت مقاومة للأمبيسيلين والأموكسيسيلين واللينكوميسي.وقد اثبتت النتائج انه يمكن معالجة اسماك الطوبار المصابة طبيعيا بنجاح في المزرعة بواسطة استخدام البيوفارم بجرعة واحدة (3 جزء في المليون لمدة 30 دقيقة) كحمام مائى قصير المدة ثم تغذيتها على جرعة من سيبروفلوكساسين (150 مجم / كجم وزن جسم السمكة) فى العليقة لمدة عشرة ايام. وقد لوحظ زيادة معدل الشفاء واختفت الأعراض الاكلينيكية وعادت الأسماك إلى حالتها الصحية الطبيعية. والهدف من هذه الدراسة هو تقييم استخدام المضادات الحيوية بعد اختبار الحساسية مع استخدام البيوفارم في علاج العدوى المشتركة للميتاسيركاريا المتحوصلة والفيبريوسيس.