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STUDIES ON LERNAEOSIS AMONG CULTURED COMMON CARP (*CYPRINUS CARPIO*) WITH SPECIAL REFERENCE TO ITS PREVENTION BY VACCINATION

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Abstract

A total number of two hundreds common carp (*Cyprinus carpio*) naturally infested with *Lernea cyprinacea* were collected a live or freshly dead and subjected to clinical signs, parasitological and pathological examinations. Heavily infested fish showed increased of mucus secretions, ulceration and rubbed their bodies against hard objects. Intensity of adult *Lernea* was ranged from 1 to 176 per fish. The light, moderate, heavy and extreme infestation rate was 7.5, 15, 40 and 37.5%, respectively. Fins were the most preferable site for infestation. Histopathologically, sloughing of most superficial layers of epidermis with edema and leukocytic infiltration. A hundred of common carp were divided into four equal groups and injected with *L. cyprincea* antigen at concentration of 10, 20, 30 and 0 µg proteins per gm fish, respectively. Poster dose was injected at the same concentration after two weeks of the first one. All fish groups were exposed to natural infestation by adding five heavy naturally infested common carp. The PVC values were significantly higher in the treated groups than control. Also, a significant protection was achieved by injection of *L. cyprincea* antigen. The infestation rates were 52, 12, 16 and 96% among the immunized groups and control one, respectively. The detection of specific antibodies was confirmed by agar gel precipitation test. Forty naturally infested common carp fingerlings were equally divided into 4 equal groups and injected by the collected serum from the second immunized group i/m at a dose of 0.1, 0.2, 0.3 ml, respectively. The fourth group was being designated as non-treated control group. The recovery rate from infestation was recorded. The recovery rates were 40, 60, 80 and 10%, respectively. Also, forty healthy common carp

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fingerlings were equally divided into four groups and inoculated with immunized serum at the same concentrations as previously mentioned. All of them were exposed to natural infestation as mentioned before. The infestation rates were 50, 20, and 10% respectively among the injected groups at 0.1, 0.2 and 0.3ml compared with 90% for control. Further research and testing of vaccine in other fish species and under field conditions is indicated.

INTRODUCTION

Cyprinids constitute the largest group of finfish species cultured worldwide comprising at least 1700 species and over 200 genera and have a global significance as a source of food (Hoole *et al*., 2001). Cyprinids have been introduced into Egypt in 1980s from Hungary as an important species for aquaculture for food security due to human over population. Unfortunately, the trans-boundary movement of living aquatic animals, facilitating the introduction of serious ectoparasitic infectious disease caused by *Lernaea cyprinacea* and led to spread in many governmental and private fish hatcheries and farms (Faisal *et al*., 1988). The economic importance of the lernaeid ectoparasites has increased due to numerous epizootics occurring among the most important farmed fish in various parts of the world (Kir, 2007).

Lernaeosis considered to be the major parasitic problem hampering aquaculture development and sustainability in many countries causing mortalities, reduced growth and low quality of fishes (Woo, 2006). Furthermore, the sites of *L. cyprinacea* attachment may become ulcerated opens the door for secondary infections, such as fungus, bacteria, virus and other parasites and lead to osmoregulatory failure.

Copepod parasites are difficult to be controlled because sclerotized exoskeleton is resistant to chemical treatment. Organo-phosphorous compounds were used in killing the free living stages of *L. cyprinacea*, thereby disrupting the developmental cycle of the parasite. So, treatment should be repeated every seven days for at least a month at 27 C to kill all

the females. However, the immune responses of fish against parasitic antigen have been largely neglected. Aquaculture requires the availability of pure, safe, potent and effective vaccine to maintain fish health and avoid sever economic losses. Attention has centered on the prospects of preventive immunization as a means to control parasitic infestation. Vaccination against parasitic infestation therefore appears promising (Tonguthai, 1997). Woo and Shariff (1990) found that all recovered and naive *Helostoma temmincki* lost their infections 30 days after homologous challenge with *L. cyprinacea.* It was suggested that there was acquired protective immunity in recovered fish. They suggested that, if no fish are introduced into a closed system for a period after an outbreak, then there will be no infective larvae in the water and the system will now be safe for restocking.

Therefore the aim of the present investigation was to through the light on lernaeosis among cultured common carp (*Cyprinus carpio*) with special reference to evaluation of vaccine as preventive measurement.

MATERIAL AND METHODS

Naturally Infested Fish

A total number of two hundreds common carp (*Cyprinus carpio*) naturally infested with *L. cyprinacea* were collected a live or freshly dead from different fish farms transferred to the laboratory and subjected to clinical signs, postmortem and parasitological examinations as described by Lucky (1977).

The detected female adult *L. cyprinacea* used to run the bioassays were picked up and counted from the naturally infested fish with the help of fine forceps, washed in sterile distilled water and placed in vials to be stored in frozen. Their attachment preferences on the naturally infested fish were recorded. Also, the intensity of infestation was recorded according to Abd El-Rahman (2000).

Experimental Fish

Apparent healthy common carp were obtained from central laboratory for aquaculture research (CLAR) hatchery ponds known to be free from lernea infection, acclimated to experimental condition for 2 weeks in clean glass aquaria supplied with dechlorinzed tap water at 25˚C ± 1˚C and continuous aeration. Fish were screened for presence of natural infestation by lernea and were found to be free from lernea and other pathogens. All experimented fish were subjected to prophylactic treatment against ectoparasites using formalin, 100mg/L for 1houre, according to Ruth (1996). They were fed on commercial feed pellets at a rate of 2% of their body weights.

Antigen Preparation

The antigen preparation technique was performed according to (Kent, 1982). Briefly, the collected parasite was washed several times by using phosphate buffer saline solution (PBS. PH 7.2) until complete removal of any impurities. Normal saline was added by 1:5 volume of the antigen and minced by ultrasonic till form complete homogenization was obtained. Purification of the prepared antigen was done by centrifugation at 3000 rpm for 5 min. The supernatant was collected for using in immunization and serum evaluation. The protein quantity in the prepared antigen was determined according to Stoscheck (1990).

Immunization Procedure

A hundred of common carp were randomly divided into four equal groups as follow:

Group 1: fish were inoculated with 0.2 ml i/m (10µg protein per gm fish).

Group 2: fish were injected with 0.2 ml i/m (20µg protein per gm fish).

Group 3: fish were inoculated with 0.2 ml i/m (30µg protein per gm fish).

Group 4: fish were kept as negative control (0.2 ml i/m of normal saline).

After two weeks, another poster dose was inoculated at the same concentration for each group. Packed Cell Volume (PVC) was recorded for each group after 2 weeks from immunization (Purves *et al*., 2004). For serum preparation, blood was drawn from tail vein (Michael *et al*., 1994) collected in serology tubes. The clot was stored overnight at 20 °C and was spun down at 400 g for 10min. The separated serum was stored in sterile eppendorf tubes at -20 °C until further use.

Challenge Test

All fish groups were exposed to natural infestation by adding five heavy naturally infested common carp. All immunized fish were kept under strict daily observation for a month for detection of *L. cyprinacea* infestation.

Passive Immunization

Forty naturally infested common carp fingerlings were equally divided into 4 equal groups and injected by the collected serum from the second immunized

group i/m at a dose of 0.1, 0.2, 0.3 ml, respectively (Stiehm *et al*., 2004). The fourth group was being designated as non-treated control group. The recovery rate from infestation was recorded.

Also, forty healthy common carp fingerlings were equally divided into four groups. All the first three groups were inoculated with immunized serum at the same concentrations as previously mentioned. The fourth group was kept as control group. Also, the passively immunized fish were exposed to natural infestation as mentioned before.

Agar Gell Immunodiffusion Test (AGID)

The AGID was performed as described by Spielberg *et al*. (1989).

Histopathological Examination

Specimens for histopathological examination were fixed and processed according to procedure described by Marudanayagam *et al*. (2006).

Statistical Analysis

The obtained results were analyzed statistically according to William (1970).

RESULTS

Clinical signs were differed according to intensity and sites of infestation, size and age of fish. Heavily infested fish showed decreased of feed intake, increased of mucus secretions, sluggish movement, loss to escape reflex, emaciated and rubbed their bodies against hard objects. Also, respiratory distress was noticed in heavy infested fingerlings. The anterior part of the parasitic female was lodged in tissues of body surface; its posterior part containing egg sacs was protruding and easily noticed by naked eyes. This penetration was associated with sever tissue damage and hemorrhagic ulcer with swollen margins which may reach to 6mm in diameter (Fig., 1).

Intensity of adult Lernea was ranged from 1 to176 per fish. The light, moderate, heavy and extreme infestation rate was 7.5, 15, 40 and 37.5%, respectively (Table, 1).While the distribution of adult metamorphosed females on the fish body surface was 3.3, 2.0, 14.3 and 8.8, 6.6, 7.9, 3.2 and 17.5, 35.1 and 1.3% in mouth, eyes, head, and dorsal, pectoral, pelvic, anal and tail fins, body sides and urogenital openings respectively (Table, 2).

Histopathologically, sloughing of most superficial layers of epidermis with edema under the dermis and leukocytic especially melanomacrophages aggregation in the dermis. Also, edema and necrosis

in the subcutaneous muscles with leukocytic infiltration was observed (Fig., 2).

The PVC values were 25.47, 23.93, 24.14 and 22.79% for the four injected groups injected with *L. cyprincea* antigens at concentrations of respectively (Table, 3). Statistical analysis showed that there was significant difference between the treated groups and controls.

The obtained results (Table, 4) showed that significant protection was achieved by injection of *L. cyprincea* antigen. The infestation rates were 52, 12, 16 and 96% among the immunized at concentration of and control groups, respectively.

Agar gel precipitation test was performed as confirmatory test for detection of specific precipitin lines in the immunized groups. It was observed from the test that precipitin lines were formed in all groups except control group.

Group acquired highly titer of immune serum against inoculated antigen was the 2nd group. Therefore, sera of the second group were used in passive immunization. The obtained results showed that the recovery rates were 40, 60, 80 and 10% among infested fish injected with immunized serum of the second group at dose of 0.1, 0.2, 0.3 and 0ml, respectively (Table 5).

With regard to the effect of immunized serum on healthy fish challenged with *L. cyprinacea*, the present results showed that the infestation rates were 50, 20, and 10% respectively among the injected groups at 0.1, 0.2 and 0.3ml compared with 90% for control (Table, 6).

DISCUSSION

Lernaeids is common an economically important copepods parasite infecting wide range of fresh water fishes, associated with high mortality especially in small fish (Hoole *et al.*, 2001 and Woo, 2006).

Naturally infested fish showed decreased of feed intake, increased of mucus secretions, emaciated, ulcer and rubbed their bodies against hard objects. Also, respiratory distress was noticed in heavy infested fingerlings. The anterior part of the parasitic female was lodged in tissues of body surface; its posterior part containing egg sacs was protruding and easily noticed by naked eyes. Similar clinical signs and postmortem change were noticed by as Abd El-Rahman (2000), Hoole *et al.* (2001), Woo (2006), Alishahi and Peyghan (2008) and Saleh (2009).

Intensity of adult Lernea was ranged from 1 to176 per fish. The light, moderate, heavy and extreme infestation rate was 7.5, 15, 40 and 37.5%, respectively. Lower intensity (10-20) was recorded by Saleh (2009). On the other hand an extreme higher intensity was noticed by Alishahi and Peyghan (2008) about 1462 in Bighead carp in Iran.

Regarding the distribution of adult metamorphosed females on the fish body surface, fins was the preferable sites fir infestation. Similar observations were recorded by Abd El-Rahman (2000) and Saleh (2009). Woo (2006) mentioned the distribution of the parasite is partly dictated by water temperature. Its optimum temperature is 26–28°C. Development is greatly reduced at lower temperatures; at 20°C nauplii take 7 days to molt into copepodids and they cease development altogether below this temperature.

The process of disease control is quite complex and depends upon the interplay of three factors: diagnosis, preventive measures and treatment. Once the diseases occur it is often too late to do anything to prevent losses. Thus preventive measures to alleviate diseases are fundamental (Tonguthai, 1997). Organophosphate insecticides were used for elimination of the infection. Treatment usually requires application at several occasions to break the life cycle of parasite because the embedded adult females are difficult to kill. Also, tissues residues associated with chemical treatment are of great concern. The development of lernaeids

resistance to insecticides, suggested the urgent need for long term solution such as vaccination (Hoole et al., 2001 and Woo, 2006).

Histopathological alterations associated with infestation showed that sloughing of most superficial layers of epidermis with edema under the dermis and leukocytic aggregation in the dermis. Similar pictures were noticed by Abd El-Rahman (2000), Hoole *et al.* (2001) and Woo (2006). The skin of fish acts as the first line of defence, protecting the animal against both the environment and infectious pathogens. Therefore, before an ectoparasite can establish itself on a host it must first disrupt the skin (Tosi, 2005). The skin epithelium of fish offers good possibilities for evaluating indirect stress effects of ectoparasites on their host (Nolan *et al*., 1999).

Quantification of blood flow in vessels provides valuable information that aids management decisions in a variety of circulatory conditions. Hematocrit is a percentage of red blood cells, so that it can compare the total volume of blood, any increase or decrease in plasma volume affects it. So, decreased hematocrit occurs during diseased conditions, which lowers the percentage of red blood cells in relation to the liquid plasma portion of blood. The obtained PVC results showed an increased in all the inoculated groups more than the control one and that is due to the stimulating of immune system given activation for the circulatory system and the immunized groups having more healthy conditions than the control agree with Caprette (1998).

The obtained results showed that significant protection was achieved by injection of L. cyprincea antigen. For confirmation the specificity of the obtained serum used AGPT as serological test determine the attraction between antigen and the specific antibodies. The obtained result showed line of demarcation between the prepared antigen and the collected serum agree with (Spielberg *et al.,* 1989). Also, histopathological finding showed the immune response against infestation

of the Lernea in form of fibrosis and infiltration of white blood corpuscles in the site of invasion of the parasite agree with Paperna (1996).

Hoole *et al*. (2001); Woo (2006) and Tasawar *et al.* (2007) mentioned that vaccination with this antigen resulted in lower attachment rates and establishment of the parasite on the host when compared to control groups. The higher antigen inoculated group wasn't more protected than lower one agree with (Stoscheck 1990).

Parasites on recovered fish produced fewer eggs and the resultant larvae were less infective than those from females on naïve fish (Woo and Shariff, 1990). Shariff *et al.* (1986) found that, after an epizootic of L. cyprinacea in a display aquarium, 18 out of 23 fish species had apparently acquired resistance by the time of a second outbreak 6 months later. Few new infections developed in experimentally infected Helostoma temmincki that had recovered from an earlier infection. Fish that became infected lost their infections rapidly.

On the contrary, no immunity was acquired by Javanese carp, Puntius gonionotus, against subsequent infection by L. minuta, possibly because the parasite does not penetrate far Phylum Arthropoda into the tissue and stimulates little or no inflammatory reaction (Kularatne *et al.*, 1994).

Further research and testing of vaccine in other fish species and under field conditions is indicated.

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Table (1): Showed intensity of *Lernaea cyprinacea* among the examined C. carpio.

Fish sp.	No.	Light		Moderate		Heavy		Extreme	
		No.	%	No.	%	No.	%	No.	%
C. carpio	200	15	7.5	30	15	80	40	75	37.5

Table (2): Showed distribution of *L. cyprinacea* on the external body surface of the examined *C. carpio*.

Body surface	Mouth	Eyes	Head	Fins					Body sides	Urogen -ital
				Dorsal	pectoral	pelvic	anal	tail		
Total No. of adult *Lernea*	15	9	65	40	30	36	15	80	160	6
% of adult *Lernea*	3.3	2.0	14.3	8.8	6.6	7.9	3.2	17.5	35.1	1.3

Table (3): Showed PCV value.

Fish Group	**1st**	**2nd**	**3rd**	**4th**
PCV	25.47** ±0.59	23.93** ±0.93	24.14* ±0.93	22.79* ±0.8

Table 4: Showed infestation rate among immunized fish challenged with *L. cyprincea* parasite.

Gp. No.	**No. inj. fish**	**Injected conc.**	**Infestation rate**	
			No.	**%**
1	25	10	13	52
2	25	20	3	12
3	25	30	4	16
4	25	0	24	96

Table (5): Shwed recovery rate of infested fish after inoculation with immunized serum.

Gp. No.	No. inj.	Injected Dose	Recovered	
			No.	%
1	10	10	4	40
2	10	20	6	60
3	10	30	8	80
4	10	0	1	10

Table 6: Showed infestation rate among health fish inoculated with immunized serum**.**

Gp. No.	No.	Injected Dose	Infestation rate	
			No.	%
1	10	0.1 ml	5	50
2	10	0.2 ml	2	20
3	10	0.3 ml	1	10
4	10	0	9	90

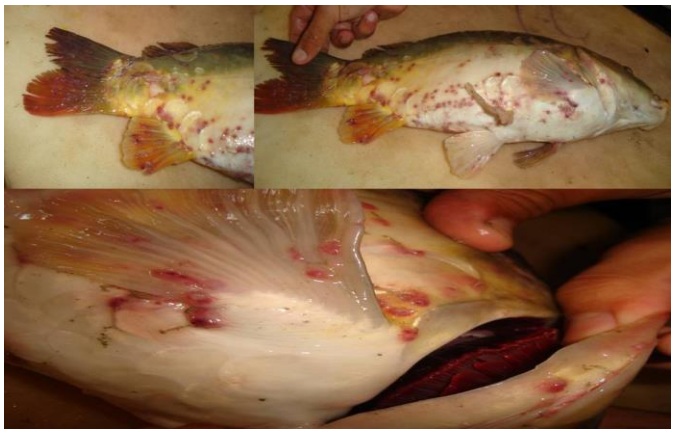

Fig. (1): *C. carpio* infested with *L. cyprincea*.

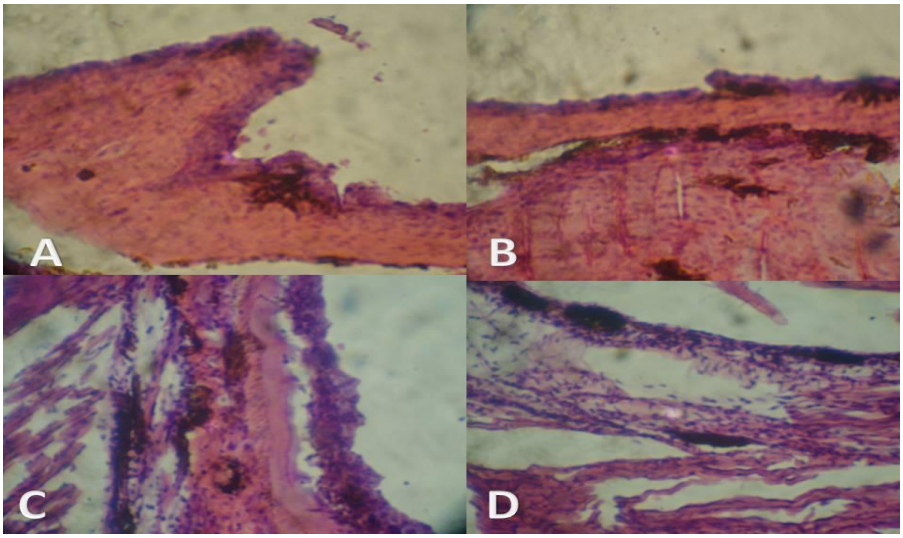

Fig. (2): Histopathological changes associated with *L. cyprincea* infestation. (A&B) Sloughing of superficial layer of epidermis and aggregation of melanomacrophages. (C&D) Edema, necrosis and leukocytic infiltration in dermis.

دراسات على الأصابة بطفيل اللرنيا بين أسماك المبروك العادى مع التركيز على تحصينها للوقاية

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قسم أمراض الأسماك – المعمل المركزى لبحوث الثروة السمكية بالعباسة–
مركز البحوث الزراعية

تعتبر الإصابة بطفيل الليرنيا من أهم المشاكل التى تواجه الاستزراع السمكى فى كثير من البلدان حيث يتسبب فى نفوق أعداد كثيرة من الأسماك المصابة ، انخفاض فى معدلات النمو ،تردى فى جودة الأسماك المنتجة. وفى الدراسة المقدمة تم تجميع طفيل الليرنيا من جسم ٢٠٠ سمكة من أسماك المبروك العادي والتي ظهر عليه من خلال الفحص الظاهرى: إنخفاض فى استهلاك العلف، وجود كميات كبيرة من المخاط على جلد السمكة، نحافة الجسم، محاولة حك الجسم بالأجسام الصلبة بالحوض. وظهر بمكان اختراق الطفيل لجسم السمكة كثير من الأنسجة المتهتكة ووجود قرح نزيفية يصل قطرها إلى ٦مم. وتراوح معدل تواجد الديدان اليافعة للطفيل من ١ – ١٧٦طفيل على السمكة الواحدة وكانت بنسبة ٧.٥% فى الإصابة البسيطة ، ١٥% فى الإصابة المتوسطة، ٣٧.٥% فى الإصابة الشديدة ،٤٠% فى الإصابات الكثيفة وكان التواجد الأكثر للطفيل على زعانف الأسماك. وأوضح فحص الأنسجة المصابة وجود تآكل لمعظم الطبقة السطحية لقشرة الجلد مع وجود انتفاخ مائي لطبقة الأدمة وتواجد كثيف لخلايا الدم البيضاء خاصة الخلايا البالعة. تم تقسيم ١٠٠ سمكة مبروك عادى إلى ٤ مجموعات متساوية وموزعة بصورة عشوائية وتم حقن ثلاثة منها بلقاح محضر من الديدان اليافعة بجرعات ١٠،٢٠،٣٠ مج من البروتين/جم من الأسماك على التوالى وترك المجموعة الرابعة كمجموعة ضابطة للتجربة. وبعد أسبوعين تم حقن جرعة منشطة بنفس التركيز. وبتعريض المجموعات إلى العدوى الصناعية تلاحظ وجود نسبة حماية معنوية للمجموعات حيث كانت نسبة الإصابة ٥٢، ١٢، ١٦% على التوالى بالمقارنة بالمجموعة الضابطة التى كانت نسبة الإصابة بها ٩٦%. تم التأكد من وجود الجسام المناعية الخاصة بالطفيل من خلال اختبار AGPT. تم تجميع المصل من المجموعة الثانية والتي أظهرت أعلى معدل من مقاومة الإصابة وتم حقنه بجرعات ٠.١،٠.٢،٠.٣ ملى، كل جرعة مجموعتين أحدهما مصابة والأخرى تعرض للإصابة بعد الحقن ومع ترك مجموعة ضابطة لكل معاملة. أوضحت النتائج نسب تعافى ٤٠،٦٠،٨٠% فى المعاملة الأولى بالمقارنة مع المجموعة الضابطة ١٠%. وفى المعاملة الثانية كان قبول الإصابة بنسب ٥٠،٢٠،١٠% بالمقارنة مع المجموعة الضابطة التى كانت ٩٠%. وتوصى الدراسة باستكمال تطوير اللقاح واختباره على أصناف أخرى من الأسماك فى ظروف التربية الحقلية.