THE RELATIONSHIP BETWEEN *LERNAEA CYPRINACEA* INFESTATIONS IN *CYPRINUS CARPIO* AND ITS CONCURRENT BACTERIAL DISEASES IN EGYPT.

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Abstract

This study will focus on the potential infestation with Lernaea *cyprinacea* for concurrent infections with bacterial infection. Prevalence, seasonal variation, parasites burden, site of infestation and mean intensity of *Lernaea cyprinacea* were monitored on *Cyprinus carpio* from six fish farms during deferent seasons in Egypt.

Two bacterial isolates were recovered from *Cyprinus carpio* which pronounced infested with *Lernaea cyprinacea* identified as *Flavobacterium columnare*, and *Pseudomonas fluorescens.*

The peak of infestation were in summer (81.7%) and then declined to (70%) in autumn follow by spring (55.7%) and no recorded infestation in winter (0%).

The differences in the infestation with copepods may be depending on the body length where the highest infection rate and intensity in 3- 8 cm which up to 35 cm with copepods in fish was moderate. The Relationship between body weight and *L.cyprinacea* in fish was also calculated. According to these results, the *L.cyprinacea* had significantly ($P<0.05$) highest prevalence in weight group of 0.5-399gm and lowest in weight groups of 400-899gm followed by <1500gm.

No bacterial isolates were found in trails to culture from gut of parasites.

Clinicopathological detection were found that *L. cyprinacea* attached to the base of the fins as a most common

side of attachments followed by gills, head and fish body .the gills were the common site of immature stages.

The objective of the study concluded that Lernaea parasites not a vector to bacterial diseases of fish but mechanically increase the transmission efficiency of bacterial pathogens by creating many portals of entry.

INTRODUCTION

Lernaeosis is caused by the parasite copepod lernaea spp. Is a universal in distribution through translocation of edible and ornamental fish mostly due to lack of adequate sanitary control during fish transportation Raghavendra *et al.* (٢٠١٢).

Economic losses due to ectoparasitic infestation not only result from direct harm to the fish, but also from disfigurement tissues which renders food fish unsuitable for sale, and thus impose a big loss to fishing industry (Piasecki *et al*., 2004).

Copepods are the most numerous among parasitic crustaceans and may be the most common group of fish parasites. They have been found parasitizing skin, gills, eyes, fins and even inside the mouth of fishes, near the palate and nostrils (Eiras, 1994).

Among the well known copepods in Egypt, the most observed species is the exotic *Lernaea cyprinacea*. *L. cyprinacea* is a cosmopolitan species that infects a wide range of fish and even tadpoles, does not reproduce at less than 14°C (Paperna 1991) its optimum Temperature is 26–28°C (Shields and Tidd, 1968).

Most bacterial pathogens of fish are aerobic gram-negative rods; diagnosis is by isolating the organism in pure culture from infected tissues and identifying the bacterial agent (Woo and Burno, 1999).

Flavobacterium columnare pathogen has been recognized to have a worldwide distribution in a wide range of freshwater fish, while *Pseudomonas fluorescence* pathogen is likely to be spread through

water, which will serve as the primary reservoir of infection (Austin and Austin, 2007).

Pseudomonades exist throughout the aquatic environment and are associated with both healthy and diseased fish. It is generally believed that these bacteria can be opportunistic pathogens or produce damaging as secondary invaders. They are aerobic Gram-negative rods, cytochrome oxidase- positive (Woo and Burno, 1999).

Recently, significant advances have been made in knowledge of the possible vector relationships of the parasitic crustacean transmitting disease organisms to fish (Ahne, 1985).

Fish themselves have been regarded as the main vectors in the transmission of bacterial and viral diseases from resistance fish for susceptible species. However, Nese and Enger (1993) isolated *Aeromonas salmonicida* from *Lepeophtheirus salmonis*, also Ahne (1985) reported that *Argulus foliaceus* to be a mechanical vector of spring viremia of carp virus (SVCV). Dombrowski (1951) presented data indicating that *Argulus* spp. as transmitting the bacterium *Aeromonas punctata* to carp.

Cusack and Cone (1986) concluded that parasite vectors increase the transmission efficiency of pathogens by creating portals of entry and/or by having the ability to transfer pathogens directly from fish to fish. Other studies suggest different mechanisms. Tully and Nolan (2002) suggested that increase host susceptibility as a result of increased stress responses by copepods infestation and linked to decreased disease resistance.

Most studies focused on a single parasite or a single bacterial pathogen. Mean while in aquaculture, the reality of a single disease agent resulting in death-loss may be small. More likely, multiple disease

agents are present (i.e., parasites, bacteria and/or a combination) which responsible for mass losses. (Shoemaker *et al.*, 2008).

The objectives of this study are focus on the potential for concurrent infection between Learnae parasite and bacterial pathogens. In addition to study of Prevalence, burden, and mean intensity of *Lernaea cyprinacea* on *Cyprinus carpio* from different fish farms during deferent seasons in Egypt.

MATERIALS AND METHODS

1- Clinical and postmortem examination of naturally infected fishes:

A total number of 335 cultured *Cyprinus carpio* were collected from different six fish farms at sharkia, beni seuif and kafr elshaikh governorates during four seasons. Fish were transported alive in plastic bags with two thirds with water provided battery air pump to restore the oxygen need to the Fish Disease Department lab in Animal Health Research Institute, Fish examined clinically in glass aquaria immediately after reached. All fish were measured for total length (cm), weighed (gm) and examined for external parasites (*Lernaea cyprinacea*). Fish were subjected to clinical signs, postmortem changes, bacteriological and parasitological examination according to Noga (1996).

2- Bacteriological examination:

A-Fish.

Samples from internal organs of examined fishes were streaked onto nutrient agar, trypticase soy agar, Rimler- Shotts medium (RS) plates then incubated at 28ºC for 24-48 hr. The growing colonies were picked up in pure form and reinoculated into trypticase soy agar for further identification. Identification of all isolates was done by cultural, morphological and biochemical characters according to Quinn *et al.* (2002) and Austin and Austin (2007) and through using API-20E (Biomérieux) for gram-negative fish pathogen.

B- *Lernaea cyprinacea* parasite.

lernaea parasites were collected from infected fish and dissected. Samples were taken under complete aseptic conditions from the gut of copepods and inoculated into Tryptic Soya agar and incubated at 22ºC for bacteriological examination according to the procedure described by Nylund *et al.* (1993).

3- In-vitro sensitivity test of pathogenic bacterial agents:

It was carried out against various chemotherapeutic agents and judgment of the obtained results in comparison to interpretive standards was applied as described by Koneman *et al*. (1992) and Quinn *et al.* (2002).

4- Parasitological examination:

External and Internal examination of collected fish for any abnormalities were done. Fish examined for the presence of parasitic copepod or its parasitic stages. The parasitic intensity and favorite site of infection were recorded .Parasites were removed out of the fish's structures, where they were attached with the help of fine forceps, and put into bottles with 10% formal saline solution. The parasites were prepared on a glass slide and identified using the characteristics given in keys by Kabata (1985).

RESULTS

1- Clinical and postmortem examination of naturally infected fishes:

Naturally infected fish with Learnaeid anchor worms showed hemorrhages and ulceration at attachment sites of parasite. Erosion of the skin layers with loss of scales was present as results of feeding activity by adult and pre-adult stages for fish gills, fins and skin.

In severe cases, anchor worms embedded deeply into skin and underlying musculature with extensive haemorrhage and underlying

muscle may be exposed. In some case deep lesions have been established till parasites reach internal organs and damage. In some case, aggregation of immature stages of parasites found on gills causing hemorrhages and damage of gill lamellae.

Naturally infected fish with bacteria *showed* hemorrhages all over the fish body especially at the base of fins, tail and fins rot, and congested gills, detachment of scales and skin ulceration and abdominal distention. Internally these fishes showed abdominal dropsy with reddish ascetic exudates, liver paleness and enlargement in some fishes and congested with necrotic patches in other fishes, spleen was congested and enlarged and hemorrhagic enteritis in some fishes.

2- The bacteriological examination.

A- Fish: Two bacterial isolates recovered from *C.carpio* infested with *Lernaea cyprinacea* were *Pseudomonas fluorescens* and *Flavobacterium columnare,* with prevalence (71)21.2 %,(30)8.9% respectively and mixed infection of parasites and two isolated bacteria were 25(7.5%) (Table, 1).

B- *L.cyprinacea* parasites.

Trail to culture from gut of lernae parasites(N=50) isolated from infected fish with parasites and bacterial infection single and/or mixed were found that there is no bacterial growth from parasites gut after applying 70% ethanol to the outer surfaces of the parasites .

3- In-vitro Sensitivity of *Pseudomonas flourescens* and *Flavobacterium columnare* to different chemotherapeutic agents.

In – vitro sensitivity tests of isolated bacterial agents revealed that all tested isolates were highly susceptible to Colistin sulphate, Danofloxacin, Gentamycin , Nalidixic acid, oxolonic acid and Oxytetracycline (Table, 2).

Table 1: The relationship between *L. cyprinacea infestations* and subsequent bacterial infection in *C. carpio.*

Total No. of examined *C. carpio*	Free of infection		Single infection						Mixed infection					
			Lernaea		Pseudomonas		Flavobacteria		Lernaea + pseudomonas		Lernaea + flavobacteria		Lernaea + Pseudo. + Flavo.	
335	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	90	26.9	60	17.9	34	10.1	25	7.5	71	21.2	30	8.9	25	7.5

Table (2): In-vitro sensitivity of *Pseudomonas flourescens* and *Flavobacterium columnare* to different chemotherapeutic agents.

Chemotherapeutic agents	Concentration per disc	Isolates	
		Ps. florescence	*Fla. columnare*
Amoxycillin	١٠µg	R	S
Cephalothin	٣٠µg	R	S
Colistin sulphate	٢٥µg	S	S
Danofloxacin	٥µg	S	S
Erythromycin	١٥mcg	R	S
Gentamycin	١٠µg	S	S
Lincomycin	١٠µg	R	S
Nalidixic acid	٣٠µg	S	S
Nitrofurantoin	٣٠٠µg	R	S
Oxolonic acid	٢µg	S	S
oxytetracycline	٣٠µg	S	S
Sulphamethoxazole 23.7ug/ Trimethoprim ١٠.٢٥ug	٢٥µg	R	S

4- The relationship between *L. cyprinacea infestations* and bacterial infection in *C. carpio*

Out of 335 fish examined, 60 (17.9%) were found to be infested with the single parasites and 59 (17.6%) were found to be infected with single bacteria. Thus, the overall prevalence of *L. cyprinacea* was (186) 55.5%, (Table 1).

5- The Relationship between body length and body weight of *Cyprinus carpio* infested with *L. cyprinacea*.

The relationship between body length of *Cyprinus carpio* and *L.cyprinacea* parasite was recorded and according to these results, *Lernaea* had highest prevalence in length group of 3-8 cm as compared to specimens with body length 8.5-20cm and 20.5-35cm . The two latter groups did not differ from each other in this index Table (3).The result of Relationship between body weight and *L.cyprinacea* in *C. carpio* showed that *L.cyprinacea* were significantly (P<0.05) highest prevalent in the body weight group of 0.5-399gm and lowest in weight group400-899 gm and >1500 (Table 4).

Table 3: Relationship between body lengths *of C.carpio* and *Lernaea cyprinacea* infestation.

parasites	***C.carpio*** **body length** **٨-٣cm**			***C.carpio*** **body length** **٢٠-٨.٥cm**			***C.carpio*** **body length** **٣٥-٢٠.٥cm**		
	No. of exam	**No. of inf.**	**%**	**No. of exam**	**No. of inf.**	**%**	**No. of exam**	**No. of inf.**	**%**
L.cyprinacea	134	106	78.5*	100	41	41	100	39	39

Significantly (P<0.05)

Table 4: Relationship between body weights *of C.carpio* and *Lernaea cyprinacea* infestation.

parasites	*C.carpio* body weight ٣٩٩-٠٠٥gm			*C.carpio* body weight ٨٩٩-٤٠٠gm			*C.carpio* body weight ١٥٠٠-٩٠٠gm		
	No. of exam	No. of inf.	%	No. of exam	No. of inf.	%	No. of exam	No. of inf.	%
L.cyprinacea	185	126	68.1*	100	43	43	50	17	34

Significantly (P<0.05)

6- The Relationship between seasonal variation and *L. cyprinacea* infestation.

Prevalence of the infection in four seasons was recorded in table (5). The peak of infestation were in summer (81.7%) and then declined to (70%) in autumn followed by spring (55.7%) and no recorded infestation in winter (0%). Most of the parasites observed in spring and autumn were Cyclops stage with adult in contrast to a single adult stage in the summer samples. Direct evidence of mortality was observed in summer only. The parasites burden and favorite site of infestation in *C.carpio* were recorded in table (6).

Table 5: Seasonal variations of lernaea infestation.

Season	No. of examined fish	No. of infested fish	%
Winter	75	0	0
Spring	70	39	55.7
Summer	120	98	81.7
Autumn	70	49	70

Table (6): Favorite site of infestation of *C.carpio* with *L. cyprinacea* and Parasites burden.

Favorite site of infestation	No. of examined fish	No. of infested fish		Mean parasite load
		Single Site	More than one site	
Bases area of fins	335	50	30	36
Fish body		6	40	48
Head		15	6	6
Gills		39	-	5/MF*

/MF* =per Microscopic Field (Cyclops stage)

DISCUSSION

Ectoparasites were as a vector and / or possible reservoir in the transmission of disease organisms to fish. Heavy infection by parasitic crustaceans may cause general debilitation of the fish, in addition the integument wounds made by the parasites often penetrate deep into the subcutaneous tissue undoubtedly aid secondary microorganisms in establishing infection in the host (Hopla *et al.,* 1994).

Lernaea species occur on all continents, with the majority in Africa. The only cosmopolitan species is *Lernaea cyprinacea*, which can infect a variety of freshwater fishes (Piasecki *et al*., 2004).

Originally, *L. cyprinacea* was not present in Egypt, but it was accidentally introduced with imported genetically selected stocks of cyprinids (Faisal *et al*., 1988).

In the present study, naturally infested fish with anchor worms showed hemorrhages, ulceration at attachment sites of parasite and Erosion of the skin layers with loss of scales. In some case, aggregation

of immature stages of parasites found on gills causing hemorrhages and damage of gill lamellae.

This results coincided with Kabata (1995); Noga (1986) and Tasawar *et al.* (2007).

From the point of our work, *L.cyprinacea* was isolated from examined fish with 186(55.5%), and about 126(37.6%) from them were mixed infection of parasites and bacterial infection single isolate and/or mixed with two isolates *Flavobacterium columnare,* and *Pseudomonas fluorescens.* No bacterial growth was found in the trails to culture from parasites. Kabata (1985) indicated that there was little concrete evidence of crustacean parasites transmitting pathogenic organisms to fish, but cited Dombrowski (1951), who reported *Argulus* spp. as transmitting the bacterium *Aeromonas punctata* to carp. Noga (1986) suggested that the wounds caused by the copepod allowed in bacteria and fungi and thus initiated disease. (Cusack and Cone, 1986) add that ectoparasites increase the transmission efficiency of pathogens by creating portals of entry and/or by having the ability to transfer pathogens directly from fish to fish.

Nese and Engero (1993) found that *Lepeophtheirus salmonis* (salmon lice) remained infected with *Aeromonas salmonicida* after applying 70% ethanol to the outer surfaces of the parasites , suggesting to these authors that *A. salmonicida* was residing inside *L.salmonis,* probably in the gut .

The relationship between body length and weight in *C. carpio* infestes with *L.cyprinacea* in was calculated and according to these results it appears that as the length and weight of the fish increased, infestation of copepod Lernae decreased. These results agreed with Bart *et al*. (2002) and Tasawar *et al*. (2007) who suggested that it may be due to the development of acquired immunity in old fish.

Regarding the seasonal prevalence of *L.cyprinacea* infestation in examined *C. carpio*, it was 98, 49, 39 and 0% in, summer, autumn, spring and winter respectively. Copepods were observed parasitizing fishes only when water temperatures exceeded 25ºC and reproduction usually occurs (Tasawar *et al.*, 2009). These obtained results were nearly similar to (Medeiros and Maltchik, 1999 and Bart *et al*., 2002) who reported that the highest peaks of *L.cyprinacea* infestation were during summer. On other hand, these results were in disagreement with Robinson *et al*. (1996) who recorded that the prevalence of Lernaea parasitism is less than 20% during the summer season.

L. cyprinacea typically were found attached to the base of the fins as a most common side of attachments followed by gills, head and fish body .the gills were the common site of immature stages. Bart *et al*. (2002) suggest that Attachment of *L. cyprinacea* at the base of fins is common because fins provide the parasite protection from water current and abrasion. Furthermore, (Bulow *et al*., 1979) added that tissues at the base of fins might be penetrated more easily than those at other locations along the body.

The severity of columnaris disease is influenced by a multiplicity of environmental stress, water temperature and host- related factors (Austin and Austin, 2007).

Pseudomonas fluorescence was isolated from half of the cases of carp fish suffering from skin ulcer. It is generally considered to be an opportunist pathogen and found as a result of secondary invasion of ulcers (wildgoose, 1998).

We concluded that the diseases problem caused by parasitic organisms is the main threat to further increase of the aquaculture industry. The single causative agent is rare, because the actual reports exist in the reviewing literature of diseases related to conditions induced by Lernae ectoparasites and bacterial infection is rare. The existence of

bacterial organisms associated with fish species in fish farming practices has been documented while the concurrent parasitic infection need further studies to clarify the possible role of vector relationships between the parasitic crustacean infestation and bacterial diseases in fish host.

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الأصابة بالليرنيا فى اسماك المبروك المستزرعة و تزامن الاصابة البكتيرية

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الملخـــص العربـــى

تم فى هذا البحث دراسة امكانية اصابة اسماك المبروك العادى بطفيل الليرنيا مع تزامن الأصابة البكترية ودور الطفيل فى نقل العدوى البكترية.

أجريت هذه الدراسة على عدد ٣٣٥ سمكة من اسماك المبروك العادى التى تم جمعها من مزارع سمكية فى محافظات مصر المختلفةعلى مدار عام كامل. وقد تم دراسة نسبه وشدة الأصابه بطفيل الليرنيا ومكان الأصابة وعلاقة الأصابة بالتغيرات الموسمية وعلاقتها بأطوال الأسماك واوزنها وعمل عزل بكتيرى للمسببات المرضية البكترية المصاحبة.

وقد تم عزل المسببات المرضية البكترية من انواع الفلافوبكتريم كولمينارى والسيدوموناس فلورسنت من الأسماك المصابة بطفيل الليرنيا مجتمعة (٧.٥%) ومنفردة (٨.٩%، ٢١.٩%) على التوالى.

تم عزل طفيل الليرنيا من الأسماك بنسبة ٥٥.٥% منهم ١٧.٩% كانت مصابه بالطفيل وخالية من المسببات المرضية البكترية.

ولمعرفة دور الطفيل فى نقل العدوى البكترية للأسماك تم الزرع البكتيرى من داخل الأنبوبة الهضمية للطفيل وكانت النتائج سالبة.

وقد أكد البحث على عدم وجود دور للطفيل فى نقل العدوى البكترية للأسماك ولكن دوره كان فتح الطريق للأصابة البكترية ونرى ضرورة عمل دراسات تكميلية لمعرفة اسباب الربط بين هذان النوعان من البكتريا خاصة والاصابة بالطفيل.