Resistance of common carp fishes *Cyprinus carpio* (L.) to re-infection by anchor worm *Lernaea cyprinacea* (L.)

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Abstract: The resistance of common carp *Cyprinus carpio* to infection with *Lernaea* cyprinacea was investigated under laboratory conditions in 10 weeks. Recovered fish group1 RG1 (fish with naturally previously infection by Lernaea cyprinacea and use later in next experiment after week from complete recovery) were most resistant to infection 50% of fishes were infected experimentally in 5th week after fishes exposure to infective stages of parasite followed by Recovered fish group2 RG2 (fish with naturally previously infection by Lernaea and use later in next experiment after month from complete recovery) 72.7% of fishes were infected experimentally in 5th week then Control fish group CG (fish without previously infection by Lernaea) 78.5% of fishes were infected experimentally in 5th week. the role of resistance on reproductive capacity of parasite and rejected for parasite by host were studied, percent of parasites have egg sacs in CG were more (84.6%) than other two groups in 5th week after fishes exposure to infective stages of parasite 44.4% and 69.2% in RG1 and RG2 respectively, 100% of parasites were lost from RG1 by 9th week after fishes exposure to infective stages of parasite, 100% of parasites were lost from RG2 by 10th week, while 91.3 of parasites were lost from CG by 10th week. these data support the hypothesis that infection with Lernaea cyprinacea produce acquired immunity to common carp fish against this parasite but this immunity does not persist for long and is not capable of preventing subsequent infection (re-infection).

Introduction

The anchor worm *Lernaea cyprinacea* (L.) causes high mortalities in culture fresh water fish, adult parasites are particularly harmful to young fish because of their relatively large size and their mode of attachment and feeding, also they may transmit viruses and bacteria which result in secondary infection (1).

In Iraq the parasite was first observed in April 1969, in Zaafaraniyah fish culture station, on the Tigris river, 15 Km south of Baghdad on seven fish species (2) and then successive it recorders in many studies.

eradication of *Lernaea* infections in fish in large water bodies is probably impossible. Many types of chemical treatments have been tried against L. cyprinacea in aquaculture ponds, but with limited success. Many of these treatments also have potential negative environmental impacts and are not suitable for use on fish destined for human consumption. However, as they are not effective against the free swimming naupliar stages or the embedded adult parasites, the treatment must therefore be directed to kill the free-swimming copepodid stages. More environmentally friendly methods include fallowing of ponds and the use of salt treatments. Based on the timing of the life cycle, Shariff et al. (3) suggested that leaving ponds fallow for at least seven days should eliminate all infective stages. In some cases, salt (NaCl) has been recommended to kill free-swimming larval Lernaea (4). However, other studies have shown that most fish cannot tolerate salt for periods long enough to kill the parasites. Almost all the above treatments require caution in application and persist undesirable. Alternative approaches might be realized by understanding the nature of immune response of fish to the parasite. Susceptibility of fish to ectoparasites can be influenced by resistance mechanisms of innate and acquired immunity (5; 6).innate resistance against ectoparasites copepods has been demonstrated for different salmonid species (7). However, the relative importance of innate and acquired immune responses in the relationship between anchor worm and their hosts is not well

understood, Some evidence of acquired immunity exists (4; 1) the aims of this study were to determine the degree of fish resistance which result in infection by parasite .

Materials and Methods

Three groups of fishes Cyprinus carpio (each group consisted of 15 fishes ranged 16-20 cm length) were sampled at Al-Furat fish farm in Hilla city, Babylon province by three times, first group named RG2 (fish with naturally previously infection by Lernaea and use later in next experiment after month from complete recovery) were collected in the 10th May 2008, second group named RG1 (fish with naturally previously infection by Lernaea and use later in next experiment after week from complete recovery) were collected in the 3rd June 2008 and third group named CG control fishes group (fish without previously infection by Lernaea) were collected in 3rd July. The first and second groups were left for one month to complete recovery. The experiment was started in 10th July, the numbers of fish in three groups in this date become as following RG2,11 : RG1, 14 : CG, 15 as result of death. Prior to the experiment, fish were checked for any attached Lernaea to make sure that they were naive in respect to Lernaea infection, the experiment were performed in aerated 30 liter glass containers, with water temperature at the start of the experiment was 20°C and 24°C at the end. The fish were maintained under a fed standard fish diet at 1% body weight once daily. The methods for obtaining the infective stages to parasite and for carrying out the infections are given in Shields (8).three sets of egg sacs were carefully removed from adult female copepods first set involve 11 egg sacs, second set involve 14 egg sacs and third set involve 15 egg sacs were design to RG2,RG1 and CG respectively (according to the number of fish in each group, one egg sac for each fish) and placed in three covered culture dish and incubated at 29°C for 24 hours to hatched and produce nauplii stages, after hatching were left for 48 hours to complete molting and produce copepods stages which regard as infective stages and introduced it into three tanks which containing RG2, RG1 and CG. The number of adult parasites and the number of egg sacs on each fish were noted weekly by visual examination and recorded for 10 weeks.

Results

Recovered fish group1 RG1 (fish with naturally previously infection by *Lernaea cyprinacea* and use later in next experiment after week from complete recovery) were most resistant to infection 50% of fishes were infected experimentally in 5th week after fishes exposure to infective stages of parasite followed by Recovered fish group2 RG2 (fish with naturally previously infection by *Lernaea* and use later in next experiment after month from complete recovery) 72.7% of fishes were infected experimentally in 5th week then Control fish group CG (fish without previously infection by *Lernaea*) 78.5% of fishes were infected experimentally in 5th week. as showing in table (1).also the number of parasites or intensity of infection for each groups of fishes were different over time both RG1 and RG2 groups had fewer parasites at five weeks 9 parasites per 6 fishes and 13 parasites per 8 fishes when compared to CG group 26 parasites per 11 fishes table (1).two fishes were died during five weeks one fish died have 2 parasites in CG group and one fish died have no parasites in RG1 group.

Time \ Week	CG				RG1		RG2			
	No. fishes	Fishes infected %	No. parasites	No. fishes	Fishes infected %	No. parasites	No. fishes	Fishes infected %	No. parasites	
1st	15	0	0	14	0	0	11	0	0	
2nd	15	13.3(2)	5	13	0	0	11	9(1)	1	
3rd	15	46.6(7)	20	13	23(3)	5	11	45.4(5)	8	
4th	14*	78.5(11)	26	13	46.1(6)	8	11	63.6(7)	12	
5th	14	78.5(11)	26	12**	50(6)	9	11	72.7(8)	13	

tabel(1):number of fishes infected and number of parasites in three groups of fishes during 5 weeks (1^{st} - 5^{th} week from start of experiment).

* fish died have 2 parasites ** fish died have no parasite

reproductive capacity represented by present of egg sacs on parasites also were different in all groups as showing in table (2), percent of parasites have egg sacs in CG were more (84.6%) than other two groups in 5th week after fishes exposure to infective stages of parasite 44.4% and 69.2% in RG1 and RG2 respectively.

Table(2):number of egg sacs (pair) and percent of parasites which have egg sacs in three groups of fishes during 5 weeks ($1^{st}-5^{th}$ week from start of experiment).

	CG		R	G1	RG2		
Time \ Week	No. of egg sacs(pair)	Parasites have egg sacs %	No. of egg sacs(pair)	Parasites have egg sacs %	No. of egg sacs(pair)	f Parasites have egg air) sacs %	
1st	0	0	0	0	0	0	
2nd	2	40	0	0	0	0	
3rd	8	40	0	0	1	12.5	
4th	20	76.9	2	25	7	58.3	
5th	22	84.6	4	44.4	9	69.2	

Number and percent of accumulate rejected parasites during five weeks (6th-10th weeks) in three groups of fishes are present in table (3), 100% of parasites were lost from RG1 by 9th week after fishes exposure to infective stages of parasite, 100%

of parasites were lost from RG2 by 10th week, while 91.3 of parasites were lost from CG by 10th week. two fishes were died during five weeks one fish died have 3 parasites in CG group and one fish died have 2 parasites in RG2 group.

	CG				RG1		RG2		
Time \ Week	No. fishes	No. accumulate rejected parasites	accumulate rejected parasites %	No. fishes	No. accumulate rejected parasites	accumulate rejected parasites %	No. fishes	No. accumulate rejected parasites	accumul ate rejected parasites %
6th	11	2	7.6	6	1	11.1	7**	1	9
7th	11	4	15.3	6	5	55.5	7	4	36.3
8th	10*	10	43.4	6	7	77.7	7	6	54.5
9th	10	15	65.2	6	9	100	7	8	72.7
10th	10	21	91.3	6	9	100	7	11	100

Table(3):number and percent of accumulate rejected parasites in three groups of fishes during 5 weeks (6th-10th week from start of experiment).

* fish died have 3 parasites

** fish died have 2 parasites

Discussion

The infection rate and total number of parasites in recovered fish group1(RG1) during five weeks were fewer than other two groups may be due to the previous infection show the development of a protective acquired resistance in common carp against *Lernaea cyprinacea*, this hypothesis supported by Woo & Shariff (1) when found similar results to this parasite on Kissing gourami *Helostoma temmincki*. on other hand the results by study Bandilla *et al.* (9) did not show the development of a protective acquired in rainbow trout *Oncorhynchus mykiss* against juvenile *Argulus coregoni* infection, and they postulate that movement of fish is probably the main factor that may have increased or decreased attachment success of *A. coregoni* during the exposure to infective stages. However all the above studies did not carried out on common carp but on other species of fishes, hence the compare between these results and the result of present study are difficult since the resistance to parasites depend on fish species (7).

The protective mechanism in the RG1 and RG2 were also directed against parasite reproduction, the parasite in these two groups have fewer egg sacs by contrast with CG group, Paperna and Zwerner (10) reported for *Ergasilus labracis* on stripped bass *Morone saxatilis* that a well developed tissue response leads to the interruption of parasites egg sac production and an apparent increase in the rate of detachment of copepods. Woo and Shariff (1) reported for *Lernaea cyprinacea* on Kissing gourami *Helostoma temmincki* that a higher proportion of egg sacs are lost from copepods growing on previously exposed fish than naive fish. Furthermore, eggs from copepods growing on previously exposed hosts either fail to develop or produce copepodids that have a low infectivity when compared to copepodids hatched from eggs of copepods growing on naive hosts.

In this study the rejection of *Lernaea cyprinacea* from skin of fishes were found in all groups during $6^{th} - 10^{th}$ week after fishes exposure to infective stages of parasite these rejection may be caused by active host rejection or mortality of the copepods independent of any host response. Host rejection of the *Lernaea cyprinacea* has been reported in both naive and previously exposed fish (**11**;**1**), but the elimination of copepods from the skin of RG1 groups were faster than other groups, the reason may belong to specific immunity that established by previously exposed to *Lernaea*. However, rejection of these copepods is believed to be due in part to cellular and inflammatory responses, these responses occurring in attachment site of parasites during infection by it (**12**; **13**) or possible removal by the fish rubbing their bodies against the tanks.

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مقاومة اسماك الكارب الاعتيادي (.L.) Cyprinus carpio للإصابة الراجعة بالدودة الكلابية cyprinaea مقاومة اسماك الكارب الاعتيادي (.L.)

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الخلاصة: تم التحري عن مقاومة اسماك الكارب الاعتيادي Cyprinus carpio للإصابة بالطفيلي Lernaea cyprinace تحت ظروف مختبرية في عشرة أسابيع. مجموعة الأسماك ألمتعافية RG1 (اسماك مصابة مسبقا بصورة طبيعية باللرنيا واستخدمت فيما بعد في التجربة اللاحقة بعد أسبوع من الشفاء التام) كانت أكثر مقاومة للإصابة 50% من الأسماك أصيبت تجريبيا في الأسبوع الخامس بعد تعرض الأسماك للأطوار المعدية للطفيلي تلتها مجموعة الأسماك المتعافية RG2 (اسماك مصابة مسبقا بصورة طبيعية باللرنيا واستخدمت فيما بعد في التجربة اللاحقة بعد شهر من الشفاء التام) كانت أكثر مقاومة للإصابة 50% من الأسماك أصيبت تجريبيا في الأسبوع باللرنيا واستخدمت فيما بعد في التجربة اللاحقة بعد شهر من الشفاء التام) 72.7% من الأسماك أصيبت تجريبيا في الأسبوع الخامس ثم مجموعة اسماك السيطرة CG (اسماك بدون إصابة مسبقة باللرنيا) 72.7% من الأسماك أصيبت تجريبيا في الأسبوع الخامس ثم در اسة دور المقاومة على القابلية التكاثرية للطفيلي وعلى رفض الطفيليات من قبل المحنيف، نسبة الطفيليات التي تماك أكياس بيض في مجموعة السيطرة CG أسماك بدون إصابة مسبقة باللرنيا) 72.7% من الأسماك أصيبت تجريبيا في الأسبوع الخامس ثم در اسة دور المقاومة على القابلية التكاثرية للطفيلي وعلى رفض الطفيليات من قبل المحنيف، نسبة الطفيليات التي تماك أكياس بيض في مجموعة السيطرة CG أعلى (84.6%) من المجموعتين الأخريين في الأسبوع الخامس بعد تعرض الأسماك أكياس بيض الطفيلي 44.4% و 2.6% في مجموعة RG1 و RG2 على التوالي، 100% من الطفيليات فقدت من مجموعة الأسبوع العاشر ، بينما التاسع بعد تعرض الأسماك للأدوار المعدية للطفيلي ، 100% من الطفيليات فقدت من مجموعة RG2 في الأسبوع التاسع بعد تعرض الأسماك للأدوار المعدية للطفيلي ، 100% من الطفيليات قدت من مجموعة RG1 في الأسبوع التاسع بعد تعرض الأسماك للأدوار المعدية للطفيلي ما 100% من الطفيليات قدت من مجموعة RG2 في الأسبوع و مجموع مالي بعلي من مجموعة الطفيلي ، 100% من الطفيليات قدت من مجموعة RG2 في الأسبوع العاشر ، بينما التاسع بعد تعرض الأسماك للأدوار المعدية للطفيلي ، 100% من الطفيليات قدت من مجموعة RG2 في الأسبوع وي الأسبوع دور القدم من الطفيليات قدت من مجموعة الطفيلي ما 100% من الطفيلي الغربي في المي م دور القدم من الطفيلييات قدت ممناعة مكسابة الساماك الكارب الاعتيادي ضد هذا الطفيلي لك