EFFICACY OF INTRAPERITONEAL INJECTION VACCINATION IN AFRICAN CATFISH, *CLARIAS GARIEPINUS*, AGAINST STREPTOCOCCOSIS

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ABSTRACT

The present study was carried out to evaluate the intraperitoneal vaccination against streptococcosis in African catfish, *Clarias gariepinus*. Forty catfish were acclimated for 2 weeks and then divided into 2 equal groups (G). G1 was kept as control and G 2 was injected by *Streptococcus faecalis (S. faecalis)* bacterin (0.1 ml). Blood samples were taken from 2 fish, weekly, in each group for 5 weeks. The remaining 10 fish, from each group, were challenged with virulent strain of *S. faecalis* one month post-vaccination. Determination of the relative percent survival (RPS) among the challenged fish revealed 88.8 %. Besides, the antibody titers were determined.

INTRODUCTION

Bacterial diseases cause up to 80 % of fish mortalities, especially streptococcal disease as the cause of significant economic losses to the world aquaculture industry (***Perera et al., 1997 and Gun et al., 2006, Garcia et al., 2007, Wuming and Aihua, 2009***).

The increasing interest in aquaculture is demanding effective control of fish diseases. Fish cultural management techniques are designed to decrease the environmental stress and these reduce the prevalence of the disease and the use of chemotherapy.

The use of antibiotics induced immunosuppression in human (***Herwig, 1979***), birds (***Panigrahy et al., 1979***) and fish (***Stoffregen et al., 1996***). The high cost of antibiotics, toxic effect on the body and the emergence of drug resistant strains of bacteria are among the drawbacks of using antibiotics (***Evans et al., 2006***). Immunization has played an important role in the control of infectious diseases (***Joyce et al., 2007***).

Therefore, several laboratories have been directed toward the development of effective vaccines, which might replace chemotherapy and the limitation in the managemental techniques. The trials for control of fish diseases were started in Egypt and succeeded for production

of vaccines against *Aeromonas hydrophila*, Pseudomonas *fluorescence* and *Edwarsiella tarda* (***Badran, 1990 & 1995***).

The present study aimed to evaluate the immune response of African catfish *Clarias gariepinus* after immunization, using *Streptococcus facealis* bacterin through IP injection via immunological examination as well as challenge test.

MATERIAL & METHODS

1- Fish:

Forty apparently healthy African catfish, *C gariepinus*, with a range of 30 to 50g body weight were obtained from a private fish farm in Dakahlia governorate. They were divided into two equal groups of 20 fish each that were kept in fully prepared glass aquaria supplied with dechlorinated tap water. The water temperature was maintained at 25 ± 1°C throughout the experimental period which extended for 8 weeks after 2 week acclimation. The fish were fed minced meat throughout the experiment at a rate of 3% of body weight.

2- Vaccine preparation:

Formalin – killed *Streptococcus faecalis* bacterin was prepared by the addition of formalin to give a final concentration of 0.3 % to well identify bacterial culture which was incubated at 30°C for 48 hours (***Sakai et al., 1984***). The formalized bacterial culture was held at room temperature overnight, then harvested by centrifugation, washed three times and emulsified in an equal volume of sterile saline solution.

3- Sterility and Innocuity of the vaccine:

The sterility test was performed according to ***Ali (1981)***, where washed bacterin cultivated on nutrient and MaCconkey agar plates, incubated at 37°C for 24 hours and examined for positive bacterial growth. The innocuity test was performed according to ***Badran (1987)*** by the IP inoculation of catfish with washed bacterin cells. The fish were kept under observation for 2 weeks post-infection and tested for reisolation of the infected organism.

4- Vaccination of fish:

The control fish were injected IP with 0.1 ml of sterile saline solution. The second group was injected IP with 0.1 ml of formalin killed bacterial cells diluted with the same volume of sterile saline solution for each fish (***Badran et al., 1993***).

Sampling was done weekly during the five weeks post vaccination.

5- Blood and serum collection:

Blood samples were collected from the caudal blood vessels (***Lied et al., 1975***) of 2 fish weekly in each group. They were kept at room temperature for 1 hour and in refrigerator for overnight. Blood samples were centrifuged at 1000g for 20 minutes and the sera were aspirated using sterile Pasteur

pipettes. The remaining 10 fish in each group were challenged by *S. faecalis* infection.

6- Antibody titeration:

The induced humeral immune responses for *S faecalis* were evaluated by micro agglutination (MA) test in standard microtiter U-shape wells. A serial two fold dilutions of serum was made in sterile saline solution, using a 0.1 ml pipette dropper. The diluted formalin killed *S faecalis* antigen (0.1 ml) was added to diluted sera. The suspension were mixed and left for 1 hour at room temperature then into refrigerator for 12 hours. The antibody titer was determined at the greatest serum dilution where agglutination occurred, as indicated by the presence of a "button" in the well and expressed as the reciprocal of that dilution.

7- challenge:

It was performed by inoculation of 100 ml of brain heart infusion broth with *S faecalis* and incubation for 18 hours. Then each 100 ml was added to 15 liters of aquarium water and each group of fish immersed for 24 hours with increasing water of the aquarium. The challenged fish were kept under observation for 4 weeks and the dead fish were removed and counted at least every 12 hours.

Relative percent of survival was measured according to ***Akhlagi et al., (1996)*** using the following equation:

$$RPS = \frac{1 - \text{mortality \% of vaccinated fish}}{\text{mortality \% of control fish}} \times 100$$

RESULTS

Sterility and innocuity test:

The sterility and innocuity tests performed on vaccine before vaccination elicit the purity of vaccine from other contaminants and the bacterial cells were inactivated perfectly with formalin.

The specific immune response of African catfish *C gariepinus* vaccinated by IP infection with 0.1 ml of formalized *S faecalis* was 3, 4, 6, 7, at 1st , 2nd , 3rd , 4th week respectively post vaccination as shown in table (1) and figure (1).

Table (1): Antibody titer of *C gariepinus* immunized with *S faecalis* bacterin by injection vaccine.

Method of vaccination	Antibody titer			
	1st week	2nd week	3rd week	4th week
Injection	3	4	6	7
Control	2	2	2	2

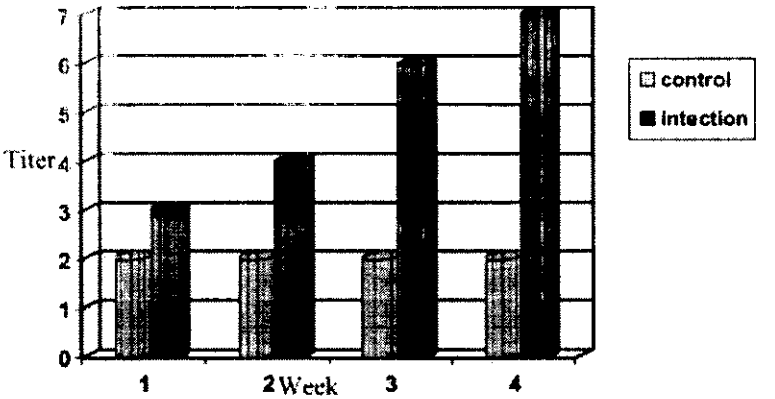

Figure (1): Antibody titer of catfish *C gariepinus* immunized with *S faecalis* bacterin by injection vaccine.

Challenge test:

The vaccinated and control groups were exposed to challenge by addition of 18 hours broth culture at level of 100 ml / 15 L aquarium water. The results showed that the mortalities were 10 % among fish vaccinated by IP injection while reached 90 % among the control group. The relative percent survival was 88.8 % among vaccinated fish (table, 2).

Table (2): Efficacy of vaccine against challenging with *S faecalis* after 30 days post vaccination.

Method of vaccination	Times (days)										Total number	Mortality %	RPS %
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th			
Injection	0	0	0	0	1	0	0	0	0	0	1/10	10	88.8
Control	0	0	0	0	3	2	2	1	1	0	9/10	90	-

***RPS: relative percent survival.**

DISCUSSION

Inmunization plays an important role in the control of infectious diseases of man and animals.

Immunization of fish proved to be effective for disease control, only on laboratory level (***Rohovac et al., 1981***).

Immunization of fish can be accomplished using a variety of antigenic preparations. The oral or parenteral administrations of the antigen beside immersion and spraying were effective. The technique used for vaccine administration on a comercial scale will be dictated by several parameters, including size, number and value of fish to be vaccinated. In the present study, we used the IP injection as a route for vaccination.

C gariepinus group's vaccinated by inactivated washed bacterial cells emulsified in sterile normal saline in ratio of 1:1 by IP inoculation of 0.1 ml/fish were protected against challenge with *Streptococcus faecalis*. This protection reached 90 % in the vaccinated catfish exposed to infection by addition of *S faecalis* into water 30 days post vaccination. These results agree with those reported by ***Iida et al., (1982) Badran et al., (2000) and, Pasnik et al., (2005)***

The antibody titers in *C. gariepinus* immunized with formalin killed *S faecalis* bacterin could be detected for 1 month post - vaccination.

Regarding the challenge test, the mortalities started from the 5th day in vaccinated and challenged fish and progressed to result in cumulative mortality percent of 10 % compared with 90 % in challenged non-vaccinated (control) group. RPS was found as 88.8% in vaccinated fish. Nearly similar results were previously mentioned by ***Akhlagi et al., (1996) and Bardan et al., (2000).***

It could be concluded that, *S. faecalis* bacterin was effective in immunization and protection of fish against infection by *S. faecalis*. The injection route gave high antibody titer and excellent relative percent survival rate .The injection route may be reliable for the immuneization of small number of highly valuable fish, but is difficult to be applied on a large scale. So, other routes of vaccination should be investigated to select an alternative method to be used on a large scale.

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الملخص العربى

فعالية التحصين بالحقن البريتونى لأسماك القط الأفريقى قرموط الجاريبينس ضد مرض الميكروب السبحي المكور

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في هذه الدراسة تم التقصى عن فعالية التحصين بالحقن البريتونى لأسماك القط الأفريقى قرموط الجاريبينس ضد مرض الميكروب السبحي المكور وذلك بتقسيم 40 من أسماك القرموط الإفريقي إلى مجموعتين متساويتين بعد الأقلمة لمدة أسبوعين. مجموعة (1) كانت المجموعة الضابطة ومجموعة (2) تم حقنها داخل الغشاء البريتوني بلقاح الاستربتوكوكس فيكالز (0.1 مل). تم تجميع عينتي دم أسبوعياً من كل مجموعة لمدة خمسة أسابيع. تم إجراء عدوى تجريبية بالميكروب السبحي المكور الضاري للعشرة أسماك المتبقية من كل مجموعة بعد شهر من التحصين لتحديد مستوى الحماية ضد العدوى في هذه الأسماك وقد أسفرت العدوى التجريبية لهذه الأسماك عن مستوى للحماية ضد الميكروب بنسبة (88.8%) ، كما تم تقدير الأجسام المضادة فى الأسماك المحصنة .