

**EFFICACY OF ORAL AND IMMERSION
VACCINATION IN AFRICAN CATFISH, *CLARIAS
GARIEPINUS* AGAINST STREPTOCOCCOSIS**

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ABSTRACT

The present study was carried out using oral and immersion vaccination in 54 African catfish, *Clarias gariepinus*, to compare the efficacy of *Streptococcus faecalis* formalin-killed vaccines (SFFKV) against infection with *S. faecalis*. The fish were divided into 3 groups each contained 18 fish. First group was kept as control while group 2 was vaccinated with SFFKV by oral route (5 mg/g of food) and group 3 was vaccinated by immersion route (2 mg/ml for 3 minutes). Blood samples were collected from 2 fish of each group, weekly, for 4 weeks for determining the level of specific immune response. The remaining fish in each group were challenged with virulent strain of *S. faecalis* one month post - vaccination for determination of the relative percent survival (RPS) among the three groups. The results of specific antibody response showed that the antibody titers of fish vaccinated by oral and immersion routes were similar to that of control group (2 by log₂) from 1st to 4th week post-vaccination. After challenge, mortality rates reached 90, 10 and 20% among the fish of the three groups. The RPS was 88.8 and 77.7% in oral and immersion vaccinated groups.

INTRODUCTION

Immunization of fish against disease is growing in fish farms and aquaculture. The use of vaccine could be a reliable approach to protect fish against bacterial infections (Kelly & Easter, 1987; Aly et al., 2000 and Gravningen and Berntsen 2007).

Nowadays, there is a great need for a practical vaccine deliv-

ery system, which exhibit high efficiency, low cost and minimum stress and is applicable to all fish. The oral route (Kusuda et al., 1978; Badran, 1991A and Aly et al., 2000) and immersion route (Badran, 1987, 1995A, Baba et al., 1988; Aly et al., 2000 and Evans et al., 2004) were reported to be suitable for vaccination. The

objective of the present study was to evaluate the immune response of catfish *Clarias gariepinus* after vaccination with *Streptococcus faecalis* formalin-killed vaccine via the oral and immersion routes employing immunological tools beside the challenge test.

MATERIAL AND METHODS

1. Fish:

Fifty four apparently healthy African catfish, *Clarias gariepinus*, with an average body weight 40 ± 10 g were obtained from a private fish farm in Dakahlia governorate. They were divided into 3 groups (each 18), group 1 served as control and groups 2 and 3 used for oral and immersion vaccination. Fish of all groups were kept in fully prepared glass aquaria supplied with dechlorinated tap water and the water temperature was adjusted to $25 \pm 1^\circ\text{C}$. They were acclimated for 2 weeks before the experiment and fed minced meat throughout the period of experiment at a rate of 3% of body weight.

2. Vaccine preparation:

Streptococcus faecalis formalin-killed vaccine (SFFKV) was prepared using well identified bacterial strain as described by *Badran (1995A)*. Sterility and safety of the prepared vaccine were tested according to *Badran (1987)*.

3. Vaccination of fish:

C gariepinus of group (1) remained without treatment and served as control. Group (2) was fed SFFKV at a level of 5mg/g of minced meat (*Fryer et al., 1976*).

Food containing vaccine was given in a ratio of 3% of the body weight of fish per day for 15 days. Group (3) were immersed for 3 min in SFFKV diluted with aquarium water in the ratio of 1:1 to give a concentration of 2mg/ml of aquarium water (*Badran, 1987*).

4. Blood and serum collection:

Blood samples were collected from the caudal vessels (*Leid et al., 1975*) of 2 fish weekly at 1st, 2nd, 3rd and 4th week post-vaccination. The sera were separated from the blood by being kept overnight in the refrigerator, centrifuged at 6000 rpm for 20 min and then aseptically collected.

5. Antibody titration:

The induced humoral immune response against SFFKV was evaluated by micro agglutination test (MA) in standard U shape microtiter wells and the antibody titer was determined as the greatest serum dilution where agglutination occurred.

6. Challenge:

Artificial infection of both vaccinated and control fish was done by addition of 100 ml of incubated 18hr *S. faecalis* cultured brain heart infusion broth to 15

liters of aquarium water and left for 24hr then aquarium water was increased. The challenged fish were kept under observation for 4 weeks.

The relative percent survival (RPS) was measured according to Akhlagi et al., (1996) as:

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

RESULTS

1) The immune responses of oral and immersion vaccination:

The specific immune response of African catfish *C gariepinus* against SFFKV used by oral and immersion routes were similar to those of control group (2 by log2) from first week post-vaccination to fourth week (Table 1).

Table (1): Antibody titers of *C gariepinus* vaccinated with SFFKV by different routes.

METHOD OF VACCINATION	ANTIBODY TITER (WEEKS)			
	1 st	2 nd	3 rd	4 th
Oral	2	2	2	2
Immersion	2	2	2	2
Control	2	2	2	2

2) Challenge test:

The mortality rates among fish of control and vaccinated groups (oral and immersion) as a result of challenge with virulent strain of *S. faecalis* were 90, 10

and 20%, respectively. The RPS was 88.8% among fish vaccinated by oral route and 77.7% among fish vaccinated by immersion route (Table 2).

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

Methods Of vaccination	Time (days)										Total numbers	Mortality %	RPS %
	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	9 th	10 th			
Immersion	0	0	0	0	0	2	0	0	0	0	2/10	20	77.7
Oral	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

The results of the present study revealed that specific immune responses of African catfish *C gariepinus* against SFFKV by oral and immersion routes were similar to those of control group (2 by log₂) through the experimental period. These results agree with those reported by *Badran (1991A, B, 1995A)*; *Azad et al. (1999)* and *El-Baz, (2001)*.

The results of the present study also revealed that although the humoral immune response of catfish *C gariepinus* vaccinated with *S faecalis* formalin-killed vaccines by oral and immersion routes were similar to those of control, they were protected against artificial infection with virulent strain of *S faecalis*. These results were indicated by the mortality rates among fish of control and vaccinated groups (oral and immersion) after challenge with virulent strain of *S. faecalis* were 90, 10 and 20%, respectively. The RPS was 88.8% among fish vaccinated by oral route and 77.7% among fish vaccinated by immersion route. These results nearly agree with those reported by *Badran (1991B)*, *Klesius et al., (2002)*, *Evans et al., (2004)* and *Brunt and Austin (2005)*. The result of protection of vaccinated fish

against infection may be attributed to the secreted antibody in the body surface and intestinal mucus that inhibit the organism to move freely and grow on the body and intestinal surfaces, consequently prevent the first step of infection (*Badran 1991A and 1995A*). This clarify why the skin of orally and immersion vaccinated fish dipped in *Streptococcus faecalis* suspension was free from the organism after 12 hr, meanwhile, the number of the organism was gradually increased, in relation to the time after challenge, in the skin of unvaccinated fish.

From the present study, it could be concluded that oral and immersion vaccination of catfish *C gariepinus* with *S. faecalis* formalin-killed vaccines are capable of protecting the vaccinated fish against artificial infection in spite of absence of humoral immune response.

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الملخص العربي

فعالية التحصين بالفم و بالغمر لأسماك القبط الأفريقي فرموط الجاريبينس ضد مرض الميكروب السبحي المكور
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في هذه الدراسة تم تقسيم 54 من أسماك القبط إلى ثلاث مجموعات تتكون كل منها من 18 سمكة لمقارنة فعالية لقاح الإسترينتوكوكس فيكالز عن طريق التحصين بالفم و الغمر ضد العدوى بالاسترينتوكوكس فيكالز. المجموعة الأولى ضابطة بينما المجموعة الثانية حصنت عن طريق الفم (5مجم/جم عليقة) و المجموعة الثالثة عن طريق الغمر في لقاح الاسترينتوكوكس فيكالز (2 مجم/مل/3 دقائق). تم تجميع عينات الدم بواقع سمكتين من كل مجموعة أسبوعياً على مدار الشهر الأول. و تم تحديد الأجسام المضادة و بعد شهر من التحصين تم إجراء عدوى تجريبية بميكروب الاسترينتوكوكس فيكالز الضار للأسماك المتبقية (10 أسماك في كل مجموعة) لتحديد مستوى الحماية ضد العدوى في المجموعات الثلاثة. و قد أسفرت العدوى التجريبية لهذه الأسماك المحصنة عن نسبة متساوية من الأجسام المضادة للأسماك المحصنة بالفم و الغمر والغير محصنة (في الأسبوع الأول و الرابع بعد التحصين). كانت نسب النفوق هي 10،90،20% في المجموعات الضابطة، المحصنة بالفم و المحصنة بالغمر على التوالي و كان مستوى الحماية ضد الميكروب هو 77,7 و 88,8% في كل من المجموعتين المحصنتين بالفم و الغمر على التوالي.