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TRIALS FOR CONTROL OF EDWARDSIELLOSIS BY IMMERSION VACCINATION (A) IMMERSION VACCINATION OF NILE TILAPIA (OREOCHROMIS NILOTICUS) WITH EDWARDSIELLA TARDA CRUDE LIPOPOLYSACCHARIDE.

(With 2 Table)

BY

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محاولات لمقاومة الأدواردسيلوزس بالتحصين بالتغطيس

(أ) تحصين البلطى النيلى بالتغطيس فى الأدواردسيلاتاردا ليبوبولى سكاريد الخام

أحمد بدران

تمت الدراسة على ٣٦٠ سمكة بلطى نيلى لمقارنة فعالية اللقاح من خلايا الأدواردسيلا تاردا بعد معالجتها بالفورمالين بالقاح المحضر من استخلاص الليبوبولى سكاريد الخام من جدر الخلايا البكتيرية فى مقاومة الأصابة بمرض الأدواردسيلوزس. وقد أثبتت الدراسة أن الأجسام المناعية فى دم البلطي النيلى المحصن بالتغطيس فى أى من اللقاحين تماثل تلك الموجودة فى الأسماك الغير محصنة. على الرغم من ذلك فإن الأسماك المحصنة تقاوم العدوى الصناعية بميكروب الأدواردسيلاتاردا وترجع هذه المقاومة الى الأجسام المناعية المفرزة فى الغشاء المخاطى المبطن لأجسام هذه الأسماك. وقد وجد أيضا أن مقاومة الأسماك المحصنة ضد العدوى تتناسب طرديا مع معدلات هذه الأجسام فى الغشاء المخاطى حيث أن معدلات البقاء للبلطى النيلى بعد العدوى الصناعية وصلت الى ١٠٠٪ عندما وصلت معدلات الاجسام المناعية فى الغشاء المخاطى الى ٩ بعد إنقضاء أربع أسابيع من التحصين فى الليبوبولى سكاريد الخام بينما وصلت نسبة البقاء ٦٥٪ عندما وصلت معدلات الأجسام المناعية فى الغشاء المخاطى ٤ بعد إنقضاء نفس المده من التحصين بلقاح الخلايا البكتيرية. وفى المقابل فإن معدلات البقاء بعد كل عدوى وصلت الى ٣٥٪ فى الأسماك الغير محصنة والتى يحتوى الغشاء المخاطى المبطن لها على أجسام مناعية طبيعية تصل الى ١ .

SUMMARY

A total of 360 Nile tilapia *(O. niloticus)* were used to compare the efficacy of formaline-killed vaccine (FKV, an insoluble antigen) and crude lipopolysaccharide (LPS, a soluble antigen) against infection with *Edwardsiella tarda. O. niloticus* vaccinated either with FKV or crude LPS by immersion (DP) method were protected against infection with *E. tarda* although the antibody titers in the sera of both groups were similar to those of the control throughout the experimen-

tal period. This protection of immunized fish was correspondence to the high level of secreted antibody in the body surface mucus. Meanwhile, the survival ratio of *O. niloticus* vaccinated with crude LPS and FKV were stayed at 100 and 65% after 4 weeks from vaccination where the secreated antibody titers were 9 and 4 respectivelly. The survival ratio of unvaccinated fish was 35% after each of the 4 challenges where the natural secreted antibody titer was 1.

Keywords: Trials, control, Edwardsiellosis,immersion vaccination, Nile Tilapia, Edwardsiella trade Crude lipopolysaccharide.

INTRODUCTION

At present there is a great need for a practical vaccine delivery system which exhibit high efficacy, low cost, minimum stress on fish and is applicable to all fish size. Indeed, oral vaccination *(KUSUDA et al. 1978, KAWAI and KUSUDA 1985, RODGERS and AUSTIN 1985 and BADRAN 1991A and 1991B)* and immersion vaccination *(CROY and AMEND, 1977; JOHNSON et al., 1982; BADRAN, 1987 and BABA et al., 1988)* are the suitable methods for the above purposes. In addition, immersion vaccination is the only method suitable for young hatcharies fishes espically fingerling at 4-5 grams body weight before transported to a potentially infected site *(JOHNSON et al. 1982)*. The antigen employed in immersion vaccination have consisted mainly of heat-or fromalin-killed bacteria *(FUKUDA and KUSUDA 1981, Song and KOU 1981,SAKAI et al., 984 and LAMERS et al. 1985)*. Nowadays, crude lipopolysaccharide of the bacteria have also been used in immersion vaccination *(BABA et al. 1988)*.

The present study was designed to compare the protective effect of immersion vaccination with FKV and crude LPS against infection with *Edwardsiella tarda.*

MATERIAL and METHODS

1-Fish:-

Three hundred and sixty *O.niloticus* each of 85 ± 5 gm body weight were used. Three hundred fish were divided into 3 groups each comprising 100 fish. The fish were maintained in glass aquaria supplied with declorinated tapwater. The water temperature was maintained at 22C° throughout the experimental period. Sixty *O.niloticus* were divided into another 3 groups each contained 20 fish placed under the same corresponding condition of the previous groups to compensate the accidental deathes during the experiment. The fish of all groups were placed under observation for 2 weeks befor starting the experiment.

2- Vaccines preparation:-

The vaccines (FKV and LPS) were prepared from *Edwardsiella tarda.* The organism was provided from

Department of Microbiology, Univ. of Maryland, U.S.A., in which crude LPS was prepared.

FKV was prepared by addition of formalin to the bacterial culture, incubated at 35C° for 48 hs, to a concentration of 3%. the formalized bacterial culture was held at room temperature overnight. Sterility and safty of the vaccine to fish was tested *(CARDELLA and EIMERS 1990).*

Crude LPS was prepared according to the method described by *SUTHERLAND (1978).* Namely, 2gm of the bacterial cells (dry weight) were suspended in 200 ml cold distilled water (10 mg/ml). The bacterial suspension was poured into 10 volumes of cold aceton (-20C°) and left for 60 min. The yellowish supernatant fluid was removed and the resude was poured on to a Buchner funnel and washed with a further 5 volumes of cold acetone under suction. The cells were transferred to a desiccator and dried under vacuum in the presence of phosphorous pentoxide. The dried bacterial cells were throughly ground and suspended at a concentration of approximately 6% (w/v) in distilled water. The mixture was stirred in a wide-necked Erlenmeyer flask in a water bath at 65C° untile the flask contents were at 65C°. An equal volume of 90% (w/v) aqueous solution of phenol at the same temperature was added to the flask and stirring was continued for 5 min. The flask contents were cooled to 5C°, poured into polystyrene tubes and centrifuged at approximately 2000g for 60 min in a refrigerated centrifuge at 0C°. The upper aqueous solution was carefully removed, dialysed for 48hs against running tapwater and concentrated under reduced pressure to volume about 1/4.

3- Vaccination process:

O.niloticus of group 1 were immersed in FKV diluted with aquarium water in a ratio of 1:1 to give a concentration of 2 mg/ml for 3 min. *(BADRAN 1987). O. niloticus* of group 2 were immersed in crude LPS at concentration of 20 ug wet weight/ml for 2 hs *(BABA et al. 1988). O. niloticus* of group 3 were remained without treatment as control.

4- Antibody response:-

Blood and body surface mucus were collected from 5 *O. niloticus* at 2, 4, 10 and 15 weeks post-vaccination as described by *KAWAI et al. (1981).* The serum was separated from the blood by being kept overnight in the refrigerator, centrifuged at 6000 r.p.m. for 20 minutes and then aseptically collected. The body surface mucus was salted out in 50% saturated solution with ammonium sulfate, then dialysed and concentrated in polyethylene glycol powder *(AUSTIN and RODGERS 1981).* The mucus was concentrated to the ratio of 0.2 ml/fish. The specific antibody titers in collected sera and body surface mucus to E. tarda were determined using bacterial agglutination test. For agglutination,

formalin- killed bac-terial cells were washed twice with phosphate buffer saline (PBS), and prepared to a concentration of 3 mg wet weight/ml in PBS *(BABA et al. 1988)*.

5- Challenge test:-

Twenty *O. niloticus* from each group were used for artificial infection by immersion method with virulent strain of *E. tarda* at 2, 4, 10 and 15 weeks postvaccination. The organism was inoculated in brain heart infusion (BHI) broth and incubated at 28 C° for 24 hs. The infection was performed by immersing the fish in the broth culture diluted by 1:5 in 0.5% NaCL solution to give a concentration of $4x10^7$- $1x10^8$ colony forming unit/ml for 10 minutes. Fish of control group were immersed in sterial BHI broth at the same dilution and for the same time. Before immersion in the broth culture, the fish were preimmersed in 1.5% NaCl solution for 5 minutes. The fish were placed under observation for 2 weeks and the dead ones were used for *E. tarda* re-isolation.

RESULTS

The results of antibody responses were reported in Table 1. The antibody titers in sera of FKV and LPS-DP vaccinated *O. niloticus* did different from those of the not control throughout the experiment. The mean titers of secreted antibody in the body surface mucus were 8, 9, 10 and 11, by Log_2' in crude LPS-DP vaccinated fish and were 2, 4, 6 and 8 in FKV-DP vaccinated vaccinated fish at 2, 4, 10, and 15 weeks respectivelly. The mean titer of secreted antibody in the body surface mucus of control group was 1 throughout the experiment.

The results of artificial infection represented by survivors percent documented in Table (2). The results revealed that, the survival ratio were stayed at 100% after 4 weeks from crude LPS-DP vaccination while reached 75% after 15weeks from FKV-DP vaccination. The survival ratio of control group was 35% after each one of the 4 challenges.

DISCUSSION

The results of the present study revealed that, the serum antibody titers at 2, 4, 10 and 15 weeks after FKV-or crude LPS-DP vacciation were not different from those of the control at the same corresponding time. This agrees with the results obtained in DP-immunized Sockeye salmon *(CROY and AMEND 1977)*, yellowtail *(FUKUDA and KUSUDA 1981)*, rainbow trout *(SAKAI et al. 1984)* and Carp *(BABA et al. 1988)*. *Lamers et al. (1985)* found that a single bath of common carp in *A. hydrophila* bacterin did not induce an increase in serum antibody levels, while a second bath at 1, 3 or 8 months resulted in clear secondary antibody responses. In contrast, Anderson *et al. 1979* reported that, the number of antibody-producing cells were increased in rainbow trout Dp-immunized with *Yersinia 0. antigen*. This difference may be at-

tributed to various factors including differences in antigen preparation and dose, fish species and degree of stress.

Lateral line, posterior body and gills of fish are the sites of antigen absorption in bath immunization *(AMEND and FENDER 1976 and AMEND and JOHNSON 1981)*. This seems to imply that penetration of soluble antigen (LPS) into the fish in DP-immunization was accompalished more easily than insoluble antigen (FKV) resulting in a larger antigen sensitization *(BABA et al. 1988)*. This phenomenon was supported by the result reported here where the secreted antibody in the body surface mucus of LPS-DP vaccinated *O. niloticus* were recorded in high levels when compared with those of FKV-DP *O. niloticus*. The mechanism by which the antibody appeared in the body surface mucus of DP-vaccinated fish could be due to the locally synthesised antibody in skin epidermal cells rather than being drived from blood (*DICOZA and HALLIDAY 1971)*.

The results of the present study also revealed that, although the humoral immune response of *O. niloticus* against FKV and crude LPS were similar to those of the control, they were protected against artificial infection with virulent strain of *E.tarda*. This was attibuted to the secreted antibody in the body surface mucus that inhibit the organism to move freely and grow on the body surface, consequently prevent the first step of infction. This clarify why the skin of orally vaccinated fish dipped in *A. hydrophila* suspension was free from the organism after 12 hs, meanwhile, the number of the organism was gradually increased, in relation to the time after challenge, in the skin of unvaccinated fish *(BADRAN 1991B)*. The protection of several fish species against artificial infection, in spite of absence of humoral antibody, were obtained in DP-immunization of yellowtail *(FUKUDA and KUSUDA 1981)*, eel *(SONG and KOU 1981)* and Carp *(BABA et al. 1988)*.

From the present study it could be concluded that:

1- Immersion vaccination either with FKV or crude LPS did not enhancing the humoral immune response.

2- Protection of DP-vaccinated fish against artificial infection was attributed to the antibody secreted in the body surface mucus.

3- Crude LPS (a soluble antigen) was capable of stimulating the immune system of fish to produce high level of secreted antibody more than FKV (an insoluble antigen).

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Table. 1: Antibody response of *O. niloticus* vaccinated with FKV and crude LPS by DP method.

Fish group	Type vaccine	Vaccine concentration	Time of sampling*	No. of fish	Mean of antibody titer by Log_2	
					Sera	Mucus
1	FKV	2mg/ml	2	5	2	2
			4	5	2	4
			10	5	2	6
			15	5	2	7
2	crude LPS	20ug/ml	2	5	2	8
			4	5	2	9
			10	5	2	10
			15	5	2	11
3	----------	----------	2	5	2	1
			4	5	2	1
			10	5	2	1
			15	5	2	1

Table. 2: Efficacy of FKV- and crude LPS-vaccination in protection of *O.niloticus* against artificial infection with *E. tarda.*

Fish group	Type of vaccine	Vaccine concentration	Time of challenge*	No. of Fish	No. of Survivors	Survivors %
1	FKV	2mg/ml	2	20	11	55
			4	20	13	65
			10	20	14	70
			15	20	15	75
2	crude LPS	20ug/ml	2	20	16	80
			4	20	20	100
			10	20	20	100
			15	20	20	100
3	-------	-------	2	20	7	35
			4	20	7	35
			10	20	7	35
			15	20	7	35