

# EFFECT OF VACCINATION ON VIBRIOSIS RESISTANCE OF *FENNEROPENAEUS INDICUS*

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## ABSTRACT

*The study aims to test the effect of vaccination on vibriosis - resistance of Indian white shrimp, Fenneropenaeus indicus. Different concentrations of vaccine viz. 0.1%, 0.5%, 1%, 5% and 10% for different durations such as 1h, 2h, 3h, 4h, 5h, 6h and 7h were tested. Vaccinated. F. indicus post larvae were challenged with a virulent Vibrio harveyi on 30 days of post vaccination. The maximum Relative Percent Survival was recorded at 1% concentration of vaccine for 5h exposure. Result showed that vaccination is highly significant and enhances the resistance of shrimp postlarvae to vibriosis.*

**Key words:** *Fenneropenaeus indicus*, vaccination and vibriosis.

## INTRODUCTION

Though shrimp culture has undergone rapid development in most Southeast Asian Countries, successful production is increasingly hampered especially by diseases in addition to environmental pollution and poor management practices. Of all the infectious diseases, bacterial infections cause serious diseases like vibriosis, ulcer disease, tail rot, necrosis etc. Vibriosis, especially luminescent disease caused by *Vibrio harveyi* leads to mass mortality in the shrimp hatcheries (de la Pena *et al.*, 1993). Post-infection therapy using antibiotics is possible now. However, there are several problems associated with the use of antibiotics including the development of potential environmental hazards and

the possible development and spread of antibiotic resistance strains (Karunasagar *et al.*, 1994). Growing awareness of the problem associated with the use of antibiotics, has led researchers towards the field of immunoprophylaxis. Effective vaccination of penaeid shrimps with a formalin – killed *Vibrio* spp. vaccine has been reported by Kou *et al.* (1989) and Itami *et al.* (1989). However, study on the development of *V.harveyi* vaccine and its effect on vibriosis in *F. indicus* was found scarce. In the present study, a more effective way of protecting cultivable penaeid shrimps against vibriosis, by

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using formalin - killed *V. harveyi* vaccine was developed and the relative percent survival (RPS) of vaccinated against non-vaccinated *F. indicus* was also investigated.

## MATERIALS AND METHODS

### Collection and maintenance of samples

Postlarvae (PL) of *Fenneropenaeus indicus* were collected from hatcheries, stocked into cement cisterns and then acclimatized for one week with good aeration and feeding with commercial feed (Growbest, size: S1&S2) at the rate of 10% of its body weight. Twenty PLs were released into a series of plastic troughs of 15L capacity filled with sea water (35ppt) for pathogenicity test.

### Isolation, identification of pathogen, *V. harveyi* and pathogenicity

Bacterium, *Vibrio harveyi* was isolated from shrimp larvae *F.indicus* (APHA, 1995), identified (West and Colwell, 1984) and the virulence was confirmed by pathogenicity test. The cells were raised in Tryptic Soy Broth, harvested by centrifugation (5000 rpm) and suspended in sterile saline. The cells (*V. harveyi*) were enumerated and simultaneously administered to the post larvae at different doses viz.  $10^3$ ,  $10^4$ ,  $10^5$  and  $10^6$  cfu/ml by immersion route to determine pathogenicity of *V. harveyi*. The temperature and pH of the culture medium were maintained at  $27\pm 2^\circ\text{C}$  and  $7.8\pm 0.2$  respectively. The larval mortality was recorded on 1d, 2d, 3d, 4d and 5d (Vera et al., 1992) and  $\text{LD}_{50}$  value was determined.

### Vaccine production and administration

*V. harveyi* cells were killed with different concentrations of formalin (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, and 1%) and the optimum concentration was standardized as 0.5% by vaccine sterility and safety test. The efficacy of formalin killed vaccine was tested by administering the

vaccine through the immersion of *F.indicus* post larvae (PL)( $85\pm 5\text{mg}$ ) with various concentrations (0.1%, 0.5%, 1%, 5% and 10%) and exposed to different durations (1h, 2h, 3h, 4h, 5h, 6h and 7h). On thirty days of post vaccination, the postlarvae were challenged with *V. harveyi* ( $6.1\times 10^5$  cfu/ml-challenge dose) and the mortality of the shrimps were recorded for a period of 5 days (Itami et al., 1989). Relative Percent Survival (RPS) was also recorded. The experimental animals were treated with 1% vaccine (standardized as optimum concentration) for different durations of exposure (1h, 2h, 3h, 4h, 5h, 6h and 7h). After 30 days, the vaccinated animals were challenged (Itami et al., 1989) with  $6.1\times 10^5$  cfu/ml (challenge dose) of *V. harveyi* and RPS was recorded for different time of exposures

## RESULTS AND DISCUSSION

### Pathogenicity

*V. harveyi* isolated from infected *F. indicus* was used in the present study as the isolates from the injected animals are naturally more virulent than the isolates from seawater (Karunasagar et al., 1994). In the present study, postlarvae of *F. indicus* infected with *V. harveyi* showed luminescence in the laboratory conditions. From the study,  $\text{LD}_{50}$  dose was found to be  $5.2\times 10^4$ cfu/ml and hence the next higher concentration ie.  $5.2\times 10^5$  cfu/ml was chosen as challenge dose (Table 1).

### Vaccine-sterility and safety tests

Among various concentrations of formalin killed vaccines tested for sterility and safety, 0.5% and above were found to be sterile *in vitro* and also safe for use in animals. These results are in agreement with Itami et al. (1989), Itami et al.(1991) Teunissen et al.(1998) and Alabi et al (1999), who have used 0.5% formalin for the preparation of vibrio vaccine.

### Effect of different concentrations of vaccine with different exposure time

Highest (70%) and lowest (25%) survival rates were observed in shrimps treated with 1% and 0.1% vaccine respectively (Table 2). Based on the observation, 1% concentration was selected as optimum concentration for vaccination. Irrespective of the concentrations, all the vaccine treated animals showed much higher survival rate than the unvaccinated ones (15%) when challenged with  $5.2 \times 10^5$  cfu/ml (challenge dose). There was a highly significant difference ( $P < 0.01$ ) between vaccinated and unvaccinated shrimps with respect to survival rate.

Highest survival of 71.67% was recorded with the vaccine exposure of 5h and lowest

survival (48.33%) was observed with 1h exposure at 1% concentration (Table 3). These results are in accordance with the observation of Itami *et al.* (1992), Teunissen *et al.* (1998) and Alabi *et al.* (1999). Results of the present study imply that vaccine treatments significantly enhance the immunity of postlarvae to vibriosis when challenged on 30 days post-vaccination (Itami *et al.*, 1989 and Teunissen *et al.*, 1998). Though there is no specific memory in shrimps, a partial specificity in immune response was observed in the case of vaccine (bactericide) treated shrimps than the immunostimulant (glucan) treated animals. To give better insight on the longevity of the vaccine efficacy, further experiments need to be carried out over 30 days of post-vaccination also.

**Table 1.**

**Accumulative mortality of *F. indicus* postlarvae challenged with *V. harveyi***

Dose of <i>V.harveyi</i> (cfu/ml)	Mortality Rate												Mean% of mortality
	Trial I						Trial II						
	0d	1d	2d	3d	4d	5d	0d	1d	2d	3d	4d	5d	
$6.1 \times 10^6$	0/20	6/20	10/20	13/20	16/20	19/20	0/20	6/20	11/20	14/20	17/20	19/20	95
$6.1 \times 10^5$	0/20	4/20	7/20	10/20	13/20	15/20	0/20	5/20	9/20	13/20	14/20	17/20	80
$6.1 \times 10^4$	0/20	3/20	5/20	8/20	9/20	10/20	0/20	2/20	5/20	7/20	9/20	10/20	50
$6.1 \times 10^3$	0/20	0/20	1/20	2/20	2/20	2/20	0/20	0/20	1/20	1/20	2/20	3/20	12.5
Control	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0

**Table 2. Effect of vaccine on the survival of *F. indicus***

Concentration of vaccine	Survival of <i>F. indicus</i> (%)			Mean % of survival
	Trial I	Trial II	Trial III	
10%	70	65	70	66.66
5%	70	70	65	68.33
1%	70	70	70	68.33
0.5%	45	45	45	45.00
0.1%	25	25	25	25.00
Control	15	15	15	15.00

**Table 3. Effect of different durations of vaccine (1%) exposure on the survival of *F. indicus* postlarvae challenged with *V.harveyi***

Duration of exposure	Survival of <i>F. indicus</i> (%)			Mean % of survival
	Trial I	Trial II	Trial III	
1h	50.0	45.0	50.0	48.33
2h	50.0	50.5	50.5	50.00
3h	55.0	55.0	55.0	55.00
4h	65.0	60.0	60.0	61.67
5h	75.0	70.0	70.0	71.67
6h	70.0	65.5	65.5	67.00
7h	65.0	65.0	65.0	65.00

### REFERENCES

- Alabi, A.O., Jones, D.A., and J.W. Latchford, (1999). The efficacy of immersion as opposed to oral vaccination of *Penaeus indicus* larvae against *Vibrio harveyi*
- APHA, 1995, Standard methods for the examination of water and wastewater, 19<sup>th</sup> Edn. American Public Health Association, Washington D.C.
- Baticados, M.C.L., C.R. Lavilla - Pitogo, E.R. Cruz- Lacierda, L.D.de la Pena and N.A. Sunaz, 1990. Studies on the chemical control of luminous bacteria, *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. Diseases of Aquatic Organisms, 9(2):133-139.
- de la Pena, I. D., Tamaki, T., Momoyama, K., Nakai, T., Muroga, K., 1993. Characteristics of the causative bacterium of vibriosis in the kuruma prawn *Penaeus japonicus*. Aquaculture 115,1-12,

- Itami, T, Y. Takahashi and Y. Nakamura, 1989. Efficacy of vaccination against vibriosis in cultured kuruma prawns, *Penaeus japonicus*. J. Aquatic Animal Health, 1:238-242.
- Itami, T. Y. Takahashi, K. Voneoka and Y. Yau, 1991. Survival of larval giant tiger prawns, *Penaeus monodon* after addition of killed vibrio cells to a microencapsulated diet, J. Aquatic Animal Health, 3(2) : 151-152.
- Itami, T., Y. Yan and Y. Takahashi, 1992. Studies on vaccination against vibrios in cultured kuruma prawn, *Penaeus japonicus*. Effect of different vaccine preparations and oral vaccination efficacy. J. Shrimonosek. Univ. Fish., 40(3): 139-144.
- Karunasagar, I., R. Pai, G.R. Malathi and Indrani Karunasagar, 1994. Mass mortality of *Penaeus monodon* larvae due to antibiotic - resistant *Vibrio harveyi* infection. Aquaculture, 128: 203-209.
- Kou, G.H., Chen, S.N., Huang, S.L., 1989. Studies on bacterial infection and vaccination trials for culture *Penaeus monodon* in Taiwan. Disease of Fish and shellfish, Abstract of the 4<sup>th</sup> EAFP International Conference, 24-28 September 1989, Santiago de Compostela, Spain, p.94.
- Teunissen, O.S.P., Faber, R., Booms, G.H.R., Latscha, T. and J.H. Boon, 1998. Influence of vaccination on vibriosis resistance of the giant black tiger shrimp *Penaeus monodon* (Fabricius). Aquaculture, 164:359-366.
- Vera, P., J.I. Navas and M.C. Quintero, 1992. Experimental study of the virulence of three species of *Vibrio* bacteria in *Penaeus japonicus* juveniles. Aquaculture, 107 (2-3) :119-123.
- West, P.A., and R.R Colwell, 1984. Identification and classification of Vibrionaceae - an overview. In : R.R.Colwell (ed.) *Vibrio in the Environment*. John Wiley. New York pp255-263.