Development of a multivalent vibriosis vaccine and its application in sea cage farming of cobia

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Abstract

The loss due to diseases in the aquaculture sector is estimated to be around 10-15% of the production cost and it can be substantially reduced if due attention is given to scientific health management. Most of the antibiotics are banned in aquaculture, so the alternative to maintain fish health is based on the supplementation of probiotics, immunostimulants and administration of vaccines. The main objective of developing a multivalent vaccine against vibriosis is to prevent the seasonal epizootics in cultured cobia *Rachycentron canadum*. The whole cell inactivated multivalent vibrio vaccine against vibriosis was developed, standardized and evaluated in cobia fingerlings. The field application of the vaccine was studied in sea cages deployed for growout and broodstock rearing of cobia. The immune responses were evaluated by challenge studies in fingerlings and serum antibody titre in cobia growout and broodstock cages using ELISA. The regular epizootics observed in cage cultured cobia, every year during the months of July to September (pre-monsoon season) was prevented by timely vaccination and proper sea cage farming management.

Keywords: *Cobia, vibriosis, multivalent vaccine, cage farming*

Introduction

Breeding, seed production and farming technologies for two marine finfishes namely cobia (Gopakumar *et al*.,2011) and silver pompano were developed by ICAR-Central Marine Fisheries Research Institute (CMFRI). The techno economic viability of farming these fishes in sea cages and ponds reached the fishermen and fish farmers through different training, awareness and frontline/participatory demonstration programmes organised by the Mandapam Regional Centre of ICAR -CMFRI. In view of the declining catches, sea cage farming can enhance the marine fish production and help to increase the income of the fishers. Currently, the institute is extending technology support to interested fishermen and entrepreneurs for cage farming and hatchery technology required for seed production of high value marine finfishes, in several maritime states such as Tamil Nadu, Karnataka, Andhra Pradesh, Kerala, Goa, Maharashtra, Odisha and Gujarat. The low-cost cage farming technology for cobia has been well accepted by the fishermen groups and entrepreneurs in several maritime states of India. In this context, fish disease prevention and management is a very crucial aspect for successful aquaculture outcomes.

Diseases in cage culture of cobia

Fish cultured in floating cages become particularly susceptible to disease when various environmental parameters such as temperature, salinity, dissolved oxygen and suspended particles fluctuate suddenly or widely, or following certain cyclic climatic conditions and handling. The prevalence and spread of diseases in marine fish farming has gained more traction in recent

years. The major diseases in mariculture are caused by a wide range of infectious organisms, including bacteria, viruses, fungi, protozoan and metazoan parasites and also nutritional and environmental problems including harmful algal blooms. Among the diseases in marine cultured finfish, vibriosis is a serious bacterial diseases characterized by exoptholmas, haemorrhagic gastritis, ascites, septicaemia and mortality (Rameshkumar *et al* 2017). After end of the nursery phase the fingerlings of cobia (each weighing 50-80g) will be stocked in the open sea cages for farming. Fish cultured in floating cages become particularly susceptible to vibriosis caused by *Vibrio alginolyticus, V.parahaemolyticus* and *V.harveyi,* whenever the juveniles are getting immuno-suppressed or its defence mechanism is lowered due to any kind of stressful conditions. The development of a suitable vaccination programme plays an important role in better health management practices. As use of most of the antibiotics are banned in aquaculture, the alternative is to maintain fish health based on supplements of probiotics, immunostimulants and administration of vaccines. Mortality details of cobia in cage culture at Gulf of Mannar and Palk Bay are listed in Table1.

Vaccine development

The first fish vaccine was developed for enteric red mouth (*Yersinia ruckeri*) in salmonids during the late 1970s. The first vaccine for prevention of vibriosis in salmonids was available in 1988 by Norvax®Vibriose (Intervet, Bergen, Norway). A killed bacterial vaccine (bacterin) is currently available for *V. anguillarum* and *V. ordalii* which has been demonstrated as effective in prevention of vibriosis in juvenile Atlantic halibut as well as for salmon. The formalin inactivated vaccines were superior than heat killed preparations, especially when the bacterins were injected with adjuvants. The vaccination strategy is a better choice to control the infection caused by vibriosis. Currently vaccines against *V.alginolyticus* are mostly made from sonicated and heat killed bacteria (Cheng, 2009) and it is reported that cobia has developed protective immunity through vaccination with inactivated *Vibrio* bacterins. Lin *et al*., (2006) developed vaccine for cobia using a polyvalent preparation comprising inactivated *V.alginolyticus, V.parahaemolyticus* and *P.damselae subsp. piscicida,* which induced appearance of specific antibody, one week after post-injection and was detected until the end of the trial at 6th week. The fish immunized

Table 1. Details of mortality during cage culture of cobia in the Gulf of Mannar

Year	Month when mortality occurred	Mortality (in numbers)	Total length (cm) range of affected fishes	Total weight of affected fishes	Diagnosis	Type of fish affected	Location
2010	August, September	670	23.5 to 43.0	73.5 to 650 g	Vibriosis	First time cage culture of cobia fingerlings.	Mandapam
2011	June, July, August	16	81 to 135	5 to 33kg	Septicaemia due to Enterobacteriacae sp and vibriosis	Brood stocks	Mandapam
2012	May, July, August	34	94 to 110	11.1 to 18.1 kg	*Trichodesmium* bloom followed by vibriosis	Sub-adults and brood stocks	Mandapam
2013	July ,August	91	90 to 102	8 to 10 kg	Typical vibriosis	Sub-adults	Mandapam
2014	December	400	16 to 19	20 to 25g	Higher stocking density followed by vibriosis	Fingerlings.	Private sea cage– Mandapam
2015	April, July, August	183	35.5 to 43.0	41.5 to 70.5 g	Higher sea surface temperature, and Vibriosis.	Fingerlings.	Private sea cage-kattumavadi and Munaikadu
2016	March, April, May, July	1775	22 to 25.5	45 to 55g	Vibriosis, and, Viral Nervous Necrosis(VNN)	Fingerlings.	Private sea cage Thangachimadam and Munaikadu
2017	July August, September	5	102 to 117	10.0 to 13.5 kg	Nonspecific	Sub adults	Mandapam
2019	March& April	62	99 to 122	8.5 to 22 kg	Photobacterium spp. due to higher sea surface temperature	Sub-adults and brood stock in sea cage.	Mandapam
2019	September	35	110-125	14.5 to25 kg	*Noctiluca scintillans* bloom	Fingerlings and sub-adults	Mandapam

either through intramuscular or intra-peritoneal vaccine use, showed protection against challenges. Bacterial vaccination was not reported earlier in sea cage culture of marine finfishes in India. Hence, a study to the develop suitable vaccine against *Vibriosis* in finfish was initiated with objectives of developing a multivalent whole cell inactivated vaccine against vibriosis, standardising the dose, route and its efficiency through *in-vivo* experiments and evaluating the protective effect of vaccine in field conditions. The three vibrio organisms (*V.alginolyticus, V.parahaemolyticus* and *V.harveyi*) were selected for multivalent vaccine preparation based on the repeated outbreaks of the vibriosis in cobia cage culture. Formalin inactivated multivalent vibrio vaccine with aluminium hydroxide (ALGEL) as adjuvant was formulated. Only those cobia fingerlings and growout stages that had not been exposed to any microorganisms and shown to be free from specific antibodies against any systemic infections were selected for the experiments where the whole cell inactivated multivalent vibrio vaccine against vibriosis was evaluated.

Twelve cobia fingerlings each weighing about 30-32 g were alloted in three groups in triplicates (n=110), namely Group I -vaccinated, Group II- Only aluminium hydroxide adjuvant and Group III-Control Phosphate Buffered Saline (PBS). For the vaccine trial, blood was collected from the caudal vein of six cobia fingerlings at 7, 14, 21 and 42 day post vaccination (DPV). At each sampling, fish were anaesthetized with a 60 μL/L dose of clove oil by dip for blood collection. The blood collected using a 1 mL tuberculin syringe was immediately transferred to an eppendorf tube was left undisturbed for two hours till the straw coloured serum separated out. This was collected by centrifugation at 3500 rpm at 4°C for 10 minutes and stored at -20°C until use.

During the challenge study, six fish from each group were challenged on the 42 DPV by intraperitoneal inoculation of 0.1 mL of *V.alginolyticus* $1x10^8$ cfu/mL ($1x10^7$ cfu/fish) cell suspension (Fig.1). The clinical signs, lesions and cumulative mortality were recorded daily for two weeks of post challenge and necropsy was conducted on dead fish to determine the cause of death. Re-isolation and the presence of *V.alginolyticus* in the tissues were determined by bacterial culture in the TSA and TCBS agars. The vaccine efficiency was evaluated by challenge methods in cobia fingerlings. In the challenge studies, all vaccinated fish survived without showing any clinical signs but in the adjuvant group (Group II) 90% mortality with 10% survival and in PBS control(Group III) 100% mortality were observed at 96 h post challenge.

The relative percent survival (RPS) was calculated (Dehghani *et al.*, 2012).

$$RPS = 1 - \left\{ \frac{\% \text{ Mortality of vaccinated group}}{\% \text{ Mortality of control group}} \right\} \times 100$$

The post vaccination immune response was detected by ELISA and antibacterial antibody titre or the OD values were analysed following the method of Gudmundsdottir *et al.* (2009). The fish serum antibody showed an increasing trend on 7th, 14th and 21st day of post vaccination (DPV) and serum antibody levels showed a decreasing trend and thereafter the immune response was maintained upto 6 weeks. The post infective response was detected by

Fig.1. Intraperitoneal vaccination of cobia with a Manual vaccinator

Fig.2. Vaccinated cobia fingerlings before stocking in the cage

Fig. 3. Intra-peritoneal administration of multivalent vaccine to cobia sub-adults in cages

ELISA and the significant change ($p<0.05$) of antibody response was noted in the vaccinated fish when compared to the control and adjuvant group placebo groups. (Figs. 3, 4 & 5)

After the standardization of multivalent vibriosis vaccine in *in-vitro* and experimental study, the field application trial was carried out in the sea cage farmed cobia fishes. Cobia sub adults (90 numbers) and brood stock (35 numbers) were vaccinated with multivalent (*Vibrio alginolyticus, V. parahaemolyticus* and *V. harveyi*) Algel adjuvant vaccine during July and September 2017. One booster dose was also given after 35 days of the first dose.

Among the six cages, one cage grow out fish (30 numbers) were kept as control without vaccination. ELISA was performed to identify the serum optical density (OD) levels in the control and the vaccinated groups. The cobia serum antibodies showed rising trends from 7th Day of Post Vaccination (DPV) and extended upto 21 DPV. Then the serum antibody OD levels showed decreasing trend after 21 DPV and serum antibodies were detected upto 6 weeks. The OD values of antibody to multivalent vaccine differed significantly ($p<0.05$) in the laboratory and in the field trial. There was a significant ($p<0.05$) increase in the OD values of antibodies from 7th to 21st day and dropped significantly ($p<0.05$) at 28 DPV. Hence, a

Fig. 4. Hyper-immune serum collected after 35 DPV

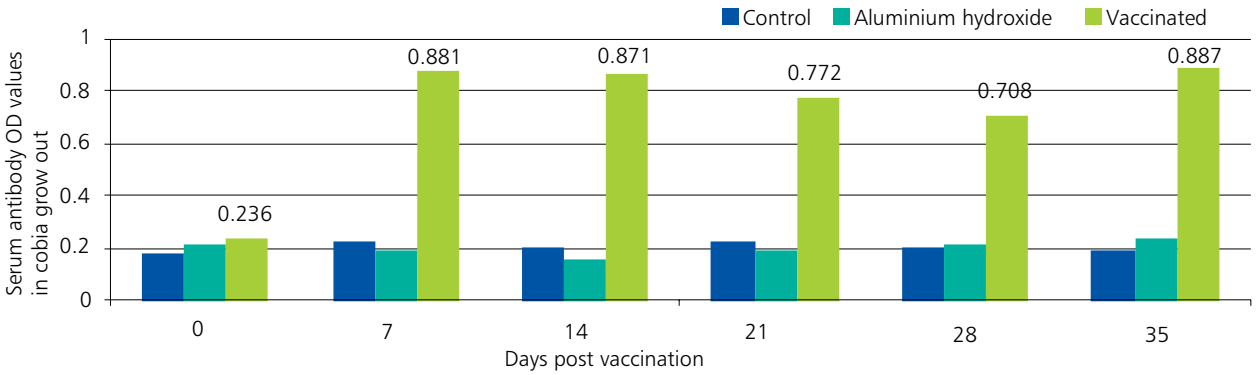

Fig.5. Hyper-immune serum analysis by ELISA

booster on 28th DPV was administered. On the 35th day OD value was higher than 21st and 28th day, indicating that serum antibody OD levels were increasing after the booster dose of the vaccine and the immune response was extended upto further 6 weeks. (Figs.4 & 5)

The first vaccination was done during April 2017 followed by a booster dose in July 2017 for the growout and broodstock cobia fishes cultured in Gulf of Mannar. No epizootics were observed during the culture period of one year. Again the next vaccination was initiated in April 2018 followed by the booster dose during July 2018. Even though the seasonal blooming (*Trichodesmium* sp) occurred during August and September, the vaccinated fish didn't show any stress condition or succumb to any diseases. But >60% mortality was observed in the control, unvaccinated fishes in the control cage. The same vaccination schedule was again followed during April 2019 with booster dose in July 2019 and vibriosis incidence was averted.

Standard guide lines

The study was performed based on the European medicines agency's standard guidelines 2013. To assess the acute safety characteristics of the vaccine, the fish should be monitored daily for mortality/ morbidity over a minimum of a 14th day period. For parenteral vaccine, necropsy examination should include investigation of the occurrence of effects such as pigmentation (Eg. Melanization) and adhesions measured using the 'Speilberg score'(Midtlyng *et al*. 1996) . The mortality is an evaluation parameter in vaccine challenge study. In our experimental finding the pathomorphological lesions were within the score of 0 and 1 grade. There was no visual appearance of lesions in abdominal cavity and no any minor opacity of peritoneum after evisceration.

Conclusion

The main objective of developing a multivalent vaccine against vibriosis is to prevent the seasonal epizootics in sea cage farmed cobia. It is concluded that in cobia fingerlings and growout fishes, the immune response can be improved against vibriosis by the timely vaccination. This might be due to the well developed detectable serological antibodies against vibriosis (Evaluated by challenge studies in fingerlings and ELISA) and establishment of acquired immunity by the vaccination method during sea cage farming activities. The acquired immunity might have developed after the vaccination, reduced the chances of life threatening infections. Thus, the regular epizootics observed in cage culture cobia, in every year during the month of July to September (pre-monsoon season) were prevented by vaccination and proper sea cage farming management. The vibriosis incidence has been successfully controlled by proper vaccination schedule. In sea cage farming of cobia, the first vaccination at the hatchery and subsequent booster injection at the cage will give the elevated antibody titres and immune response for more than 3 months. So, the fisherman or the cage farmers would not get any losses during the entire culture period, due to the seasonal epizootics of vibriosis.

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