Passive Maternal Antibody Transfer to Eggs and Larvae of Tiger Grouper (*Epinephelus fuscoguttatus*)

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Abstract. The immune response of Tiger grouper (*E. fuscoguttatus*) broodstocks and its passive transfer of maternal antibodies to eggs and larvae were evaluated following vaccination with an inactivated *V. harveyi*. Tiger grouper broodstock (mean BW 8.66 ± 0.09 kg, n=19) were vaccinated intraperitoneally (IP) and followed by a booster two weeks post vaccination, while Controlled Non-vaccinated (CG) broodstock were IP injected with PBS. The serum antibody level against *V. harveyi* was monitored for two weeks on post-vaccination and monthly up to 5 months post-vaccination. This study showed that the Vaccinated Group (VG) broodstock induced significantly (P<0.05) higher in specific IgM antibody level against *V. harveyi* as compared to the CG, which in turn induced a marked increased (P<0.05) in specific IgM in eggs and larvae produced from VG broodstock at 14 weeks post vaccination. The findings from this study suggested that inactivated *V. harveyi* vaccines were able to stimulate the immune response in broodstock and passively transferred the maternal antibody to their eggs and larvae.

1. Introduction

A number of bacterial diseases in grouper aquaculture have been reported in the Asian Pacific region including Malaysia, China, Korea, Thailand, Taiwan and Japan [1]. Major causes of fish disease are excess present of environmental pathogens, low resistance of the fish stock and unsatisfactory of water environment. Among bacterial diseases, vibriosis is a common problem in the intensive culture farms of grouper [2,3]. A wide range of vibrio bacteria were detected as pathogen of vibriosis such as *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum* and *V. vulnificus* [4]. The transmission of disease easily occurred among cultured fish as the habitat is in trapped and limited area such as in the fish rearing tanks or in cage culture and caused of mass mass mortality in cultured fish [5]. *Vibrio* related infections occur frequently in hatcheries, but epizootics and mass mortalities also commonly occur in pond reared fish [6]. Vibriosis caused by *V. harveyi* has shown clinical sign such as anorexia, darkening of the whole fish, local hemorrhagic ulcers on the mouth or skin surface, tail and fin rot, focal necrotic lesions in the muscle and swollen intestine, and eye opacity [7]. In many cases, vibriosis happens when the host organism is immuno-compromised cause by poor management in intensive culture and environmental stress conditions [8]. Therefore vaccination plays an important role in large-scale commercial fish farming [9]. Where, stimulation of host defenses can increase resistance to infectious disease by triggering specific immune responses to enhance defense system [10]. The effectiveness of vaccination strategies in developing an immune response depends upon the immunological capabilities and maturity of the host’s immune system. The development of lymphoid organs starts only after birth in all animals and in neonates, where the immune competence is achieved only after several weeks depending on the species. However, during the early phases of development, neonates are often exposed to a wide range of pathogens causing various types of diseases. Similarly, the ability to mount a specific immune response against a pathogen starts on 3 weeks onward of an age in fish, before which they either depend on non-specific immunity or maternally derived specific...
immunity [11,12]. Since the fish at their early stages of development are prone to several infectious diseases, significant loss due to larval mortalities in various fishes has been reported throughout the world [13,14,15]. Similarly massive mortalities of tiger grouper juvenile occurred in Malaysia due to vibriosis. To check this mortality, it is desirable to increase the immune status of these larvae. Transfer of immunity from mother to offspring has been reported from various fish species like trout, catfish and tilapia. In the process, an active immune response developed in mother through immunization is transferred to offspring through placenta or egg yolk during embryogenesis [16]. However little information is available on the immune response and transfer of maternal antibody of grouper to their eggs and larvae. Hence, this study was aimed to investigate the immune response by broodstock of grouper following vaccination to fight against vibriosis for 22 weeks period and evaluate passive transfer of maternal antibodies exist in egg and larvae of tiger grouper.

2. Materials and Methods

2.1. Killed-vaccine preparation

Virulent V. harveyi (VHJR7) obtained from the the Fish disease laboratory Borneo Marine Research Institute, University Malaysia Sabah. This strain selected on basic antigenic characteristic from the previous work by [17]. The bacteria were prepared and cultured overnight on thiosulfate citrate bile salts sucrose agar (TCBS) and grown in tryptone soy broth (TSB, Merck) supplemented with 1.5 NaCl at 30°C for 24 hours. In the following incubation, the bacterial concentration was determined using standard plate count techniques and inactivated in 0.5% (V/V) formalin in 24 hours at 40°C [18]. The bacterial cells were washed with phosphate buffer saline (PBS) and centrifuged at 2,415 x g rcf for 10 minutes to remove residue from culture. The inactivated V. harveyi were killed [18].

2.2. Vaccination of broodstock

The broodstock of tiger grouper were obtained from the Fish hatchery in Tanjang bedak Tuaran, Sabah and reared for 22 weeks. Thirty eight of tiger grouper broodstock grouper (n=38) divided into 2 group as Vaccinated Group (VG=19) and Control Non-vaccinated Group (CG=19) with the average body weight of 8.66 ± 0.09 kg were kept into 100 tonnes of marine water tank, with flow through system, the water temperature maintained at 28-30°C, with oxygen 5-6 mg/L, salinity 28-30 ppt and pH 7-8. The broodstock were fed 2 times per day with trash fish. Broodstock of VG were injected intraperitoneally with 1.0 ml/kg of 10^6 CFU/ml inactivated V. harveyi vaccine with booster after 2 week, while broodstock of CG were injected with 1.0 ml/kg PBS (phosphate-buffered saline).

2.3. Serum sampling

A random fish from VG and CG broodstock blood was collected from the caudal vein in 22 week up intervals, the blood are allowed to clot at room temperature for 1-2 hours then at 4°C overnight, the sera were collected and stored at -80°C.

2.4. Egg collection and larvae rearing

The egg were collected from the VG and CG broodstock from natural spawning. The fertilized eggs were washed with sterile salt water and incubated at the density of 30 eggs per litter in two 250 L polyethylene tanks at 28-30°C. The tanks were supplied with aerated, sand filtered, and UV treated sea water. Hatched larvae were fed live fry starter feed containing Nannochloropsis (10^6 cell ml^-1) and rotifer (Brachionus plicatilis) (10-12 ind ml^-1). The water quality was monitored daily for bacteria count and pH, as well as dissolved oxygen, nitrite, nitrate and ammonium. They were sampled randomly on weekly basis from 0 dAH until 20 dAH.
2.5. Extraction of egg and larvae

Eggs and larvae collected were washed three times with sterile PBS, pH 7.4. Homogenised with (1:1 v/v) in PBS centrifuged at 604 x g rcf for 10 min at 4°C. The supernatants were collected, centrifuged again for 3 times and stored at -20°C for next analysis.

2.6. Enzymes Linked Immunosorbent Assay (ELISA)

Antibody titre in broodstock, egg and larvae was detected by indirect ELISA according to [19] with some modification. The coating antigen was prepared by the cultured V. harveyi into TSB supplemented with 1.5% sodium chloride (NaCl) incubated for 24 h in shaker incubator at 30°C. The bacterial concentration was determined using Optical density at 1.0, were centrifuged and washed with PBS and suspended in carbonate-bicarbonate buffer (pH 9.6) to a concentration 1.2 x 10^5 CFU/ml. The suspension was boiled in water bath 97°C for 30 min to kill bacteria and cool at room temperature. The flat-rounded microtiter plates were coated with 100 ul of inactivated V.harveyi and was left overnight at 4°C before washed twice with washing soultion (PBS + 0.05% Tween 20). 200 uL of 1% bovine serum albumin (BSA) were added as blocking buffer and was incubated at 37°C for 1 hours. After incubation, 100 uL sampled (broodstock serum, egg and larvae extracts) were added (1:100) and incubated again at 37°C. Then, 100 uL of anti-grouper IgM (Aquatic Ltd) (1:1,000) was added and incubated at 37°C. A total of 100 uL of conjugated anti-mouse horseradish peroxidase (Immunocoujugated) (1:5,000) was added and incubated at 37°C. Next, the microtiter plates were washed again for three times with PBST and added 100 uL of TMB substrate solution before reaction was stopped with 0.2 mol/L sulphuric acid. Finally, the reading absorbance at 450 nm wavelength.

2.7. Statistical methods

The analysis of the data was done by using IBM SPSS statistic 20.0 under independent T-Test to observe the significant different of control sample and vaccinated sample p<0.05 (sig 2-tailed)

3. Results

The serum IgM antibody level as showed in Figure 1 of the VG broodstock showed non significant (P>0.05) difference from CG at the beginning of experimet before vaccination (0 week) at 0.3 (OD_{405}). VG showed significantly (P< 0.05) higher IgM level as compared to CG from 2 weeks post vaccination until the end of experiment. The antibody level of VG showed an increasing pattern in OD_{405} values from 0.3 at pre vaccination to 0.8 at 2^{nd} week and reached peak to 0.9 at week 6^{th} before it started to decrease significantly (P< 0.05) to 0.7 (OD_{405}) at 10^{th} week to 0.6 (OD_{405}) at 22^{nd} week post vaccination.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** The specific antibody level (IgM) (OD_{405}) against *V.harveyi* of the VG and CG broodstock from pre-vaccination (0 week) up to 22 weeks post vaccination.
Level of the specific antibody (IgM) in the eggs (0.15 at OD_{450}) in Figure 2 for both VG and CG broodstock showed a non-significantly difference (P>0.05) on 0th week before vaccination but at 14th weeks of post-vaccination, the antibody level showed significantly (P<0.05) higher with 0.27 (OD_{450}) in eggs of the VG broodstocks as compared to eggs of CG, 0.18 (OD_{450}) . The antibody level in eggs of the VG decline to 0.20 (OD_{450}) in 22nd week post-vaccination and showed non-significant different (P>0.05) to CG antibody level 0.17 (OD_{450}).

Figure 2. The variation of specific antibody (IgM) level (OD_{450}) in the eggs produced by the VG and CG of broodstock at pre-vaccination (0 week), 14 weeks and 22 weeks post vaccination.

The antibody level of the larvae Figure 3 showed non significant (P>0.05) difference at pre-vaccination (week 0) was 0.09 (OD_{450}) for both VG and CG for all larve stages at 7dAH (1st wkk), 0.08 (OD_{450}) at 14 dAH (2nd week) and 0.07 (OD_{450}) at 21 dAH (3rd week). While, it was observed the antibody level in larvae of the VG (0.27, 0.18, 0.15 OD_{450}) were significantly higher (P<0.05) then CG (0.15, 0.13, 0.10 OD_{450}) on 14th week post-vaccination for the larvae at 7dAH, 14 dAH and 21 dAH respectively. Similarly on 22nd week post-vaccination the antibody level for in larvae of the VG (0.15, 0.12, 0.10 OD_{450}) were significantly higher (P<0.05) as compare to CG (0.13, 0.07, 0.06 OD_{450}) in the 7 dAH, 14 dAH and 21dAH larvae stages respectively. However, all larvae showed declined pattern of the antibody level in the 7 dAH, 14 dAH and 21dAH.

Figure 3. Specific antibody level (IgM) (OD_{450}) against V. harveyi in larvae produced by the VG and CG of broodstock at pre-vaccination (0 week), 14 weeks and 22 weeks post vaccination.
4. Discussion

The increase production of grouper cause the intensive aquaculture and plague by disease associated with crowding stress triggering infection and disease transmission, lead economic loss in aquaculture industry [20]. In early stages of development, fish embryos (eggs) and larvae lack ability to synthesize immune and lymphoid organs are not yet full maturated [21,22], at this stages the maximum of mortalities caused several infectious disease. An alternate method used immunisation is one availability to protect the seed from infectious disease from parents and transfer of the maternal immunity in several species [23, 24, 25, 26]. The humoral immune response of Grouper broodstocks was investigated frollowing injection IP of the inactivated V. harveyi vaccine and measured by Indirect ELISA.  

In the present study, on the day of egg collection, although the level of IgM in the eggs was only half of the IgM level in the plasma of broodstocks, the findings from this study supports the interpretation that IgM in the eggs is derived from the circulation of the broodstock. The result clearly demonstrated that vaccination of the broodstocks induced a significant increased in the concentration of IgM specific antibody level within 22nd week post vaccination indicates that immunisation are able to evoke the immune response in the vaccinated grouper broodstock which was then transfer its maternal antibody into the eggs and larvae similarly as reported by [25] the existence of maternal antibodies in eggs, larvae and fry of the vaccinated Indian major carp broodstocks. Where, the successful transfer of antibody from mother to offspring has also documented in several teleosten fishes like plaice (Pleuronectes platessa), tilapia (Oreochromis aureus), Carp (Carpio, labeo rohita), sea bream (Sparus aurata), seabass (Dicentrarchus labrax) and rainbow trout (O.mykiss) [27]. Total IgM level in all samples (serum, eggs and larva extracts) originating from VG broodstocks were higher in comparison to similar activities from samples of the CG broodstocks. The level of IgM specific antibody in egg extracts from pre-vaccination broodstocks were indistinguisable among the two groups but significantly (P<0.05) high at 14 weeks post vaccination. This is in agreement with the observation in the significantly higher (P<0.05) antibody level of larvae extracts that were hatched from the eggs of VG broodstocks at 14-week and 22-week post-vaccination. Lillehaug et al., (1996) indicate that specific maternal antibodies are transfererred from Atlantic salmon mothers to eggs and fry at very low levels [28]. It is possibly due to the antibody level in fish embryo has been reported to a vary according the time intervals between the vaccination of mother and spawning [29]. Hence, in this study it was observed the antibody titre was found to be slightly higher in eggs and larvae of VG broodstock at 14-week post vaccination as compared to 22-week post vaccination. While other researcher have been reported that significant transfer of antibody from mother to offspring by immunising the mother one month prior to spawning [29].  

The Ig within the eggs yolk were absorbed by the larvae and provide for the maternal transfer of immunity [30]. 

However, the result showed the antibody level gradually declined from broodstock to eggs and larvae, this is in agreement to the finding on previous study by [25]. The low level of antibody was found to be transferred from mother to larvae [26] and the levels in the embryos varied with the time between immunization of the mother and spawning [31]. This maternal antibody transfer is affected by many elements, including biological factors and environmental conditions experienced by broodstock [32]. The first appearance IgM in lymphocytes varies considerably in different fish species and are more dependent on size than age [33,34]. In oviparous fishes, maternal IgM is initially transferred via yolk to immature oocytes during vitellogenesis and then to eggs and yolk sac larvae in a sequential manner [35]. The Ig levels increased to a maximum at around hatching [36] and gradually decreased from hatching until complete yolk absorption [30]. Nevertheless, it was observed in this study that within the same group, all larvae showed general decline in the IgM levels from 7 dAH, to 14 dAH and 21 dAH for both VG and CG group of 14 weeks and 22 weeks post vaccination of the broodstocks.
5. Conclusion

In conclusion, the present investigation revealed that the vaccination with inactivated *V. harveyi* has significantly increased in specific IgM in tiger grouper broodstock and able to transfer its maternal antibodies to eggs and larvae. Therefore, the vaccination of broodstocks prior to spawning season could be used as an effective way to protect larvae against disease before their specific immune system is fully developed. This will increase specific immunity of larvae in nursery rearing practices towards preventing mass mortalities due to *V. harveyi* infections. Hence, this strategy could overcome antibiotic usage in aquaculture by practicing vaccination of broodstock for a better health management for a more sustainable aquaculture industry.

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