In-vitro responses of peritoneal macrophages of marine red hybrid tilapia (*Oreocromis* spp.) model to vibriosis: A comparative study between vaccinated and non-vaccinated fish

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ABSTRACT

Vibriosis, an infection caused by Gram-negative bacteria of the genus Vibrio, is a major disease in global aquaculture. Vaccination is an effective preventive measure against vibriosis. Phagocytosis, the fundamental cellular mechanism that eliminates invading pathogens is governed by phagocytic cells like macrophages and neutrophils. This study evaluates the effect of formalin-inactivated feed-based *Vibrio harveyi* vaccine on macrophage activities to challenged live Vibrio spp. A total of 345 marine tilapias were divided into three equal groups and were vaccinated at weeks 0, 2, and 6 with adjuvanted (Group 1) and non-adjuvanted (Group 2) vaccine, while unvaccinated fish were fed with PBS and served as control group (Group 3). Serum samples were collected at 2-week intervals while peritoneal macrophages were collected on weeks 0 (pre-vaccination) and 10 (post-vaccination). Serum IgM against *V. harveyi* and *V. alginolyticus* were measured by indirect ELISA. The cultured macrophages were exposed to *V. harveyi* or *V. alginolyticus* and harvested at 0-, 30-, 60- and 120-minutes post-infection. The rate of phagocytosis, intracellular killing rate of bacteria and rate of macrophage cell death were calculated. The primary and first booster vaccinations enhanced the serum IgM levels that lasted 4 weeks, while the second booster maintained the high IgM levels until week 12 in both vaccinated Groups 1 and 2. However, Group 1 developed significantly ($p<0.05$) higher IgM levels against both *V. harveyi* and *V. alginolyticus* than Groups 2 and 3. Similarly, Group 1 demonstrated significantly ($p<0.05$) higher rate of macrophage phagocytosis than unvaccinated Group 3 at 30 min and 60 min after exposure to *V. harveyi* and *V. alginolyticus*, respectively. Group 1 also showed significantly ($p<0.05$) higher intracellular killing of *V. harveyi* and *V. alginolyticus* than Groups 2 and 3 at 30-, 60- and 120 min but non-significant ($p>0.05$) lower macrophage death rates at 30 min after *V. harveyi* challenge and at 60 min after *V. alginolyticus* challenge compared to Groups 2 and 3. In conclusion, oil-adjuvanted formalin-killed *V. harveyi* feed-based vaccine could induce effective systemic immunity that enhances the phagocytosis and killing of *V. harveyi* and *V. alginolyticus* by macrophages of marine tilapia.

Keywords: *Vibrio harveyi*, *Vibrio alginolyticus*, vibriosis, marine tilapia, fish peritoneal macrophages, immunofluorescence

INTRODUCTION

Vibrio spp., causes economically important vibriosis in many marine fish species causing fatality. The disease by Vibrio is primarily associated with many risk factors such as stress caused by handling, density, transportation, skin damage, water parameters and the presence of other pathogens. Vibrio organisms have been reported frequently in vibriosis outbreaks in Malaysia. Vaccination is regarded as an effective method of prevention against vibriosis in cultured fish (1).In bony fish, the phagocytosis is a fundamental mechanism that eliminates invading pathogens and it is governed by phagocytic cells like macrophages and neutrophils The macrophages provide a first line of cellular defense against pathogens (2). In this study, we evaluate the effects of an inactivated feed-based vaccine in stimulating systemic and innate immunities in controlling infections by *Vibrio harveyi* and *V. alginolyticus* in marine tilapia model. We assessed and compared the antibody response in serum and the *in-vitro* efficiency of macrophages from vaccinated and unvaccinated marine tilapia against *V. harveyi* and *V. alginolyticus*.

METHODOLOGY

Figure 1. Vaccine incorporated feed types

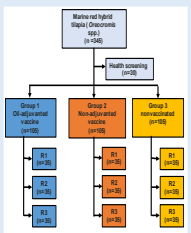

Figure 2. Experimental design

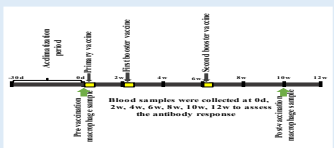

Figure 3. Vaccination and sampling timeline

RESULTS AND DISCUSSION

Antigen-specific antibodies in serum

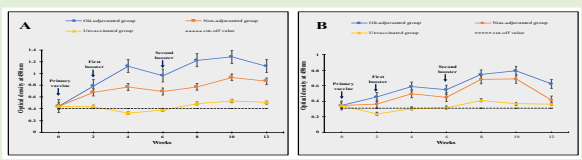

Figure 4. IgM antibody titer in the serum against *V. harveyi* (left) *V. alginolyticus* (right) in the oil-adjuvanted vaccine (blue line), non-adjuvanted vaccine (orange line) and unvaccinated control (yellow line) group.

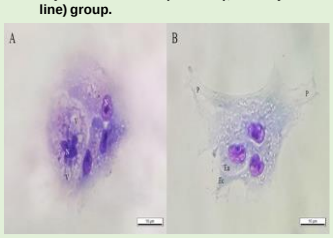

Figure 5. Photomicrographs of a cluster containing several macrophages (Wright stain, bar = 10 µm). Note the variable size and surface modifications. A. Macrophages at 6 h post-incubation. B. Macrophages at 24 h post-incubation. N=Nucleus; V=Vacuole; P=Pseudopodia; En = Endoplasm; Ec = Ectoplasm).

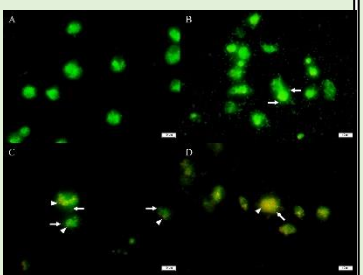

Figure 6. Photomicrographs of macrophages of marine tilapia after exposure to *V. harveyi* or *V. alginolyticus* (acridine orange, bar = 10 µm). A. Viable macrophages at 0 min of incubation emitting green fluorescence, without observable phagocytosis of Vibrio sp. B. Viable macrophages containing viable phagocytosed Vibrio sp. (arrows). C. Viable macrophage (green) containing phagocytosed viable (arrows) and non-viable Vibrio sp. (arrowheads). D. Non-viable macrophages with viable (arrow) and non-viable Vibrio sp. (arrowheads).

In-vitro challenge with *V. harveyi*

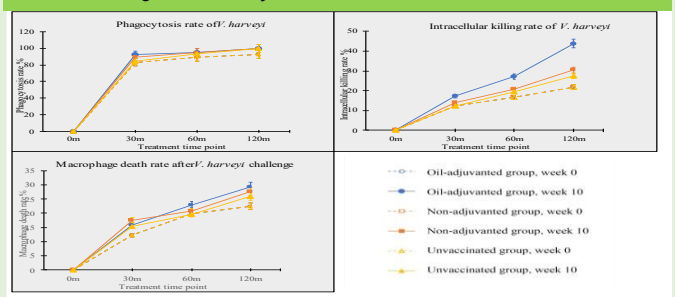

Figure 7: *In-vitro* rates of phagocytosis, intracellular killing and cell death of macrophages after the *V. harveyi* challenge of vaccinated and unvaccinated marine tilapia.

In-vitro challenge with *V. alginolyticus*

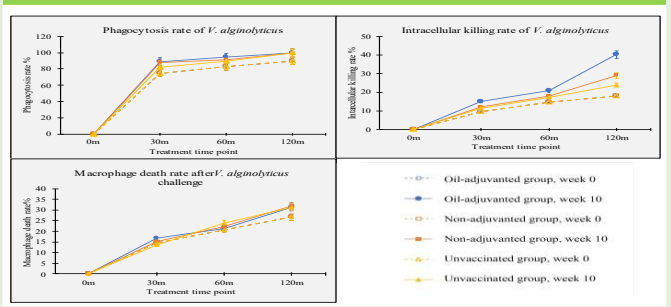

Figure 8: *In-vitro* rates of phagocytosis, intracellular killing and cell death of macrophages after the *V. alginolyticus* challenge of vaccinated and unvaccinated marine tilapia.

CONCLUSION

Oil-adjuvanted vaccine showed more efficient immune response, phagocytosis, intracellular killing, and resistance compared to non-adjuvanted vaccinated and unvaccinated tilapia, following infection by *V. harveyi* or *V. alginolyticus*. Although the mean rate of phagocytosis is non-significant between the two vaccine groups, the intracellular killing is significantly higher after administration of the oil-adjuvanted vaccine. In terms of macrophage efficiency, vaccination using killed *V. harveyi* was proven to be beneficial to control infections by *V. harveyi* and *V. alginolyticus*.

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