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Efficacy of live attenuated vaccine derived from the *Streptococcus agalactiae* on the immune responses of *Oreochromis niloticus*

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ABSTRACT

Streptococcus agalactiae species have been recognized as the main pathogen causing high mortality in fish leading to significant worldwide economical losses to the aquaculture industries. Vaccine development has become a priority in combating multidrug resistance in bacteria; however, there is a lack of commercial live attenuated vaccine (LAV) against *S. agalactiae* in Malaysia. The aim of this study is to compare two methods using attenuated bacteria as live vaccine and to evaluate the efficacy of selected LAV on the immune responses and resistance of *Oreochromis niloticus* (tilapia) against *S. agalactiae*. The LAV derived from *S. agalactiae* had been weakened using the chemical agent Acriflavine dye (LAV1), whereas the second vaccine was weakened using serial passages of bacteria on broth media (LAV2). Initial immunization was carried out only on day one, given twice-in the morning and evening, for the 42 day period. Serum samples were collected to determine the systemic antibody (IgM) responses and lysozymal (LSZ) activity using ELISA. On day 43 after immunization, the fish were injected intraperitoneally (i.p) with 0.1 mL of *S. agalactiae* at $LD_{50} = 1.5 \times 10^5$ (CFU)/fish. Fish were monitored daily for 10 days. Clinical signs, mortality and the relative percent of survival (RPS) were recorded. Trial 1 results showed a significant increased ($P < 0.05$) in serum IgM titers and LSZ activity as compared to LAV2 and the control group (unvaccinated fish). The efficacy of LAV1 was proven effective as determined by the RPS values, LAV1 at 81.58% as compared to LAV2 at 65.79%. Trial 2 of LAV1 and control group were further determined by administering primary and booster doses revealed a RPS value for LAV1 of 82.05%, with the significant enhancement on the immune responses of tilapia as compared to control group. In conclusion, LAV revealed to elevate antibody IgM levels, LSZ activity and provide long-term protection when added to feed. LAV is a low-cost vaccine shown to rapidly increase the immune response of fish and increase survival rates of fish against *S. agalactiae* infection.

1. Introduction

Nile tilapia (*Oreochromis niloticus*) is the second most important fish species in aquaculture in terms of quantity. Higher production outputs through intensified farming systems involving high stock densities has rendered fish to be highly susceptible to diseases and infections. . In Malaysia, *Streptococcus agalactiae* is recognized as an important causative agent of mortality in tilapia [1,2]. Treatment of such fish infections with antibiotics often leads to bacterial drug resistance, rendering the treatment ineffective. Vaccination greatly reduces the need for drugs and chemicals and is an environmentally friendly disease control strategy to combat significant fish diseases.

As of today, the 3 widely used vaccines in controlling *S. agalactiae* infection include formalin-killed vaccine [3], live attenuated vaccine [4,5], and DNA and subunit vaccines [6]Different strategies can be used in developing modern vaccines for the prevention of *S. agalactiae* infections in fish diseases. Vaccination can be carried out before exposure to the pathogen, thus allowing adequate time for an immune response to develop. However, infectious disease of fish is most validly prevented through the use of live attenuated vaccines (LAV), which provides the most effective immunity [7]. LAV induce mucosal, cellular, and humoral immunity in the host [8]. Achieving desirable features of live vaccines requires the attenuated vaccines to be safe, efficacious and capable of stimulating a strong cellular immune response leading to

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