

TITLE: BIVALENT VACCINE FOR TILAPIA: PHASE I ANALYSIS

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ABSTRACT:

Nile Tilapia (*Oreochromis niloticus*) is the most important species of fish farmed in Brazil, representing 55.4% of the total national fish production in 2018. One of the main factors limiting the increase of tilapia production in Brazil is the spread of pathogens by immunosuppression of the fish defense system, causing large economic losses. Vaccination of fish can prevent infections against major pathogens. Here we performed phase I analysis of a vaccine against *Aeromonas sobria* and *Streptococcus agalactiae* in Nile tilapia from the western region of Paraná, Brazil. A total of 10 commercial fish were selected from the western region of Paraná and maintain in aquariums in the laboratory until the juvenile phase. The vaccine was developed with 1×10^7 cells/mL of *A. sobria* and *S. agalactiae* isolated from the region with an oily adjuvant. The fish were divided into two groups, the control, which received a dose of 5 μ L sterile saline and the vaccinated group that received 5 μ L of the vaccine. The agglutination test was conducted on Kline's plate, 30 days after vaccination. The test was carried out with the antigen of both bacteria, *S. agalactiae* and *A. sobria*, separately, with 20 μ L of the bacterial antigen and 20 μ L of the sera from groups that were added at 1:10, 1:100 and 1:1000 dilutions. The visualization of the agglutination was performed with an optical microscope. Fish sera from the vaccinated group showed strong agglutination at all dilutions tested, whereas fish sera from the control group showed no agglutination (Figure 1). Agglutination tests enable rapid identification of precipitates resulting from the antigen-antibody interaction. The results demonstrate that after 30 days of vaccine application, the fish in the vaccinated group have antibodies against the bacterial antigens of both bacteria, *S. agalactiae* and *A. sobria*, indicating that the vaccine developed is able to stimulate fish against the development of diseases caused by these pathogens. Post-challenge testing are in progress.

Keywords: *Aeromonas sobria*; *Streptococcus agalactiae*; Agglutination test.

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Figure 1. Visualization of agglutination test with the *Aeromonas sobria* and *Streptococcus agalactiae* antigens and control and vaccinated fish sera at 1:10, 1: 100 and 1: 1000 dilutions.

