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## LONG-TERM EFFICACY OF AN IMMERSION VACCINE PROTOTYPE AGAINST FLAVOBACTERIUM OREOCHROMIS IN TAMBAQUI (COLOSSOMA MACROPOMUM)

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### RESUMO

The effective protection of farmed fish against bacteriosis is fundamental in the context of One Health. Immersion vaccines with inactivated pathogens is considered an elective method to prevent bacteriosis in aquaculture due to practicality, safety, and effectiveness for long-term periods, which combines contribution to fish health, human food security and the health of the aquatic environment. In this context, the objective of this study was to determine the long-term efficacy of an immersion vaccine prototype and optimize the protocol to protect tambaqui against *Flavobacterium oreochromis*. To prepare the vaccine prototype, a strain from the most prevalent genetic group of *F. oreochromis* (previously determined by REP-PCR, non-published data) was prospected from farmed diseased tambaqui. The selected-strain was reactivated on G agar (28°C/48h), and transferred to G broth (28°C/24h/140rpm). The inoculum was resuspended in sterile-PBS, until  $1.6 \times 10^{12}$  CFU/ml, and then inactivated with 1% formalin. The assay (CEUA003/2021) included 336 tambaquis ( $4.2g \pm 0.90$ ), distributed into 12 experimental units of 90L (3 groups/4 replicates/28 juveniles each): vaccinated with single-dose, vaccinated with booster-dose and control (non-vaccinated). At day 0, vaccinated fish were immersed for 2h in the respective inoculum, containing 10L of water and 3.5mL of bacterin ( $5.6 \times 10^8$  CFU/L in vaccination-tank). The bacterin was replaced by sterile-PBS in the control group (at day 0 and 21) and in the single-dose vaccinated group (at day 21). After immunization, fish returned to experimental units and were maintained with routine standardized management for 5 months (up to  $\pm 300g$ ). To determine the vaccine efficacy, at the end of the experimental trial (5 months after first immunization), fish from all groups were anesthetized and intraperitoneally challenged with 0.1mL of the homologous strain at  $2.75 \times 10^8$  CFU/ml for each 10g of live weight. Mortality was analyzed until the plateau (15 days). For diagnostic, the bacteria identity of recently-dead fish was confirmed by re-isolation from muscle and kidney on G agar at 28°C/48h, followed by proteomic analysis in MALDI-TOF. A relative percentage of survival (RPS) >80% was considered effective. The results demonstrated that the groups vaccinated with single-dose and booster protocols presented long-term efficacies of 83.33% and 100%. Although efficacy was achieved with the single-dose protocol, due to the biological risk of the pathogen, the protocol with booster is the most suitable for long-term protection of tambaqui against Flavobacteriosis. This study provides an efficient immersion vaccine prototype against a key-pathogen of tambaqui, contributing to the

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**PALAVRAS-CHAVE:** Bacteriosis, Flavobacteriosis, Prevention

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