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PRODUCTION OF POLYCLONAL ANTIBODIES ANTI - IgM OF NILE TILAPIA, *Oreochromis niloticus* FOR STANDARDIZATION OF IMMUNOENZYMATIC ASSAY

PRODUÇÃO DE ANTICORPOS POLICLONAIS ANTI - IgM DE TILÁPIA-DO-NILO, Oreochromis niloticus PARA PADRONIZAÇÃO DE ENSAIO IMUNOENZIMÁTICO.

P. B. CLISSA[1*]; D. P. OREFICE[1], M.COLOMBINI[1]; M. C. GUIMARÃES[2]; E. E. BACH[3]; D. C. DIAS[2]; L. TACHIBANA[2]

Dear Editor,

According to *Anuário 2021 Peixe BR da Piscicultura*, in 2020 the Brazilian fish culture reached 802,930 tons, maintaining growth of around 5.93% compared to the previous year. Tilapia is the most popular aquaculture species item farmed in Brazil, representing 60,6% of the national production, with 486,155 tons in 2020, an increase of 12.5% over the previous year. As a result, Brazil consolidates itself in the 4th position among the largest Tilapia producers in the world. Among the largest producing states in Brazil, Paraná stands out, which leads the national ranking, with 166,000 tons, followed by São Paulo, with 70,500 tons, and Minas Gerais with 42,100 tons.

The intensification of fish production increases outbreaks of infectious diseases that affect the aquaculture sector. Streptococcosis is a fish disease that is horizontally transmitted by direct contact between infected and healthy animals or indirect contact with water and fomites, resulting in disseminated disease in fish production systems (MIAN et al., 2009). *Streptococcus species* are considered the pathogenic bacterium with the highest occurrence and economic importance for intensive fish culture (MISHRA et al, 2018).

One of the most used ways to treat and prevent bacterial infections in a fish farm is the administration of antibiotics; however, the abuse of these drugs represents a risk to the environment, besides causing drug resistance to future infections (CARUSO, 2016), A good alternative strategy for the control of this disease is the administration of vaccines in animals, which has proven to be a successful strategy (SECOMBES & BELMONTE, 2016). Fish immunization can be performed by intraperitoneal injection, immersion, or oral administration (DADAR et al, 2017). Although these methods have the same purpose, there are advantages and disadvantages concerning the efficiency practicality, side effects, and cost/benefit of immunization. Therefore, it is interesting for fish producers to develop methods capable of identifying and thus confirming those animals that have been immunized.

In this letter, we describe the development of an immunodiagnostic assay capable of detecting specific IgM in the blood circulation of experimental Nile tilapia that received the vaccine against *S. agalactiae*.

Rabbit immunization protocol: The Immunization procedure was approved by the Animal Ethics Committee of the Butantan Institute (CEUA Protocol number 3124080618). A female rabbit, weighing 2.5 kg, was immunized intramuscularly with a solution containing 500 µg of purified tilapia IgM/500 µL of PBS, emulsified in 500 µL of Marcol-Montanide®. After 30 days, the animal was injected *intramuscularly* with 500 µg of IgM, following the same protocol as before. At the end of 30 days after the second immunization, two more doses of 150 µg of previously purified IgM/300µL of PBS were injected *intradermally (i.d.)* at intervals of 15 days. Blood was collected after the last immunization by central artery ear bleed. The serum was separated from the red cells by centrifugation (2,000 RPM/10 min) and stored at -20 ºC until further use.

IgG Rabbit anti-IgM Purification: The total sera were delipidated with 20 µL/mL of a 10% dextran sulfate solution and 100 µL/mL of $CaCl_2$ 1.0 M, stirred for 15 min, centrifuged at 5000 g for 10 min, and the supernatant dialyzed against PBS overnight. Rabbit serum IgGs were isolated by affinity chromatography using the High Trap Protein A column (5 mL) and borate-saline buffer (pH 8.5) for sample application and adsorption of IgGs to protein A. The adsorbed IgGs were eluted with 0.2 M glycine / HCl buffer containing 0.15 M NaCl (pH 2.8). The eluate was collected in 1 mL fractions, immediately neutralized with 1 M Tris, and then dialyzed against PBS (pH 7.4). The IgGs were then concentrated in the Amicon Diaflo 8050 System and the concentration was determined by the molar extinction coefficient factor for rabbit IgG (HARLOW & LANE, 1988).

Reactivity of rabbit IgG against Streptococcus agalactiae inactivated evaluated by Enzyme-Linked Immunosorbent Assay (ELISA): ELISA was standardized using inactivated *S. agalactiae* as the substrate to identify

[1] Butantan Institute, Av. Vital Brasil, 1500, Butantã, CEP 05503-900, São Paulo, SP, Brazil.
[2] Fisheries Institute/APTA/SAA, Av. Francisco Matarazzo, 455, Perdizes, CEP 05001-970, São Paulo, SP, Brazil.
[3] Biological Institute, Av. Conselheiro Rodrigues Alves, 1252, CEP 04014-002, São Paulo, SP, Brazil.
* Patricia Bianca Clissa,. Butantan Institute, Av. Vital Brasil, 1500, Butantã, CEP 05503-900, São Paulo, SP, Brazil. patricia.clissa@butantan.gov.br

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Tilapia groups that were vaccinated or not. The bacterin was diluted to a concentration of 10^5 CFU in carbonate buffer pH 9.6 and allowed to adhere to the Maxisorb ELISA plate (NUNC®) overnight at 4°C. After that period, the plate was washed 3 times with washing buffer (PBS / 0.05% TWEEN 20) and was blocked with 3% nonfat bovine milk/PBS Tween for 2 h at 37°C and then washed with washing buffer again. Tilapia sera submitted to vaccination protocol was diluted 40 times in diluent solution (1% nonfat bovine milk/PBS/ 0,05% Tween 20) and incubated for 1 hour at 37°C. After the incubation period, the plate was washed again and incubated with IgG Rabbit anti-IgM diluted 1000X in diluent solution for 1h at 37°C, and after another wash step incubation was performed with the anti-rabbit antibody HRP (SIGMA®) diluted 1000X in diluent solution, for 1h at 37°C. After a new 3-wash step, the colorimetric substrate Tetramethylbenzidine (TMB - BD®) was added and the solution was blocked with 30% sulfuric acid. The absorbance was read at a wavelength of 450 nm (HARLOW & LANE, 1988).

Tilapias vaccination schedule: The experiments were performed at the Development Aquaculture Research Center/Fisheries Institute/APTA/SAA, SP, Brazil. The project was approved by the Ethics Committee of Fisheries Institute (protocol No. 01/2018). The vaccination was carried out using a completely randomized design with three treatments and five replicates per treatment: Not vaccinated (Tilapia injected with PBS); Adjuvant (Tilapia injected with adjuvant); Vaccinated (Tilapia injected with vaccine - Biocamp Laboratory, Campinas, Brazil). Fish were anesthetized by immersion with eugenol solution (75 mg/L) and injected intraperitoneally with 100 μL of respective inoculum. The procedure was repeated 14 days after the 1st vaccination (for booster).

Results: The ELISA protocol was successfully standardized and was shown capable of detecting different levels of IgM anti-*S. agalactiae* in the Tilapia serum (Figure 1). The vaccine increased the levels of specific IgM when compared to the administration of adjuvant.

Our results are promising and indicate that it is feasible to continue the studies in order to develop a commercial trial to identify those animals that have been immunized against the bacterium *S. agalactiae*, being interesting for fish producers.

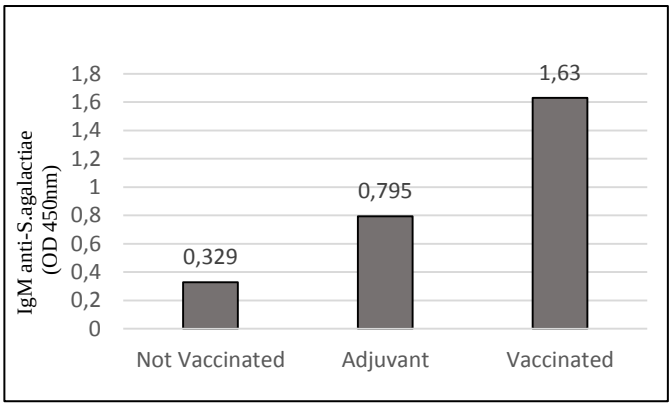

Figure 1 - Quantification by ELISA of IgM anti - *S. agalactiae* on serum samples from Nile tilapia (*O. niloticus*) collected 14 days after receiving: PBS (Not Vaccinated); Marcol-Montanide (Adjuvant) and Vaccine against *S. agalactiae* (Vaccinated and booster).

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