TITLE: DEVELOPMENT OF IMMUNOREAGENTS FOR BRAZILIAN *RANAVIRUS* DIAGNOSTICS: RECOMBINANT MAJOR CAPSID PROTEIN (rMCP) AND ANTI-rMCP POLYCLONAL ANTIBODIES

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ABSTRACT:
Ranavirus are pathogens capable of infecting three classes of ectothermal vertebrates (fish, amphibians and reptiles). In Brazil, the occurrence of outbreaks caused by *Frog virus* 3 (FV3) has increased, mainly in commercial bullfrog breeding. Additionally, ranavirus infection pose a risk to other sectors of aquaculture, such as fish farming. So far, there is only a single Brazilian isolate of FV3 in cell culture. Aiming at the production of immunoreagents for use in diagnostic tests, the objectives of the present study were to produce the recombinant *Major capsid protein* (rMCP) of the Brazilian *Ranavirus* FV3-like isolate and anti-rMCP rabbit polyclonal antibodies (CEUA/FZEA nº 2265041116). To this end, the MCP gene was cloned into pGEM®-T that was further submitted to digestion with restriction enzymes with the recovered fragment being cloned into expression vector pET28a(+). Plasmid pET28a/MCP was transformed into *Escherichia coli* strain Rosetta™(DE3) and the protein was expressed after induction with IPTG. The protein purification was performed using a column packed with Talon® Metal Affinity resin, followed by dialysis. For the production of anti-rMCP polyclonal antibodies, three immunizations of two young male New Zealand White rabbits were carried out via intramuscular and subcutaneous routes, at intervals of 14 days. As a control, prior to the first inoculation, pre-immune sera were collected and analyzed by Immunoblot as well as serum aliquots collected previously to the third inoculation and at 14th day after. Then, plasmids pGEM-T/MCP and pET28a/MCP carrying the 1398-bp insert and overexpression of the rMCP protein at 4 h of induction were obtained. However, the rMCP was in insoluble form and its recovery was carried out under denaturant purification and dialysis. However, the protein precipitated at the refolding process; therefore, low concentrations of the soluble rMCP were obtained. The three rabbit immunizations were carried out with 80µg/mL, 60µg/mL and 40µg/mL respectively. Immunoblot revealed increasing titres of anti-rMCP polyclonal antibodies through second to last blood collections. In view of the above, it is expected that the developed immunoreagents can contribute to the diagnosis of ranavirus infection in fish, as well as amphibians, aiming to the improvement of sanitary conditions of Brazilian aquaculture. Additionally, these immunoreagents may support future studies for the production of vaccine immunogens against *Ranavirus*.

Keywords: FV3, Heterologous expression, Immunobiologicals, Ranaviral infection

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