TITLE: ISOLATION, PHENOGENOTYPIC IDENTIFICATION AND EVALUATION OF THE INDUCTION OF APOPTOSIS BY BRAZILIAN STRAIN OF *FROG VIRUS 3-LIKE* FROM *LITHOBATES CATESBEIANUS*

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ABSTRACT:
Ranaviruses (family *Iridoviridae*) are a growing worldwide threat to populations of amphibians, fish and reptiles. In this sense, it was proposed the isolation and characterization of a pathogenic *FV3-like* strain associated with an outbreak with high mortality of adult amphibians (*Lithobates catesbeianus*) in a frog farm in Brazil. In addition, we aimed to verify the possible induction of apoptosis by this strain. Virus isolation was performed from organ fragments of animals, which were inoculated into BF-2 (*Lepomis macrochirus*) cells. The polymerase chain reaction (PCR) technique was carried out with primers directed to MCP and ORF53R, followed by nucleotide sequencing and phylogenetic analysis. Other diagnostic techniques, including transmission electron microscopy, indirect immunofluorescence (IFA) and Western blot (WB) were used in the characterization and confirmation of the isolation. Two apoptotic markers were used to investigate the possible activation of apoptosis in infected BF-2 cells that were sampled from 4 to 16 hours, including the activation of effector caspases and the fragmentation of cellular DNA by the TUNEL assay. We obtained the isolation of an *FV3-like* strain with typical cytopathic effects for ranavirus. PCR confirmed the presence of viral DNA in cultures of infected BF-2 cells, with positive results for MCP and ORF53R oligonucleotides. Analysis of the nucleotide sequence obtained for MCP revealed high homology (99%) with *Frog virus 3*, type species member of the genus *Ranavirus* and the isolated strain showed to be closely related to other ranaviruses detected in Brazil in phylogenetic reconstruction. Electron micrographs showed icosahedral particles in infected BF-2 cells, with a nucleocapsid measuring about 150 nm, similar to ranaviruses. Infected BF-2 cells showed positive staining in the IFA test for MCP, while MCP was demonstrated by WB by the identification of a polypeptide estimated at 50 kDa. Finally, we verified that the FV3-like isolate is able to induce apoptosis in BF-2 cells, since effector caspases were detected at all experimental times, suggesting to be a caspase-dependent mechanism. The fragmentation of cellular DNA, clearly observed at all experimental times, confirmed the induction of apoptosis by the Brazilian FV3-like strain. The results obtained here become the basis for several future studies, and may contribute as a subsidy to better understand the outbreaks caused by these viruses in the country.

Keywords: Amphibians, Brazil, Cell culture, *Ranavirus*

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