

TITLE: MOLECULAR SCREENING OF *RANAVIRUS* IN WILD ANURAN SPECIES IN THE EAST-CENTRAL REGION OF THE STATE OF SÃO PAULO, BRAZIL

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ABSTRACT:

Amphibians play key roles in several habitats; however, they are in massive decline and extinction across the globe. Emerging diseases are implicated in this population decline, especially those caused by viruses of the genus *Ranavirus*. Ranavirus infections have alarmed the world over the past few decades because they affect not only amphibians, but also fish and reptiles, therefore, pose a potential threat to global biodiversity and aquaculture production. In Brazil, information about the occurrence of ranaviral disease in wild animals is scarce; however, there are few reports about *Frog virus 3* (FV3)-ranavirus circulation in bullfrog farms in Brazil. In this context, the aim of this work was to investigate the occurrence of FV3 infection in wild specimens of whistling frog (*Leptodactylus fuscus*) from the cities of Pirassununga and Porto Ferreira, located in the east-central region of the state of São Paulo, Brazil. This work was previously approved by the institutional ethics committees (Sisbio protocol no. 72166447; and CEUA-FZEA protocol no. 3503101116). A total of 40 animals were captured and tissue samples from liver, spleen and kidney of each specimen were submitted to pooled DNA extraction. DNA extraction was carried out by the SV Genomic DNA Purification System kit (Promega, EUA), using 20 mg of samples. PCR was then performed using the primers described for the MCP gene, which encodes the main viral capsid protein, highly conserved within the *Ranavirus* genus and recommended by the International Organization of Epizootics (OIE). PCR products obtained were submitted to agarose gel electrophoresis. All the analyzed samples were negative for the ranaviral MCP gene, not detecting the presence of FV3 in the evaluated cities. This approach can contribute to the epidemiological monitoring of the occurrence of ranavirus in wild anurans, in view of the presence of these viruses in bullfrog farms in different states of Brazil, which certainly represents a potential risk of ranavirus spreading to the natural environment.

Keywords: *Ranavirus*, FV3, *Leptodactylus fuscus*, molecular diagnostics.

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