Title: Molecular investigation for Iridoviral and Herpesviral infections associated with mortality in fishes from São Paulo, Brazil

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Abstract:

Megalocystivirus (MV), a genus belongs to family *Iridoviridae*, is composed of double-stranded DNA virus responsible for many outbreaks of great ecological and economic impacts on freshwater and marine fish of importance in aquaculture around the world, including Brazil. The genus *Megalocystivirus* includes five species of virus: orange-spotted grouper iridovirus (OSGIV), rock bream iridovirus (RBIV), red sea bream iridovirus (RSIV), large yellow croaker iridovirus (LYCIV) and infectious spleen and kidney necrosis virus (ISKNV). *Cyprinid herpesvirus* 3 (CyHV-3), also known as *Koi herpesvirus,* is a double-stranded DNA virus and belongs to genus *Cyprinivirus* and family *Alloherpesviridae*. CyHV-3 is very pathogenic and is responsible for a high rate of morbidity and mortality in common carp (*Cyprinus carpio carpio*) and koi carp (*Cyprinus carpio Koi*) causing large-scale losses in worldwide aquaculture. Additionally, it has been suggested that other species of fish can be asymptomatic carriers of CyHV-3. The main goal of this study was to investigate the cause of death of four species of fish *(Poecilia reticulata, Pygocentrus nattereri, Cyprinus carpio and Trichogaster leeri)* from Sao Paulo/SP. For this purpose, fragments of gill, eye, liver, intestine, kidney and spleen of 14 fishes were sampled and stored at -80°C. Aliquots of 50mg (pool of tissues) were submitted to DNA extraction followed by PCR (polymerase chain reaction) for sequential amplification of 409bp- and 348bp-fragments of the CyHV-3. In addition, the same samples were subjected to sequential amplification of 1362bp- and 369bp-fragments of the MV with primers to the iridoviral MCP gene. Twelve samples were PCR positive for *Megalocystivirus* and the virus identity was confirmed by nucleotide sequencing. Although no amplicons of TK gene could be obtained with the primers set used in this study, concomitant infection of virus of family *Iridoviridae* and CyHV-3 is not a rare event, indicating that different molecular diagnostic approaches can be further used to better understanding the etiological nature of these infections. For this purpose, further studies with specimens of other species including common carps and Koi carps will be performed using a new pair of degenerated primers based on the DNA polymerase gene. The priority will be for sick fishes with symptoms of Koi herpesvirus disease. The same approach will be used for the already tested fish samples.

Keywords: Molecular diagnostics, fish, *Cyprinid herpesvirus 3*, *Megalocystivirus*

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