First report of megalocytivirus in red piranhas (pygocentrus nattereri) by molecular diagnosis in Brazil

Abstract

The genus Megalocytivirus belongs to the family Iridoviridae and due to the high mortality rates among infected animals, represents a significant threat to world aquaculture. The affected fish present general and nonspecific symptoms, which makes the early diagnosis of the infection difficult. Nine samples from ornamental fish of the species Poecilia reticulata and Pygocentrus nattereri with nonspecific symptomatology were submitted to molecular diagnosis for Megalocytivirus through the PCR technique. Infection by this genus was detected in 100% of the analyzed samples, and three of them were sent for sequencing. All samples showed identity values of 100% with the Megalocytivirus Sabah/RAA1/2012 strain BMGIV48 described in Malaysia. These results indicate, for the first time, the circulation of Megalocytivirus among Brazilian ornamental fish, which emphasizes the necessity of larger studies regarding this important viral genus.

Keywords: megalocytivirus, ornamental fish, live fish food, molecular diagnosis

Introduction

The family Iridoviridae is composed of five genera that affect a wide variety of animal species, including freshwater and saltwater fish and invertebrates. Two genera, Iridovirus and Chloriridovirus, contain viruses of invertebrates, while three genera, Ranavirus, Megalocytivirus and Lymphocystivirus, contain viruses that infect lower vertebrates. The genera Megalocytivirus and Ranavirus are emerging pathogens of global distribution that cause systemic diseases of high severity. Ranaviruses are also important amphibian pathogens. The genus Lymphocystivirus causes lesions on the surface of fish epidermis and is rarely responsible for economic losses.

Infections by Megalocytivirus have been reported in several species of ornamental fish, including Trichogaster leeri, T. microlepis, Colisa lalia, Poecilia sphenops, Poecilia reticulata, Astronotus ocellatus, Hyphessobrycon innesi, Pterophyllum scalare, Pterapogon kauderni, Platax orbicularis, Xiphophorus helleri, Aphyosemion gardneri, Xiphophorus maculatus, Poecilia latipinna, among others and can cause significant economic losses worldwide.

Clinical signs are nonspecific and resemble the clinical signs observed in many other diseases in fish. Darkened skin, irregular swimming (including rotating movements) or positioning on the surface of the water, increased respiratory movements, distention of the abdominal cavity, ulceration, hemorrhages (including punctate hemorrhages in the skin and gills), pallor of the gills, anemia, erosion of the fins, whisth stools and high mortality rates are the most common clinical signs. Necropsy shows necrosis in many internal organs such as spleen, kidneys, and liver. Other organs and tissues, including muscles, gonads, heart, gills and gastrointestinal tract may also be affected. Some fish may have visible hemorrhagic fluid in the body cavity. The aim of the study was identify red piranhas (Pygocentrus nattereri) used as ornamental fish suspected of viral infection by molecular diagnosis, and to alert to the presence of Megalocytivirus in Brazil.

Material and methods

Fifty red piranhas (Pygocentrus nattereri), originating from the Amazon basin through extractive fishery and acquired by a wholesaler of ornamental fish in Sao Paulo, showed nonspecific clinical signs after being fed with live fish food (Poecilia reticulata). Clinical signs included loss of appetite, irregular swimming and positioning on the surface of the water (Figure 1A), lethargy and respiratory movements that started three days after feeding with live fish, which progressed to 94% mortality within five days after the onset of clinical signs.

![Figure 1](image_url)

Five specimens of red piranhas (Pygocentrus nattereri) were sacrificed and necropsied (Figure 1B). At necropsy, hemorrhagic...
points in the organs were observed, more evident in the gastrointestinal tract (Figure 1C) and gills (Figure 1D) observed under 4X microscopy. On suspicion that live fish food was the source of the problem, immediately, the responsible veterinarian sent a total of eight samples of red piranhas (Pygocentrus nattereri) and a batch of approximately 30 frozen guppies fish (Poecilia reticulata) to the Laboratory of Zootechnical Hygiene of FZEA-USP for molecular diagnosis through the technique of nested-PCR and nucleotide sequencing in order to identify possible viral agents as cause of these alterations in the animals studied.

In the laboratory one specimen of the species Poecilia reticulata and eight specimens of the species Pygocentrus nattereri were submitted to molecular diagnosis. For the extraction of DNA, fragments of approximately 50mg of these tissues (pool) were collected; DNA extraction was then performed using the NucleoSpin Extract II kit (Macherey-Nalgel, Germany). The extracted DNA was measured by spectrophotometry (DS-11, DeNovix, USA) according to the absorbance ratio A260/A280. PCR was performed from the DNA extracted for the partial amplification of the Megalocytivirus Viral Major Capsid Protein (MCP) gene. The primers used in nested-PCR are described in Table 1.

Samples with band pattern close to that expected in the nested-PCR were excised from the agarose gel, and the DNA fragments contained therein were extracted using the illustra™ GFX™ PCR DNA and Gel Band Purification (GE Healthcare, USA) kit, According to the manufacturer’s recommendations. For nucleotide sequencing, the BigDye® Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems/ Thermo Fisher Scientific, EUA), containing AmpliTaq DNA Polymerase, was used according to the manufacturer’s specifications. The BioEdit program, version 7.0.9.4 was used to edit the sequences while to the search for the similarity of the sequences generated and edited, the BLAST program, version 2.0.9 was used. Editing and multiple alignments of the nucleotide sequences obtained were performed in the ClustalW program, version 1.4,19 and implemented in the BioEdit Sequence Alignment Editor program, version 7.0.2.9 Visualization of the alignment, using the deduced amino acid sequences, was performed through the Jalview program, version 2.8.1.11

| Name  | Orientation | Sequence (5'-3')                      | Genus        | Product (Bp) |
|-------|-------------|---------------------------------------|--------------|--------------|
| MV-F  | Sense       | ATGTCTGCAATCTCAGGTG                    | Megalocytivirus | 1,362a       |
| MV-R  | Antisense   | TTACAGGATAGGGAAGCCTGC                  | Megalocytivirus |              |
| nMV-F | Sense       | CACCGCAACGTGCAAGGCAA                   | Megalocytivirus |              |
| nMV-R | Antisense   | TTGACTGCAATAACGACCAGTTCAAC             | Megalocytivirus | 369a         |

Results and discussion

All the nine processed samples (a sample of Poecilia reticulata and eight samples of Pygocentrus nattereri) were positive for the molecular diagnosis for Megalocytivirus, and 3 of them presented ideal DNA concentration for nucleotide sequencing. BLAST analysis of the 369 bp sequences obtained revealed that 3 of the 2 Brazilian sequences analyzed showed 100% identity with the collinear region of the MCP gene of Megalocytivirus Sabah/RAA1/2012 strain BMGIV48 (GenBank Access Number: JQ253374), originating from Malaysia (Figure 2), and also with the strain described as belonging to the species infectious spleen and kidney necrosis virus (ISKNV), which is one of the species within the genus Megalocytivirus (KX354219). The red piranha is a common native species in the region of the Amazon basin and is in a vulnerable conservation status. This was the first report of molecular diagnosis of Megalocytivirus in Brazil in red piranhas, which draws attention due to the problems that the virus can cause.

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needed including histopathology and electron microscopy of the samples.

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**Conflict of interest**

The author declares no conflict of interest.

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