APPLICATION OF POLYMERASE CHAIN REACTION (PCR) TECHNIQUE TO DETECT KOI HERPES VIRUS (KHV) INFECTION IN CARPS

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Introduction

Disease outbreaks have been identified worldwide as a significant constraint to the development of aquaculture production and ornamental fish industry. Effective treatment and control of aquatic animal diseases requires access to diagnostic tests that are rapid, reliable and sensitive. Conventional diagnostic methods are time consuming, costly and often lack specificity and sensitivity to detect pathogens present in low numbers. To overcome the above constraints molecular diagnostic techniques are required to cater for the increasing demand that arises from the expanding aquaculture industry.

Koi Herpes Virus (KHV) infection was first described in 1996 and it is listed in the world organization for animal health (OIE) since year 2006 as a serious reportable disease. KHV is capable of causing significant economic losses in the ornamental fish industry throughout the world. KHV is spreading rapidly around the world through international trade of ornamental carp. The objective of this study was to apply polymerase chain reaction (PCR) amplification techniques for the detection of KHV infection in ornamental carps.

Materials and Methods

Carp fish samples were collected from 40 locations distributed in various parts of Sri Lanka to represent various sectors such as importers, breeders, exporters, commercial aquariums and hobbyists. Fish were dissected to obtain pooled organ samples (brain, kidneys, spleen, gills). Total DNA was extracted using a commercial DNA extraction kit (*Fermantas life sciences*, Italy) following manufacturer's instructions. A PCR reaction was performed targeting the conserved, specific thymidine kinase (TK) gene region of the KHV. The primer sequence used for the DNA amplification was designed according to the OIE Aquatic Animal Health Standard Commission report, 2007. Non infectious KHV DNA obtained from the National Reference Laboratory for fish and shellfish diseases, Lelystad, Netherlands was used as the positive control. PCR products were analyzed on 2% Agarose gels and the diagnosis was given based on the visualization of specific DNA product of 409bp.

Results

All the positive controls manifested a clear band at the 409bp region. All the negative controls indicated very clear gel lanes without any bands. None of the 40

samples analyzed indicated any bands at the anticipated 409bp region.

Discussion

KHV disease status in Sri Lanka was unknown until year 2007 (Haenen *et al*, 2007). Clinical signs of KHV infection in ornamental fish include lethargy, disorientation, gasping, pale necrotic patches to extensive discoloration of the gills, enlarged or haemorrhagic kidney and liver ultimately resulting in extensive mortality. Although similar clinical signs were reportedly observed in some areas of the country specially during harvesting from the mud ponds, none of the samples were positive for KHV DNA. The above described molecular diagnostic technique has now been successfully established and is being regularly utilized by the National Animal Quarantine Service to screen imported ornamental carp consignments to the country.

One of the best ways to prevent the transmission of this virus is adhering to biosecurity protocols including adequate quarantine. All new carp should be quarantined for a minimum of 30 days before introduction to an established population (Lewbart, 2005). Other control measures adopted include stamping out, disinfection, stopping fish movements, raising the water temperature to 28-30^{0}C and vaccination (Haenen *et al*, 2007).

Conclusion

Presently KHV seems not to be present in Sri Lanka and can be considered as an exotic disease. It is currently reported in many countries in the region and Sri Lanka regularly imports carps from these countries. The above described single step PCR techniques can be conveniently used for the rapid diagnosis of KHV. All imported carp consignments should be tested to keep the country free of KHV. As suggestions for future research there is a need to establish PCR based molecular diagnostic procedures for the detection of other aquatic animal diseases, especially viral diseases.

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