DETECTION OF INFECTIOUS SPLEEN AND KIDNEY NECROSIS VIRAL DISEASE IN SEA BASS BY HISTOPATHOLOGY AND MOLECULAR TECHNIQUES

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Abstract

Infectious Spleen and Kidney Necrosis Virus is a devastating virus causing mass mortality in fish. Detection of the virus is crucial due to confusing clinical manifestations with many other bacterial and viral diseases. Pathology is an old and promising method for diagnosis of viral diseases. However, molecular methods are used as a confirmatory diagnosis method. Twenty moribund fish samples of marine sea bass with the average body weight around 80 to 100 g were collected from selected farms in Trincomalee district. Fish were selected based on the clinical signs suspected for ISKNV such as anorexia, abnormal swimming, loss of body pigmentation, pale gills, petechial hemorrhages in the operculum, mandible, fin base and abdomen. Spleen, Kidney, Liver, Gill and Brain samples were fixed in 10% neutral buffered formalin (NBF) for Histopathalogical analysis. Spleen and Kidney tissue samples were collected from the diseased fishes and kept in 90% ethanol for PCR testing. Extracted DNA products were quantified and PCRs were conducted using relevant primers. DNA sequencing was carried out in PCR products which are positive for the selected primers at the molecular laboratory, Faculty of Science, University of Peradeniya. Phylogenetic tree was built by Mega 7 software following maximum likelihood method. The formalin-fixed tissues were processed routinely, embedded in paraffin wax, and cut at 4 μm, stained with hematoxylin and eosin (H&E). Postmortem finding of the study documented as the presence of pale liver, pale gills, splenomegaly and fin rot. Degenerative and necrotic lesions in the kidney (mainly tubular epithelia) were observed in 15 fish (75%). Inflammatory cell (granulocytes, macrophages and lymphocytes) aggregates were observed in 10 fish (50%). Seventeen fish (85%) showed degeneration and necrosis of hepatocytes. Inflammation of the gills was observed in 10 fish (50%). This study found 100 % homology with Infectious spleen and kidney necrosis virus strain RSIV-Ku, complete genome, Gene bank Accession number KT781098.1 and KX354215.1 from Taiwan. This is the first study reporting the presence of ISKNV in Sri Lanka with these pathological and molecular findings. Further studies are needed to study the detail molecular and pathology of ISKNV in different species of fish.