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Immunolocalisation of nervous necrosis virus indicates vertical transmission in hatchery produced Asian sea bass (*Lates calcarifer* Bloch)—A case study

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Abstract

A probable vertical mode of piscine nodavirus transmission is reported in the present investigation based on a case of nodavirus associated larval mortalities in hatchery produced Asian sea bass. Polyclonal rabbit anti-SJNNV antibodies (SGWak97) detected the viral antigens in the tissue sections from the eggs and the larvae at different time intervals from −1 to 42 days post hatch (dph). Immunopositive ovarian connective tissue associated with the oocytes along with the progressive localization of the viral antigens in the brain, spinal cord, liver, stomach and dermal musculature during the larval development indicates a probable vertical transmission of nodavirus in the Asian sea bass. The surviving larger larvae, from the batch suffering mass mortalities, produced very intense immunofluorescent positivity in the liver, stomach and dermal musculature. Results of this investigation demonstrating a possibility of vertical transmission of the nodavirus emphasize the need for screening of eggs and larvae for evolving suitable preventive and prophylactic health management strategies.

Keywords: *Lates calcarifer*; Nodavirus; Vertical transmission; Immunofluorescence; Immunoperoxidase

1. Introduction

Aquaculture of food fishes is on the rise the world over with the success of captive breeding and standardization of larval rearing technology. Asian sea bass (*Lates calcarifer* Bloch) is an important food fish in Asia. This fish species is found to be susceptible to various pathogens of parasitic, bacterial and viral origin (Wong and Leong, 1989; Anderson and

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Norton, 1991; Subhasinghe and Shariff, 1992; Soltani et al., 1996; Azad et al., 2004). Viral nerve necrosis due to the piscine nodavirus is the single most important viral fish disease that attracted the attention of fish health workers during the last decade. The virus is recorded from over 40 fish species covering different geographic locations. Since then several aspects of VNN have been reported (Yoshikoshi and Inoue, 1990; Mori et al., 1992; Comps et al., 1996; Munday and Nakai, 1997; Nishizawa et al., 1997; Castric et al., 2001; Starkey et al., 2000, 2001; Breuil et al., 2001; Johansen et al., 2003, 2004; Azad et al., 2005).

Glazebrook et al. (1990) accounted for the first report of picorna-like viruses affecting the Asian sea bass or barramundi. The association of nodavirus with the brain and retinal lesions and resultant mortalities in this species were reported by Renault et al. (1991). Comparison of nodavirus strains isolated from the barramundi and the European sea bass (Comps et al., 1994), nodavirus infection in the hatchery reared sea bass from Indonesia (Zafran et al., 1998), phylogeny and genetic characterization of nodaviruses from different geographic locations and fish species (Skliris et al., 2001) have all contributed immensely to events related to VNN in the Asian sea bass.

Investigations on the possible routes of VNN infection have yielded interesting information and many of them suggest a probable vertical transmission of the virus in various fish species (Arimoto et al., 1992; Grotmol et al., 1999; Grotmol and Totland, 2000; Breuil et al., 2002). The work of Arimoto et al. (1992) was probably the first, which aimed at detecting the nodavirus through antibody-based ELISA technique in the fertilized eggs. Grotmol and Totland (2000) showed that the nodavirus in artificially infected eggs of Atlantic halibut (*Hippoglossus hippoglossus*) could be inactivated through ozonation, thus preventing vertical transmission of the virus. Breuil et al. (2002) inoculated live nodavirus to the brood sea bass (*Dicentrarchus labrax*) and observed nodavirus-associated lesions in the brain and retinal tissue of the larvae suggesting a probable vertical transmission of VNN. However information on the sequence of events relating to the appearance of VNN leading to a clear indication on the vertical route of infection in the Asian sea bass (*L. calcarifer*) is scanty.

The present work was carried with an objective of investigating the probable route of nodavirus infection in hatchery produced Asian sea bass through immunohistochemistry and histopathology.

2. Materials and methods

2.1. Fish

The fish sample selected in the present study was from captive brood stock maintained in 500 ton outdoor cement tanks for the breeding programme. The brood stock was collected (2–4 years-old) from the coastal waters off Chennai, India and domesticated (5–6 years under captive conditions) at the fish hatchery of the institute. There was no known history of the VNN outbreak in the hatchery previously. Daily water exchange of 25% using filtered seawater (34–36 ppt) was carried out. Eggs from a randomly selected potential female fish were collected using a catheter. As a part of the general procedure, the brood is examined for the ripeness of the ovary by pressing the abdomen for free ova and collecting a sample of eggs using a catheter inserted into the vent leading to the ovary. The sampled eggs corresponded to, roughly, 24 h prehatch. The female fish was induced to spawn using hormonal induction. Fertilized eggs were hatched and reared separately, but in the hatchery environment where several batches of eggs/hatchlings are generally held. Eggs and hatchlings sampled at −1, 0, 2, 4, 6, 10, 15, 20, 25 and 42 dph were fixed in 10% buffered formalin and processed for histology. Mass mortality of the hatchlings was observed at 18–21 dph with typical clinical manifestations of viral nervous necrosis such as anorexia, blackening, lack of swimming coordination and settling to bottom. Paraffin embedded sections of eggs and larvae were used for standard histological staining (haemotoxylin and eosin; H&E) and immuno-staining (immunoperoxidase and immunofluorescence). Moribund larvae were tested positive by RT-PCR for the presence of the piscine nodavirus (Azad et al., 2005).

2.2. Light microscopy

Paraffin embedded tissue sections of eggs and whole larvae, sampled at different time intervals,