A Viral Disease in Hatchery-reared Larvae and Juveniles of Redspotted Grouper

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Recently, new viral diseases associated with mass mortalities have been recorded among hatchery-reared larvae and juveniles of marine fish such as Japanese parrotfish (Oplegnathus fasciatus)1), barramundi (Lates calcarifer) 2, 3) and turbot (Scophthalmus maximus)4), all of which seem to have a similar viral etiology. These diseases were characterized by the affected central nervous system with picorna-like viruses; however the viral etiology has not been established yet mainly due to the unsuccessful isolation of the virus.

This paper describes a similar viral disease among hatchery-reared larvae and juveniles of redspotted grouper, Epinephelus akaara.

Materials and Methods

A disease occurred in rearing ponds of Hiroshima and Tokushima Prefectural Fisheries Experiment Stations in August to September, 1990, both resulting in mass mortality. In Hiroshima, the disease was initiated at the larval stage, 7–8 mm in total length (14 day-old), followed by heavy mortality at 9–10 mm, and continued until at 20 mm, while in Tokushima mass mortality occurred in juveniles of 26 to 39 mm. In both cases, fish were reared at 25–27°C and the mortality reached approximately 80%.

Abnormal behavior of fish represented by listless swimming near the surface of water, abrupt whirling, and sinking to the bottom was observed in either case.

Moribund larvae and juveniles were fixed in Bouin’s solution and embedded in paraffin wax. Sections were stained with haematoxylin–eosin (H & E). For electron microscopy, fish were fixed in a 2.5% glutaraldehyde-2% paraformaldehyde mixture in phosphate buffer (0.2 M, pH 7.4) and post-fixed in 1% osmium tetroxide. Ultrathin sections were stained with uranyl acetate/lead citrate and examined with a JEM 1200 EX electron microscope. One gram of frozen samples (−80°C) of diseased fish was homogenized with 1 ml of Hanks’ balanced salt solution, centrifuged, and filtered through a membrane filter (0.45 µm). The filtrate was used as the source of virus for an experimental infection and virus isolation in cell lines. The experimental infection was carried out against normal redspotted grouper (average 82 mm in total length) by intraperitoneal injection or bath challenge with the filtrate. Fish were kept in 20 l tanks at 23.4–25.8°C for 30 days.

Results

In all specimens examined by light microscopy, conspicuous vacuolation was observed in nuclear layers of eye retina and in various parts of brain (Fig. 1), but no significant histopathological changes were found in other tissues such as gills, spleen, kidney, liver, skin, and skeletal muscles. Transmission electron microscopy revealed necrotic and lytic degeneration of neurons and unidentified cells in the affected retina and brain, and numerous hexagonal virus particles in the heavily vacuolated cytoplasm and in the extracellular spaces but not in the nucleus (Fig. 2A). In the cytoplasm, the regular-shaped nonenveloped particles, about 28 nm in diameter, distributed randomly or arranged in paracrystalline arrays in a membraneous structure, forming inclusion bodies (Fig. 2B).

A preliminary trial for virus isolation in two fish cell lines, FHM and EPC, and a primary culture of the brain cells from a normal grouper was made, but resulted in no formation of cytopathic effects.

In the experimental infection, though mortalities were relatively low (10 to 30%) irrespective of virus source and infection method, the same clinical signs and histopathological changes as those in the naturally affected fish were reproduced. Abnormal swimming behavior appeared 10–14 days after the virus exposure and
Numerous virus particles, morphologically indistinguishable from those seen in naturally affected fish, were also found in the affected retina and brain.

**Discussion**

The above mentioned findings indicate that the present grouper disease is quite similar to the disease, so-called viral nervous necrosis (VNN)\(^1\) or encephalomyelitis\(^4\). Although the causative viruses were suggested to belong to the family Picornaviridae based on its size, replication site, association with membraneous structures, and RNA content\(^2-^4\), taxonomical position of the present grouper virus could not be settled because of no data on nucleic acid of the virus. Mass mortalities due to the viral infection in Japanese parrotfish, barramundi, and turbot were restricted to their larvae, though slight susceptibility of juvenile fish was shown in parrotfish\(^5\). However, in redspotted grouper, VNN could occur and produce significant mortality even in juvenile stage, as evidenced by one of the present cases. It was also supported by the observation that there were no significant difference between larval and juvenile stages in the severity of affected tissues. It could not be concluded whether the discrepancy comes from the difference in causative agent or in host fish.

**References**