Diagnostic and Preventive Practices for Iridovirus in Marine Fish

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ABSTRACT

The first outbreak of red sea bream iridoviral disease (RSIVD) caused by red sea bream iridovirus (RSIV) was recorded among cultured red sea bream (*Pagrus major*) in 1990 in Ehime, Shikoku, Japan. Since then, the disease has caused mass mortalities of many cultured marine fishes. From 1990-2000, RSIVD was detected in 31 cultured marine fish species, including 28 Perciformes, 2 Pleuronectiformes and 1 Teteraodontiformes, in 18 prefectures in the southwestern part of Japan. The infected fish are lethargic and show severe anemia, petechiae of the gills, and enlargement of the spleen. Histopathologically, the disease is characterized by the presence of enlarged cells in the spleen, heart, kidney, liver and gills that are deeply stained with Giemsa solution.

Diagnostic methods for RSIV, such as the observation of stained imprints or tissue sections, an immunofluorescent (IF) test with a monoclonal antibody (MAb) and a polymerase chain reaction (PCR) technique have been developed. The IF test with MAb is commonly used in the rapid diagnosis of RSIV-infected fish. For an effective control measure against RSIVD, a formalin-killed vaccine has been developed and this showed a significant effect in red sea bream under both experimental and field conditions.

INTRODUCTION

Iridoviruses, which are recognized as causative agents of serious systemic diseases, have been identified from more than 20 fish species in recent years (Hyatt *et al.*, 2000). The family Iridoviridae comprises of large isometric viruses with icosahedral symmetry, 130-300 nm in diameter, with a genome of double stranded DNA, replicating only in the cytoplasm.

Red sea bream iridoviral disease (RSIVD) is one of the newly emerging major diseases in the aquaculture industry. The first outbreak of RSIVD caused by red sea bream iridovirus (RSIV) was recorded among cultured red sea bream, *Pagrus major*, around Shikoku Island, Japan in 1990. Since 1991, the disease has caused mass mortalities of many species of cultured marine fish. This paper briefly reviews RSIVD and recent studies on its diagnosis and vaccination trials against the disease.

RED SEA BREAM IRIDOVIRAL DISEASE

The affected fish are lethargic and exhibited severe anemia, petechiae of the gills and enlargement of the spleen (Inouye *et al.*, 1992). The disease is characterized by the appearance of enlarged cells which stained deeply with Giemsa solution in tissue sections of the spleen, heart, kidney, liver, and gills of infected fish (Inouye *et al.*, 1992). The most typical histological change observed in affected fish is the appearance of enlarged cells in the spleen.

The causative agent is a large, icosahedral, cytoplasmic DNA virus classified as a member of the family Iridoviridae (Inouye *et al.*, 1992). The virus was first isolated in red sea bream and thus was designated as RSIV. Each virion consists of a central electron-dense core (120 nm) and an electron-translucent zone, measuring 200-240 nm in diameter. The biological and physico-chemical properties of the virus have been reported (Nakajima and Sorimachi, 1994). The RSIV can grow on GF, BF-2, CHSE-214, FHM, JSKG, KRE-3, RTG-2 and YTF cell lines. The titers of the virus are higher on GF, BF-2 and KRE-3 than on other cell lines. The virus can replicate at 15, 20, 25 and 30°C but not at 37°C. The optimum temperature for viral growth is 20°C or 25°C. The virus is sensitive to acid (pH 3), chloroform, ether, and heat but is not sensitive to ultrasonic treatment and repeated freezing and thawing. Treatment with 5-iodo-2-deoxyuridine (IUdR) reduced the titer of the virus.

The virions of RSIV contain linear double-stranded DNA. The complete nucleotide sequence of RSIV has been determined. The genome of RSIV is about 112,000 base pairs (bp) in length and contains about 90 potential genes (Kurita, unpublished data).

The antigenic relationship between RSIV and two iridovirus-like agents associated with systemic infection in fish, the epizootic haematopoietic necrosis virus (EHNV) and iridovirus isolated from sheatfish (SFIV), has been examined. Although cross-reactions were observed between RSIV and other fish iridoviruses by immunofluorescence (IF) or immunoprecipitation test using anti-RSIV serum, none of the monoclonal antibodies (MAbs) against RSIV reacted with EHNV- or SFIV-infected cells by the IF test (Nakajima *et al.*, 1998). Pathogenicity tests of EHNV or SFIV to red sea bream have not been shown by experimental challenge (Nakajima and Maeno, 1998).

The RSIVD has affected 31 species of cultured marine fish in 18 prefectures in the southwestern part of Japan from 1990-2000 (Matsuoka *et al.*, 1996; Kawakami and Nakajima, 2002). The infected fish include species belonging to Perciformes, Pleuronectiformes and Tetradontiformes (Kawakami and Nakajima, 2002). The affected fish are yellowtail, sea bass, Japanese parrotfish, amberjack (*Seriola dumerili*), goldstriped amberjack (S. *aureovittata*), striped jack (*Pseudocaranx dentex*), horse mackerel (*Trachurus japonicus*), albacore (*Thunnus thynnus*), Japanese flounder (*Paralichthys olivaceus*), and tiger puffer (*Takifugu rubripes*).

Transmission of the disease to healthy red sea bream has also been established by co-habitation or through the rearing water of RSIV-infected fish (Nakajima, unpublished data). These suggest a possibility of horizontal transmission of RSIVD. Until now, RSIVD has not occurred in hatcheries; thus, the possibility of vertical transmission seems to be little.

DIAGNOSIS OF RSIVD

Diagnostic methods such as the observation of stamped or sectioned specimens stained with Giemsa, an IF test with a MAb and a polymerase chain reaction (PCR)-based technique have been reported (Inouye *et al*., 1992; Nakajima and Sorimachi, 1995; Nakajima *et al*., 1995; Oshima *et al*., 1996, 1998; Kurita *et al*., 1998). The diagnosis of RSIVD by an IF test with a MAb is done either by isolation of RSIV in cell culture followed by its identification with an anti-RSIV M10 MAb or direct demonstration of antigens in infected tissue using M10 MAb. The indirect IF test using an M10 MAb revealed that specific fluorescence was observed in tissue imprints or frozen sections of spleen of red sea bream and other fish (Nakajima *et al*., 1995). Thus, the indirect IF test with a MAb is commonly used for the rapid diagnosis of RSIV-infected fish in the field.

The localization of the reactive antigen with M10 MAb has been examined by immuno-electron microscopy. The reactive antigen was not found in the virion with M10 MAb, but an antigen was detected on the surface of the virus-infected cells (Nakajima, unpublished data). These results suggest that the MAb recognizes a virus-induced polypeptide located on the surface of the virus-infected cells.

A PCR technique was developed to detect RSIV using primers based on the sequence data of RSIV. Four oligonucleotide primer sets based on the ATPase gene, DNA polymerase gene and a Pst I-restriction fragment of RSIV genomic DNA were synthesized to amplify the RSIV DNA of 563-570 bp length (Kurita *et al*., 1998). Furthermore, since the target region among RSIV was successfully amplified from diseased fish other than red sea bream, the PCR using primers designed for RSIV has a broad application for the diagnosis of RSIVD in a number of species (Kurita *et al*., 1998).

VACCINATION AGAINST RSIVD

The effectiveness of vaccination against RSIVD has been evaluated using two kinds of vaccines in red sea bream under experimental conditions (Nakajima *et al*., 1997). For vaccine preparation, RSIV-infected GF cells or its cell culture supernatant were inactivated with formalin (1.0% v/v or 0.3% v/v) for 10 days at 4°C. Juvenile red sea bream were intraperitoneally injected with the vaccine, and after 10 days the fish were RSIV-challenged by intraperitoneal injection. Statistical analysis showed significantly higher survival rates in the vaccinated groups than that of the respective control groups, indicating the protection was induced by vaccination.

In addition, the expression of the virus specific antigens in the spleen was monitored by IF test for both the vaccinated and control fish after RSIV challenge. The results show that the expression of antigens was weaker in the vaccinated fish compared with the control fish supported the efficacy of the vaccination.

To establish control measures for RSIVD, the effectiveness of a formalin-killed viral vaccine was evaluated in a field trial (Nakajima *et al*., 1999). Two groups each consisting of 1000 juvenile red sea bream were either intraperitoneally inoculated with the vaccine (vaccinated group) or were not vaccinated (control group). After vaccination, the fish were held in tanks for one week, then transferred to a marine net pen and observed for 12 weeks. The cumulative mortalities

caused by RSIVD in the vaccinated group and control group were 19.2 and 68.5%, respectively. Additionally, the presence of virus antigen in the spleen was investigated and body weight was measured 6 and 12 week post-vaccination. In the vaccinated group, viral antigen was not detected. In contrast, viral antigen was detected in the control group at 6 week post-vaccination, but was not detected at 12 week post-vaccination. The increase in body weight of vaccinated fish was significantly ($p<0.05$) greater than that of control fish. These results suggest that the vaccine against RSIVD was effective in the field trial.

The vaccine was also found to be effective in other cultured marine fish such as the yellowtail (*S. quinqueradiata*). The vaccine for red sea bream and yellowtail is now commercially available and is currently used for vaccination against RSIVD in Japan.

DISCUSSION

The outbreak of RSIVD was first reported in 1990. Although the original source of infection of the disease is not clear, importation of the seedling fish from foreign countries without any quarantine is suspected to have introduced the new pathogen into Japan. In relation to infectious source, comparison between RSIV and other iridovirus isolated in foreign countries especially in Southeast Asia where Japan has imported a lot of seedlings is needed.

Control measures against infectious diseases of cultured fish include the following: avoidance of exposure to the pathogen, environmental manipulation, immunization, activation of non-specific defense, development of disease resistant-strains, health maintenance, and chemotherapy. Among them, activation of non-specific defense and specific immunization based on general health maintenance seem to be the most promising prophylactic methods for the control of RSIVD. Breeding of red sea bream for disease resistant-strains has been left for further studies.

REFERENCES

Hyatt, A.D., Gould, A.R., Zupanovic, Z., Cunningham, A.A., Hengstberger, S., Whittington, R.J., Kattenbelt, J. and Coupar, B.E.H. 2000. Comparative studies of piscine and amphibian iridoviruses. Arch. Virol., 145:301-331.

Inouye, K., Yamano, K., Maeno, Y., Nakajima, K., Matsuoka, S., Wada, Y. and Sorimachi, M. 1992. Iridovirus infection of cultured red sea bream, *Pagrus major.* Fish Pathol., 27:19-27.

Kawakami, H. and Nakajima, K. 2002. Cultured fish species affected by red sea bream iridoviral disease from 1996 to 2000. Fish Pathol. (in press)

Kurita, J., Nakajima, K., Hirono I. and Aoki, T. 1998. Polymerase chain reaction (PCR) amplification of DNA of red sea bream iridovirus (RSIV). Fish Pathol., 33:17-23.

Matsuoka, S., Inouye, K. and Nakajima, K. 1996. Cultured fish species affected by red sea bream iridoviral disease from 1991 to 1995. Fish Pathol., 31:115-119.

Nakajima, K. and Maeno, Y. 1998. Pathogenicity of red sea bream iridovirus and other fish iridoviruses to red sea bream. Fish Pathol., 33:143-144.

Nakajima, K. and Sorimachi, M. 1994. Biological and physico-chemical properties of the iridovirus isolated from cultured red sea bream, *Pagrus major.* Fish Pathol., 29:29-33.

Nakajima, K. and Sorimachi, M. 1995. Production of monoclonal antibodies against red sea bream iridovirus. Fish Pathol., 30:47-52.

Nakajima, K., Maeno, Y., Fukudome, M., Fukuda, Y., Tanaka, S., Matsuoka, S. and Sorimachi, M. 1995. Immunofluorescence test for the rapid diagnosis of red sea bream iridovirus infection using monoclonal antibody. Fish Pathol., 30:115-119.

Nakajima, K., Maeno, Y., Kurita, J. and Inui, Y. 1997. Vaccination against red sea bream iridoviral disease in red sea bream. Fish Pathol., 32:205-209.

Nakajima, K., Maeno, Y., Yokoyama, K., Kaji, C. and Manabe, S. 1998. Antigen analysis of red sea bream iridovirus and comparison with other fish iridoviruses. Fish Pathol., 33:73-78.

Nakajima, K., Maeno, Y., Honda, A., Yokoyama, K., Tooriyama, T. and Manabe, S. 1999. Effectiveness of a vaccine against red sea bream iridoviral disease in a field trial test. Dis. Aquat. Org., 36:73-75.

Oshima, S., Hata, J., Segawa, C., Hirasawa, N. and Yamashita, S. 1996. A method for direct DNA amplification of uncharacterized DNA viruses and for development of a viral polymerase chain reaction assay: application to the red sea bream. Anal. Biochem., 242:15-19.

Oshima, S., Hata, J., Hirasawa, N., Ohtaka, T., Hirono, I., Aoki, T. and Yamashita, S. 1998. Rapid diagnosis of red sea bream iridovirus infection using polymerase chain reaction. Dis. Aquat. Org., 32:87-90.