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ISOLATION OF AN AQUATIC BIRNAVIRUS FROM SEA BREAM (*SPARUS AURATUS*)

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Abstract

An aquatic birna-like virus was isolated from adult sea bream (*Sparus auratus*), which were being farmed in Greece. The virus was transmitted to *Salmo trutta* via injection, reisolated and induced focal pancreatic necrosis.

Introduction

Sea bream (*Sparus auratus*), as one of the most important species in Mediterranean aquaculture, have been fortunate in that, in general, they have not been affected by significant viral disease conditions. The viruses that have been associated with sea bream include those of the family *Iridoviridae* , which has resulted in lymphocystis (Paperna *et al.* 1982) and those of the family *Reoviridae* which were isolated from bream suffering moderate mortalities (Bandín *et al.* 1995). Virus-like particles resembling those of the family *Nodaviridae* have recently been observed in larval sea bream suffering high mortalities (Comps & Raymond 1996) and various microorganisms including a picorna-like virus, a parvo-like virus and a reo-like virus have been associated with some farmed sea bream suffering "winter disease", a condition of unknown aetiology (Bovo *et al.* 1995). This communication reports the isolation of a birna-like virus from farmed adult sea bream in Greece.

Materials and methods

Fifteen adult sea bream were sampled from a broodstock population held in sea cages in a farm in Greece. These were sampled to assess stock health and to screen for significant pathogens. Fresh skin, gill and gall bladder smears were examined by light microscopy for parasites and kidney swabs were inoculated onto tryptone soya agar plus 1.5% sodium chloride for bacteriology. Skin, muscle, gills, heart, kidney, hepato-pancreas, intestine, liver and spleen samples were fixed, processed and sectioned for histology. Spleen samples were taken for virological analysis, pooled in three lots of five and homogenised in Hank's balanced salt solution (HBSS) supplemented with 2% foetal bovine serum to give a 1:10 dilution of crude extract. A further 1:5 dilution was performed using the same diluent and this then filtered (0.45µm) to produce a 1:50 fluid extract. CHSE-214, BF-2 and EPC cell lines were simultaneously inoculated with 1/10 volume of the fluid extract and incubated at 15°C. Following development of a cytopathic effect (CPE) the flask supernatant was harvested, cell monolayers scraped off and Karnovsky's fixative added. These were then centrifuged at 1500g for 10 minutes. The spent fixative was poured off, fresh fixative added and following two hours this was replaced by cacodylate rinse. These samples were embedded in epoxy resin, sectioned, stained with uranyl acetate and lead citrate, and examined by transmission electronmicroscopy (TEM).

A transmission trial was established using two tanks, each containing 16 brown trout (*Salmo trutta*) of an average weight of 20g. The fish in one tank were each injected intraperitoneally (i/p) with 0.1ml of a 1:10 dilution of the cell culture fluid extract and

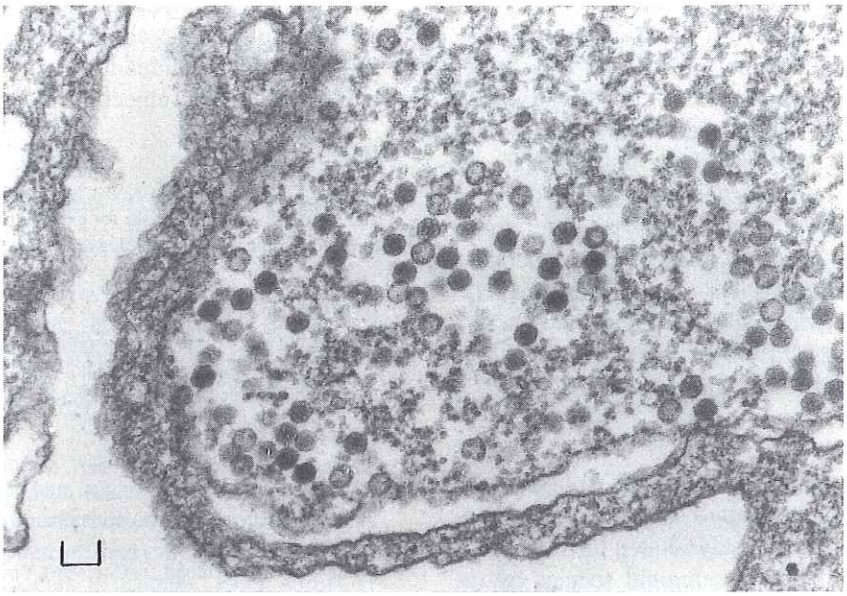

Figure 1. Transmission electronmicrograph of a birna-like virus isolated on a BF-2 cell line from farmed sea bream (*Sparus auratus*), bar = 100nm.

the fish in the control tank were inoculated i/p with 0.1ml of HBSS plus 2% serum. These were monitored for clinical signs and changes in behaviour and subsamples taken on a weekly basis for histology and virology, for a period of four weeks.

Results

One of the fifteen sea bream sampled had white splenic nodules and another had pericarditis, but otherwise there were no gross pathological abnormalities and clinically the stock had appeared healthy and were not suffering from any apparent clinical problem. Parasitology revealed a low level of gill flukes (*Dactylogyrus* spp.) and the fish with the splenic nodules proved positive for the presence of *Pasteurella piscicida,* which was isolated on the bacteriological media. Histopathology revealed granulomas and pathology consistent with Pasteurellosis in two fish and a low level of epitheliocystis but no other significant pathology.

A cytopathic effect appeared in the BF-2 cell lines inoculated with one of the three pools of tissues after 19 days incubation. On passage this appeared at day four and on the second passage at day three. A positive result was obtained following a serum neutralisation test, conducted using Infectious Pancreatic Necrosis Virus (IPNV) Sp serotype antisera. Transmission electronmicroscopy revealed icosahedral virus particles of approxmately 60nm in diameter (Figure 1).

In the transmission trial, one week after the initial challenge, two of the 16 trout which had been challenged with the viral extract appeared dark in colour and feeding behaviour, in general, was depressed. Kidney and spleen samples taken from these fish and those in the controls were inoculated onto BF-2 and CHSE-214 cell lines. The samples taken from the fish inoculated with the viral extract consistently gave a CPE on both cell lines, four days after inoculation. The control samples showed no CPE on

either cell line. The samples which produced a CPE gave a positive result on serum neutralisation to IPNV Sp serotype. Histology samples taken concurrently with the virology samples, on a weekly basis, did not show any pathology until the fourth week of the trial, when two out of the four fish sampled in the virus positive group exhibited focal necrosis of the pancreatic acinar tissue, typical of that seen in IPNV infection (McKnight & Roberts 1976). The control fish did not exhibit any histopathology consistent with this condition.

Discussion

This case report confirms the isolation of a previously unreported virus in sea bream, which appears closely related to IPNV. It is transmissable to a salmonid species by injection and appears to induce pathology in brown trout consistent with that described associated with IPN infection. This fact, coupled with the positive reaction to IPNV Sp antisera and the morphology of the virus indicate that the virus is most likely a member of the family *Birnaviridae* and we propose the name sea bream birnavirus (SBBV) for this isolate. The clinical significance of this virus for sea bream is not established and should be the subject of further study.

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