FIRST REPORT OF LYMPHOCYSTIS DISEASE IN *SPARUS AURATA* (LINNAEUS) IN SPAIN

By Basurco, B., Marcotegui, M.A., Rueda, A., Tiana, A*., Castellanos, A*., Tarazona, J.V., Muñoz, M.J. and Coll, J.M.

Introduction

Lymphocystis is a benign viral fish disease (Wolf, 1962), though it can cause economic losses because of rejection of the affected fishes for human consumption, reduced growth rate in high density marine culture, and dissemination of the infection to healthy fish. Only two reports exist of lymphocystis in *Sparus aurata* under aquaculture conditions, in Israel and Italy (Paperna et al, 1982; Masoero et al, 1986). This is the first report of lymphocystis disease occuring in cage-reared *Sparus aurata* in Spain.

Materials and methods

Fish. The infected fish were from a cage culture site in the South of Spain. Fish of 5-10 cm length were kept in 30 l aquaria in groups of 10 fish in reconstituted sea water (Sera, Heinsberg, Germany) with 20° C, recirculating conditions and monthly water renewal. Non-infected fish of 3-5 cm were obtained from a hatchery in the North of Spain. For cohabitation studies 10 fish recovered from infection (2 months in aquarium tanks) were kept in the same tanks as 5 healthy fish for 6 months.

Microscopy. Tumour tissue for light microscopy was fixed in 10% buffered formaldehyde, sectioned, stained with hematoxylin and eosin and mounted as described by Walker and Weissenberg, 1965. Tumour specimens for electron microscopy were fixed in 6% buffered glutaradehyde, post-fixed with 1% osmium tetroxide, dehydrated in ethanol, embedded in Epon-araldite and stained with uranyl acetate and lead citrate.

Virus. Lymphocystis tissues (1g) were homogenized at 10% (w/v) in TNE buffer (10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 7.4) at 4° C. The suspension was clarified by centrifugation at 3000g for 10 min. and the supernatant was layered over a sucrose gradient of 25-60% in TNE. After centrifugation at 25000g for 5 h the virus band was harvested and analyzed by polyacrylamide gel electrophoresis as described previously (Basurco and Coll, 1989).

Cell cultures. Cell lines used were the BF-2 (bluegill fibroblast), EPC (epithelioma papulosum carpio), RTG-2 (rainbow trout gonad) and CHSE-214 (chinook salmon embryo). Cell culture medium and conditions were as described previously (Basurco and Coll, 1989).

One ml vols. of 10% tumour tissues, homogenized in cell culture media were added to cells in 25 cm^2 flaks (Costar) filled with 10 ml of cell culture media, gased with 5% CO_2 in air and incubated at 20° C (BF 2 or EPC) or 14° C (RTG-2, CHSE) for a month.

Results and Discussion

The outbreak occurred at the end of 1988 in a netcage farm located on the south coast of Spain. Symptoms started in one cage in November and spread to the other cages in the following 4-5 months as the fish in the first infected cage were recovering. Nearly 90% of the population in a cage showed lesions but no significant mortalities were observed. A reduced growth rate was also noticed probably due to the high level of water pollution by industrial sewage of that area.

Samples were taken for electron microscopy and virus isolation after bacteriological tests were negative.

The fish were densely covered by tumours on the skin and fins. Microscopic examination of biopsies from the skin and tips of the fins revealed aggregates

of typical hypertropic cells identical to those of lymphocystis cells in other hosts (Faisal, 1989; Masoero et al. 1986; Paperna et al, 1982; Samalecos, 1986).

Tumours were dissected from the skin lesions and processed for electron microscopy (Figure 1). The size and morphology of the observed virions coincide with the data already published for lymphocystis virus (Samalecos, 1986; Walker and Weissenberg, 1965).

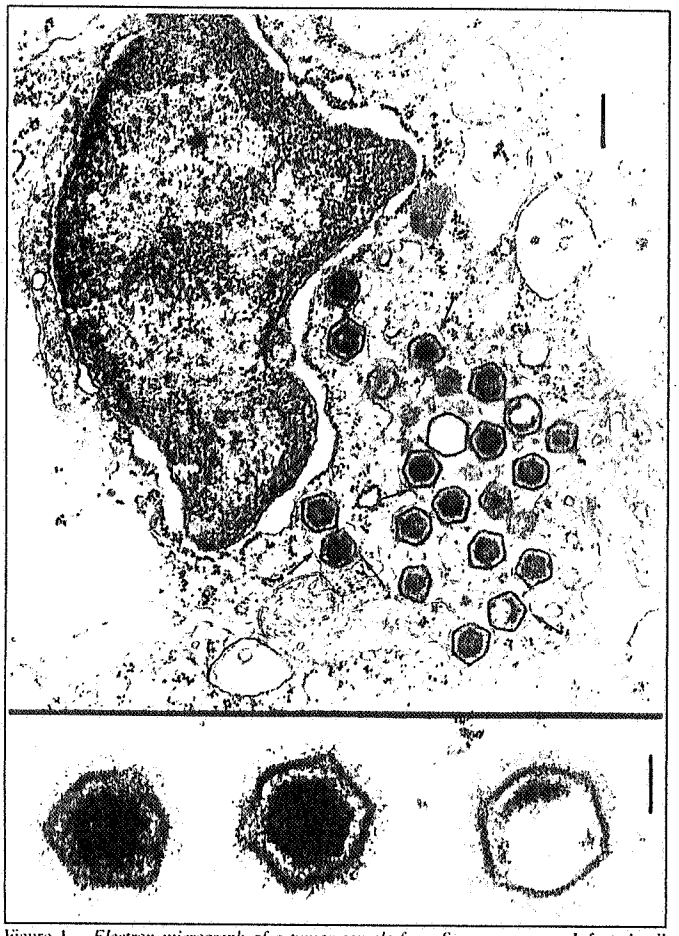

Figure 1. - *Electron micrograph of a tumor sample from Sparus aurata.* Infected cell located in the peripheral area of the tumour. The viral factory is surrounded by a thin membrane (arrow) and contains viral particles in different stages of maturation. Cytopathic effect on the cells is evident. Bar = 300 nm. Inset, viral particles found free in the tumour showing their icosahedral symmetry and the crown of fibrillar material characteristic of this virus. One particle is in early stage of maturation. Average diameters were 220 nm. Bar = 100 nm.

Polycrylamide gel electrophoresis of the ultracentrifuged virus band shows that some of the major virus polypeptides are 70, 45 and 20 KDa as described previously for lymphocystis virus from marine flat fish (Flugel et al, 1982).

Cell lines were inoculated with preparations of tumours and examined daily for cytopathic effects. Only the BF 2 cells showed cytopathic effect. The cytopathic effect was variable, ranging from complete cell lysis (not described before) in 2-3 days of culture to distortion of the cells as descibed by others (Walker and Hill, 1980; Faisal, 1989), depending on the experiment. No cytopathic effects were observed by using the BF 2 cell line inoculated in parallel with either kidney extracts from the infected fish or skin extracts from non-infected fish.

The fish growth rate was extremely slow since affected fish were only 5-8 cm in total length after 6 months of netcage culture (average temperature of 18° C). The highest proportion of affected fish was about 90% but after 2 months either in the aquarium (20° C) or on the farm (15° C) it decreased to 0%. Several specimens showed skin scars indicating a healing tissue reaction to the disease and a resumption of the normal growth rate (about 2 cm/month).

In sea bass (*Lates calcarifer*, Bloch) raised in netcages, the disease could be transmitted either by direct application to scarified fins or by cohabitation (Chao, 1984). However, in *Sparus aurata*, once the affected fish recovered from the disease, they were unable to experimentally transmit the disease to healthy fish, during 6 months of cohabitation. Presumably, only infected fish with external symptoms were able to infected others.

Summary

Sparus aurata (Gilthead seabream) from netcage culture on the south coast of Spain, were densely covered by tumours on the skin and fins. Microscopic examination of the tumours revealed aggregates of hypertrophic cells, confirmed to be lymphocystis infected cells by electron microscopy and inoculation into BF 2 cells in culture. Ultracentrifuged virions showed similar major polypeptides to those described for lymphocystis virus. The affected fish recovered from infection when kept in aquaria and subsequently did not transmit the infection by cohabitation to healthy fish. This is the first report of an infection of *Sparus aurata* with this virus in Spain.

Aknowlegments.

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Author's address

Departamento de Sanidad Animal. INIA. c/Embajadores, 68, 28012, Madrid, Spain.

* Dibaq. Diproteg.