SHORT COMMUNICATION

Infectious pancreatic necrosis virus: isolation from rainbow trout, *Salmo gairdneri* Richardson, imported into Chile

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Infectious pancreatic necrosis (IPN) is a viral infection primarily of salmonids, although IPN and IPN-like viruses have been isolated from non-salmonid fishes and marine invertebrates (Hill 1982; McAllister 1983). In salmonids, acute infection occurs in fry and fingerlings and can cause high mortality. Subclinical or inapparent infection can occur in fish 6 months old or older, but mortality seldom results. Although epizootics have been reported throughout the world, some areas are at present apparently free of IPN. Australia and New Zealand have avoided introduction of salmonid viruses by consistent enforcement of rigorous fish health policies. South America was described by Conroy (1981) as free of salmonid viruses, but few reports of virus surveys of hatchery and natural populations have been published. The prevalence of salmonid viruses in mainland Asia or Africa has not been documented.

Salmonid fishes were introduced into South America in the early 1900s. Recent emphasis has focused on the development of aquaculture resources, and the planning and implementation of salmonid husbandry programmes have progressed steadily (Conroy 1981; Food and Agriculture Organization of the United Nations 1976, 1978a, b, c). These salmonid programmes currently depend on eggs, fry, and brood stock supplied from Europe, Japan, and North America (geographical areas with significant viral disease problems). The potential for introduction of viral diseases and subsequent contamination of captive stocks of fish and natural populations is a great concern, and some South American countries are formulating fish health regulations. This note documents the first isolation, in Chile, of IPN virus from hatchery-reared rainbow trout, *Salmo gairdneri* Richardson, and constitutes the first report of the virus from South America. Circumstantial evidence suggests that IPN virus was introduced with eggs imported from North America.

Three lots of eggs certified as free of virus at their points of origin (one from Europe and two from different sites in North America) were imported into Chile in 1981. The egg lots were held in separate incubators and subsequently reared in separate raceways. No unusual mortality occurred during maturation of the fish. At about 18 months of...
age, the three lots were surveyed for virus. Samples of liver, kidneys, and spleen were
removed from 60 fish of each lot and processed as 5-fish pools (McDaniel 1979). Tissues
were homogenized, resuspended in phosphate buffered saline (pH 7.2) containing peni-
cillin (100 iu/ml) and streptomycin (100 µg/ml), and centrifuged at 1500g for 20 min at
4°C to pellet tissue debris. Drained monolayers of chinook salmon embryo (CHSE-214)
cells were inoculated with a 1/100 (vol:vol) dilution of the original tissue, and the inocu-
likum was allowed to adsorb for 1 h at 15°C. The monolayers were then overlaid with
Eagle’s minimum essential medium (Earle’s salts) containing 2% foetal bovine serum,
16 mM tris buffer (pH 7.8), and penicillin (100 iu/ml) and streptomycin (100 µg/ml)
(Wolf & Quimby 1973). Cultures were incubated at 15°C, and cells were observed for
10 days. The medium was harvested from cultures that showed cytopathic effects (CPE),
filtered through a 0.45 µm membrane filter, and passaged at 1/1000 (vol:vol) dilution
in CHSE-214 cells. Serum neutralization assays were performed for virus identification
(McDaniel 1979).

Material from three of the 12 5-fish pools from one lot of fish of North American
origin produced CPE in cell cultures. Preliminary screening, in which a polyvalent IPN
virus antiserum was used, indicated that IPN virus was isolated. Because fish of both
European and North American origin were held at the hatchery, additional serum
neutralization assays were performed with antisera against three IPN virus serotypes
(Ab, Sp and VR-299). The virus was not neutralized by antiserum against serotype Ab
and only partly neutralized by antiserum against serotype Sp (Table 1). The virus was
most clearly related to the North American VR-299 serotype. Furthermore, differential
cell culture susceptibility assays showed that the isolate grew readily in FHM cells, an
indication that the isolate was not serotype Ab (Hill 1982).

<table>
<thead>
<tr>
<th>IPN virus isolates</th>
<th>Antiserum</th>
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<tbody>
<tr>
<td></td>
<td>Ab*</td>
</tr>
<tr>
<td>Chilean</td>
<td>0†</td>
</tr>
<tr>
<td>Ab</td>
<td>5.9</td>
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<tr>
<td>Sp</td>
<td>0.5</td>
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<tr>
<td>VR-299</td>
<td>0.5</td>
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</tbody>
</table>

* Antisera to IPN virus serotypes Ab and Sp and homologous virus were provided by B. J. Hill (Ministry of Agriculture, Fisheries and Food, Fish Disease Laboratory, The Nothe, Weymouth, England).

† Results are expressed as Log10 of the neutralization index.
No significant virological surveys had been performed previously on the lots of fish, but neither had unusual mortality been observed. A fish health certification survey performed 1 year before the arrival of these lots of eggs indicated that no virus was detected in any of seven separate lots of fish reared at the hatchery (X. Reyes & J. Plumb, unpublished data). The occurrence of IPN appears to have been confined to one lot of fish from North America because no isolations were made from other lots of fish from North America or Europe. Initially, eggs had been treated with iodophor at the point of origin and on arrival at the hatchery. Egg-associated transmission occurs with IPN virus (Fijan & Giorgetti 1978; Wolf, Quimby & Bradford 1963), and iodophor treatment of eggs does not reliably control IPN virus transmission (Bullock, Rucker, Amend, Wolf & Stuckey 1976). Circumstantial evidence suggests that IPN virus was introduced in association with rainbow trout eggs imported from North America. The lot of fish from which virus was isolated was destroyed, and the hatchery area disinfected.

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References


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