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A RHABDOVIRUS ISOLATED FROM BROWN TROUT (*SALMO TRUTTA* m. *LACUSTRIS* (L.)) WITH LESIONS IN PARENCHYMATOUS ORGANS

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In 1987 an outbreak of disease occurred in a single tank of brown trout (*Salmo trutta* m. *lacustris* (L.)) fingerlings at a freshwater fish farm in northern Finland. Mortalities in the affected tank began in August when the water temperature was 16°C and continued for about one month thereafter. The accumulative mortality reached 2.2% compared with only 0.2-0.9% in the 7 other tanks containing brown trout fingerlings at the farm.

Moribund fish were dark and lethargic and swam near the outlet of the tank. On post-mortem examination, the liver was seen to be coloured mustard-yellow, the kidney was bright red or greyish-red, and in some of the fish, intra-muscular haemorrhages were visible.

In histological sections stained by Harris' haematoxylin and eosin (Luna, 1968), massive coagulative necrosis was seen in the liver parenchyma, with focal damage of the vascular endothelium (Fig. 1). Large intracytoplasmic inclusions were visible in the hepatocytes in sections stained by Page-Green method for inclusion bodies (staining was done according to Luna, 1968, but blueing of nuclei with ammonia solution was excluded). There was also extensive necrosis of the haematopoietic tissue of the kidney and spleen and necrotic changes were also evident in the excretory tissues of the kidney and in exocrine pancreatic tissue. No signs of ectoparasites were found in direct microscopical examination of gill, pectoral fin and skin smears. Kidney swabs on tryptone soya agar incubated at room tem-

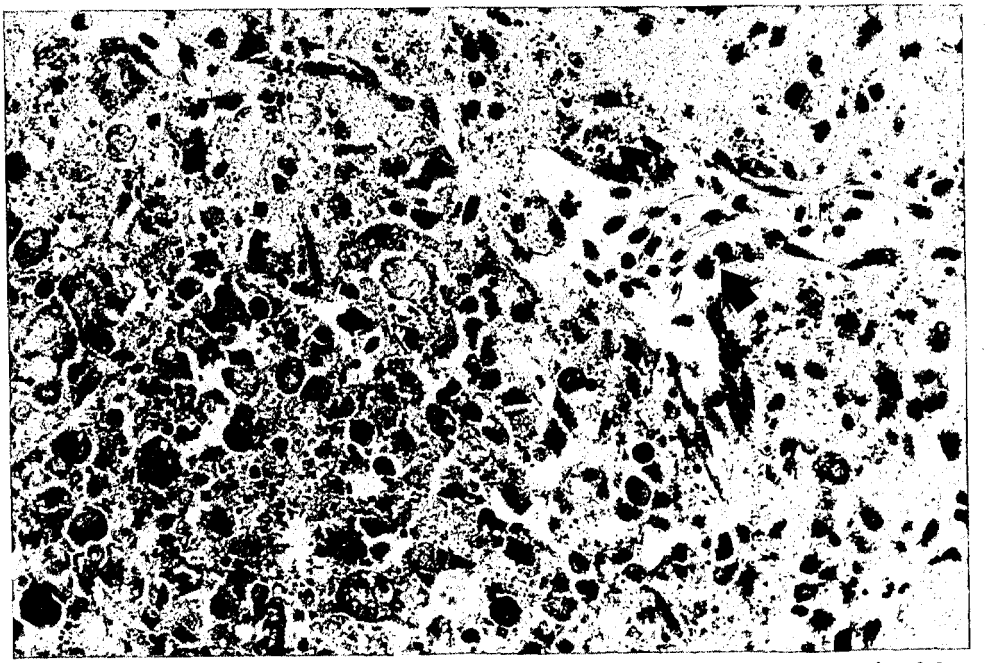

Figure 1 Histological section of a liver showing massive coagulative necrosis of the hepatocytes and loss of integrity of endothelial layer of blood vessel (arrow). H & E; x 300.

perature gave no evidence of bacterial infection.

To test for virus, pooled anterior kidney, spleen and liver tissues from 10 moribund fish were homogenised in 9 volumes of cell culture medium (Eagle's MEM + 10% foetal bovine serum, pH 7.4-7.6) containing penicillin (200 I.U./ml) and streptomycin (1000 µg/ml). Following clarification by centrifugation (2000 rpm for ½ hr in a bench centrifuge) and treatment with high levels of antibiotics (10,000 I.U./ml penicillin and 2000 µg/ml streptomycin), the extract was further diluted in cell medium to final dilutions of 10^{-2}, 10^{-3} and 10^{-4} for inoculation onto 1-day old monolayer cultures of BF2, FHM and RTG2 cells. Inoculated cultures were incubated at 15°C. Cytopathic effects (CPE) developed in all 3 cell lines within 2-5 days, with BF2 cells being the most sensitive and RTG2 the least sensitive.

For electron microscopy, infected BF2 cultures showing complete CPE were harvested, partially purified by PEG 6000 (Way & Dixon, 1988), and concentrated by centrifugation at 35,000 g for 90 min through a cushion of sucrose (25% in phosphate buffered saline) and the final pellet re-suspended in a small volume of distilled water. After negative staining (1% methylamine tungstate) the preparation was seen to contain virus particles of typical rhabdovirus morphology and an average (15 particles) size of approximately 170 nm x 102 nm (Fig.2). The isolate was designated "virus 903/87".

Plaque titration of infectivity and plaque neutralisation tests of virus 903/87 were performed in BF2 cells at 20°C (48 hr incubation). Reference rhabdoviruses and their respective antisera used for comparison were viral haemorrhagic septicaemia virus (VHSV), infectious haematopoietic necrosis virus (IHNV), pike fry rhabdovirus (PFRV), European eel virus (EVX), spring viraemia of carp virus (SVCV) and perch rhabdovirus (PRV). The origins and methods of growth, titration of infectivity, purification and antiserum production for these viruses have been described previously (Hill *et al.*, 1975, 1980: Way & Dixon, 1988).

In plaque neutralisation tests with reference antisera there was no evidence that virus 903/87 is antigenically-related to any of the other rhabdoviruses tested (Table 1). The antiserum raised against virus 903/87 was found to have a very low neutralising titre (< 1:1000), so cross-neutralisation tests with this serum against the reference rhabdoviruses was not attempted. However, the same antiserum was found to have suffi

Figure 2 Electron micrograph of negatively-stained (1% methylamine tungstate) semi-purified virus 903/87 (Bar = 200 nm).

Table 1 Neutralisation titres* of reference rhabdovirus antisera against virus 903/87.

Virus	Antiserum against:				
	VHSV	IHNV	PFRV	EVX	SVCV
Homologous	11,000*	4,500	30,000	380,000	23,500
903/87	<100	<100	<100	<100	<100

* Reciprocal of serum dilution giving 50% plaque neutralisation.

Table 2 A_{405} values of 903/87 with reference rhabdovirus ELISAs.

Virus	ELISA system (A_{405} value)				
	VHSV	IHNV	PFRV	EVX	SVCV
Homologous	1.45	1.43	1.20	1.30	1.78
903/87	0.07	0.09	0.03	0.20	0.04

Table 3 A_{405} values of reference rhabdoviruses with 903/87 ELISA

Virus						
903/87	VHSV	IHNV	PFRV	EVX	SVCV	PRV
1.22	0.04	0.04	0.03	0.07	0.06	0.05

Table 4 A_{405} values of VHSV serotypes and 903/87 with the VHSV ELISA

Virus				
VHSV I	VHSV II	VHSV III	VHSV IV	903/87
1.71	0.77	1.05	0.60	0.02

ciently high levels of binding antibody toallow an ELISA to be developed using the method described previously for SVCV (Way, 1991) and the standard ELISA procedure. ELISAs for VHSV and IHNV (Way & Dixon, 1988), EVX (Dixon & Hill, 1984), SVCV (Way, 1991) and PFRV (Way, unpublished) gave only weak reaction with virus 903/87 (Table 2) and the ELISA against virus 903/87, although giving a strong homologous reaction, gave no significant reaction with the same reference rhabdoviruses nor with PRV (Table 3). Furthermore, the only other rhabdoviruses to have been isolated from brown trout, i.e. the PFRV isolate 84/4 (Adair & McLoughlin, 1986) and VHSV type III (de Kinkelin & Le Berre, 1977), showed no reaction in the 903/87 ELISA system. Also, like type III, reference strains of types II and IV of VHSV showed no reaction with the 903/87 ELISA, although all 3 types react strongly in the VHSV type I ELISA system (Table 4), further indicating that virus 903/87 is not another type of VHSV.

These results, although by no means conclusive, indicate that virus 903/87 could be a previously unknown rhabdovirus, since it shows no antigenic relatedness to the known salmonid rhabdoviruses (VHSV and IHNV),

nor to PFRV which has been isolated from brown trout, nor to any of the other non-salmonid rhabdoviruses isolated so far on the European Continent. However, further comparative studies are necessary to confirm that the virus is a "new" rhabdovirus of fish. Also, although the histopathology is typical of that caused by rhabdovirus infections of fish, it cannot be certain at this stage that the isolated virus was the cause of the clinical condition observed in the infected brown trout. The infectivity and pathogenicity of virus 903/87 for brown trout and other salmonids, as well as non-salmonid fish species, need to be deteremined experimentally.

So far, the virus and the disease has been found in only one farm and that is located in the northern part of Finland on the River Oulujoki which was dammed some 30 km below the farm in 1948, since when there has been no migration of wild salmonids from the Bothnian Bay to the stretch of river on which the farm is situated. Upstream from the affected farm there are numerous other fish farms containing salmonids and there are many large stocks of several different wild fish species in the river. The infected brown trout originated from a broodstock which had been on the farm for several years. All the fish in the affected tank were slaughtered, since when there have been no further cases of the disease on the farm and all subsequent virological tests for the virus have been negative: the origin and host/geographical distribution of the rhabdovirus is, therefore, unknown, at present.

Summary

A rhabdovirus was isolated from diseased brown trout fingerlings at a fish farm in northern Finland. In neutralisation and ELISA tests the virus showed no antigenic relationship to known rhabdoviruses from salmonids (VHSV and IHNV), nor to several rhabdoviruses of non-salmonids (SVC, PFRV, EVEX and PRV). This indicates that the isolate may be a "new" rhabdovirus, but further comparative characterisation work is necessary to confirm this. Although associated with mortalities in brown trout fingerlings, the pathogenicity of the virus needs to be determined.

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