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Isolation of an iridovirus from pike-perch *Stizostedion lucioperca*

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ABSTRACT: We have isolated a large virus from pike-perch *Stizostedion lucioperca* fingerlings with no signs of disease. The biochemical, structural, and serological properties of this newly isolated virus suggest that it belongs to the family *Iridoviridae*. The virus multiplied and was cytopathogenic in several cultured fish cell lines. The virus has a DNA-containing genome and is assembled in the cytoplasm. When viewed in electron micrographs, the assembly sites showed a paracrystalline array of hexagonal nucleocapsids. The ultrastructure of the pike-perch virus resembled that of previously isolated fish iridoviruses. It is an enveloped icosahedral DNA virus. The diameter of the nucleocapsid in thin sections was 127 ± 3 nm; in negatively stained preparates the size of the enveloped virus varied from 147 to 187 nm. In immunofluorescence the virus was stained by rabbit antisera against EHN (epizootic haematopoietic necrosis) virus, sheatfish iridovirus and cod iridovirus. The pathogenicity of the virus isolate was studied by inoculation into juvenile rainbow trout *Oncorhyncus mykiss*. Experimental infection under aquarium conditions suggested that the virus is apathogenic to rainbow trout. The infective virus could be recovered from the viscera of inoculated fish during the first week post-infection, after which the proportion of virus-positive fish declined over time. A small proportion of the fish still carried the virus 24 d post-inoculation.

KEY WORDS: Iridoviridae · Iridovirus · DNA virus · Pike-perch · Fish virus

INTRODUCTION

Iridoviruses are large DNA viruses that are known to cause disease in insects, molluscs, amphibians and fish. The family *Iridoviridae* comprises the genera *Iridovirus*, *Chloriridovirus*, *Ranavirus* and *Lymphocystivirus*, and an additional suggested genus of goldfish iridoviruses (Francki et al. 1991). The iridoviruses found in fish and amphibians are classified either in the *Ranavirus* (type virus FV 3), the *Lymphocystivirus* (type virus fish lymphocystis disease virus, LCDV) or the Goldfish virus group (Goldfish iridoviruses 1 and 2). The most common fish iridoviruses cause mild infections. LCDV has a broad host range and a world-wide distribution causing papillomatous tumours on skin and fins (Wolf 1988). Examples of other iridoviruses are the viral erythrocytic necrosis virus (VENV) and white sturgeon *Acipencer transmontanus* iridovirus (Hedrick et al. 1990). An iridovirus etiology has been also suggested in the cod ulcus syndrome (Jensen et al. 1979) and in an incident of high mortality in turbot fry *Scophthalmus maximus* (Bloch & Larsen 1993) in Denmark.

Recently, previously unknown iridoviruses have been isolated in different parts of the world in association with serious disease outbreaks of cultured fish. The epizootic haematopoietic necrosis virus (EHNV) was isolated in Australia in association with fatal disease outbreaks in redfin perch *Perca fluviatilis* (Lang-

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don et al. 1986, Langdon & Humphrey 1987) and rainbow trout *Oncorhyncus mykiss* (Langdon et al. 1988). Iridoviruses causing high mortality in sheatfish *Silurus glanis* and catfish *Ictalurus melas* were discovered in Germany (Ahne et al. 1989) and in France (Pozet et al. 1992), respectively. A recent study from South Carolina, USA, describes the isolation of an iridovirus from a diseased wild population of large mouth bass (Plumb et al. 1996). In addition, pathogenic iridoviruses affecting several fish species have been isolated in Asia (Inouye et al. 1992, Miyata et al. 1997).

Comparisons among the fish iridoviruses isolated from redfin perch (EHNV; Langdon et al. 1986) and amphibian (ranavirus: FV3; Bohle iridovirus: BIV), as well as sheatfish and catfish iridoviruses, suggest that all belong to the genus *Ranavirus*. The ranaviruses were found to be distinct from the other piscine iridoviruses, namely LCDV and the goldfish iridoviruses, when these viruses were compared both at the DNA and protein level (Essani & Granoff 1989, Hedrick et al. 1992, Hengstberger et al. 1993, Mao et al. 1997).

The pike-perch is an original inhabitant in Finnish lakes, but has been cultivated for restocking purposes since 1978. Spawners are captured from natural populations from special brood-fish lakes. Spawning occurs in anchored floating net cages; after spawning the females are replaced, and the males remain and fan water above the nest with their pectoral fins to keep the eggs clean until hatching occurs at a water temperature of >18°C. Hatching time is minimised by raising the temperature to near 22°C; thereafter 2 to 5 d are usually required until the fry swim horizontally and can be transferred to water ponds for the summer and restocked early in autumn. An iridovirus was isolated from a batch of apparently healthy pike-perch fingerlings (mean size 5 cm) collected just before restocking.

MATERIALS AND METHODS

Cell culture and original isolation of the virus. The following cell lines were used: bluegill fry fibroblast (BF-2), rainbow trout gonad (RTG-2), fathead minnow epithelium (FHM), *Epithelioma papulosum cyprini* (EPC) and chinook salmon embryo fibroblast (CHSE). The growth medium used was Eagle's MEM supplemented with 10% foetal bovine serum, 1% glutamine, 100 IU ml^{-1} penicillin and 40 µg ml^{-1} streptomycin. The growth temperature was 20°C. Cells used in infection experiments were routinely inoculated 24 to 48 h after trypsinization. After inoculation of virus the growth temperature was 15°C. The cells were grown in either 80 cm^2 tissue culture flasks or 24-well dishes (Nunc A/S; Roskilde, Denmark). For titration of infective virus, 10-fold dilutions of virus were inoculated into subconfluent BF-2 cells grown on 96-well dishes (Nunc); titrations were performed in triplicate.

The virus was isolated from a tissue homogenate of pike-perch fingerlings diluted 1:10 in cell culture medium. The suspension was precleared by low speed centrifugation and then used to inoculate BF-2 and EPC cells at 2 dilutions (10^{-1} and 10^{-2}).

Determination of the biophysical properties of the virus. The temperature sensitivity of the virus isolate was determined by treating the virus for 30 min at 50°C, followed by titration on BF-2 cells. The pH sensitivity was studied by lowering the pH of the virus suspension in Hank's balanced salt solution (Gibco BRL) to 2.9 with 0.1 M citric acid. After 4 h in low pH at room temperature the virus was titrated in BF-2 cells seeded in 96-well plates.

The effect of lipid solvents on infectivity of the virus was investigated by incubating 4 ml of virus dilution in Hank's balanced salt solution with 1 ml of diethyl ether for 1 h at room temperature. Thereafter the solution was incubated on ice overnight. Next day the diethyl ether was removed by evaporation at room temperature, and the virus solution was titrated on BF-2 cells after removing diethyl ether. When determining the temperature, pH or lipid-solvent sensitivity, a preparation of control-treated virus was titrated simultaneously. The amount of virus was determined as tissue culture infective dose $(TCID)_{50}$ ml^{-1}.

Determination of nucleic acid. The virus was titrated in growth medium containing iododeoxyuridine (IUDR; 50 µg ml^{-1}; Fluka AG, Switzerland). Two RNA viruses, the infectious pancreatic necrosis virus ($IPNV_{Ab}$) and the European lake trout rhabdovirus (Koski et al. 1992, Björklund et al. 1994) were treated simultaneously. The results were compared with titrations of the same virus preparations in normal growth medium lacking the nucleotide analogue.

Preparation of virus for negative staining in electron microscopy (EM). The virus was inoculated in BF-2 cell cultures, and the growth medium and cells were collected when the cytopathic effect (CPE) was complete (cells detached from the bottom of the flask). The intracellular virus was released by freezing and thawing 3 times. Of this virus-containing medium, 30 ml was precleared by low-speed centrifugation. Glutaraldehyde (Electron Microscopy Sciences, Washington, PA, USA) was added to attain a concentration of 0.05%, and the virus-containing supernatant was centrifuged through a 30% sucrose cushion in a Beckman L8-60M SW 41 rotor at 20 000 rpm for 60 min at +4°C. The virus pellet was dissolved in 50 µl of TN buffer (50 mM Tris-HCl, pH 7.5; 150 mM NaCl), and samples were applied to carbon-reinforced, formvar-coated grids and stained negatively for 10 s with 1% aqueous uranyl acetate, pH 4.1.

Preparation of thin sections for EM. EPC cells grown on 5 cm^2 culture dishes (Nunc) were infected with the virus. Cells were collected on Days 1 to 4 postinfection by adding 0.7 ml 2.5% glutaraldehyde in 0.1 M Na-cacodylate pH 7.2 on cells without overlay medium, followed by detaching the cell layer with a 'rubber policeman'. The cells were pelleted and fixed for 30 min at room temperature. The glutaraldehyde fixative was substituted with 0.2 M sucrose in 0.1 M Na-cacodylate buffer (pH 7.2), postfixation was performed with 1% osmium tetroxide for 1 h followed by staining *en bloc* with 1% aqueous uranyl acetate overnight. After dehydration in an ethanol series, the samples were embedded in Epon 812. Thin sections were cut with a Sorvall MT-6000 ultramicrotome and thin sections poststained with lead citrate. A Jeol CS-100 II electron microscope was used for both thin-sectioned and negatively stained samples.

The size of the naked intracellular nucleocapsid in thin sections was estimated by measuring the diameter of 14 nucleocapsids, vertex to vertex dimension, and calculating the mean size and standard deviation of the diameter. The size on the enveloped virus after negative staining was estimated by measuring the mean diameter of 10 negatively stained virus particles, respectively.

Hoechst stain. EPC cells grown on cover glasses were infected with the virus isolate; uninfected cells served as negative controls. The cells were fixed on Days 0 to 3 postinfection (25% acetic acid, 75% methanol). The fixed cells were stained with Bisbenzamid fluorochrome H 33258 (Reiden De Haen AG), 0.05 µg ml^{-1} in phosphate-buffered saline (PBS).

Staining of cells for immunofluorescence. Coverglass cultures of FHM cells were inoculated with the virus strain at dilutions of 500^{-1} and 50^{-1}, respectively. The incubation time was 24 h and temperature 15°C. Coverglass cultures were rinsed, fixed in 80% acetone and stained for immunofluorescence as previously described (Jørgensen et al. 1989) using dilutions of rabbit serum in the first reagent layer and rhodamine-conjugated swine antibodies to rabbit immunoglobulin (Dako, Copenhagen, Denmark) as the second reagent layer in dilution 1:100. Rabbit antisera against the following viruses were used: (1) sheatfish iridovirus (Ahne et al. 1989), (2) perch iridovirus (EHNV), Lake Nillahcootie (Langdon et al. 1986), (3) cod iridovirus (Jensen et al. 1979), (4) lake trout rhabdovirus (Björklund et al. 1994), (5) IPN virus strain Sp (Jørgensen & Grauballe 1971). The antisera against sheatfish iridovirus (221Aα62.90) and EHNV (221Bα13.91), respectively, were kindly provided by Dr P. de Kinkelin, INRA, France. Antiserum against cod iridovirus, lake trout rhabdovirus and IPN virus were produced at the Danish Veterinary Laboratory. The antiserum against cod iridovirus was produced in 1986 by immunization of 2 rabbits (K16 & K18) with crude cod iridovirus grown in Eagle's MEM without serum, concentrated 30× by ultracentrifugation, and mixed with equal amounts of Freund's Incomplete Adjuvant. Each rabbit was given 6 injections subcutaneously at intervals of 2 wk. Due to background staining in the immunofluorescence, the cod iridovirus antiserum was absorbed *in vivo* before use.

Preparation of virus for experimental infection. The virus was plaque-purified twice; for plaque purification BF-2 cells grown in 12-well dishes were infected with 10-fold dilutions of the 4th passage of the virus for 1 h at 15°C after which 1 ml of 0.25% agarose (SeaPlaque GTG agarose; FMC BioProducts, Rockland, ME, USA) in growth medium was layered on the cells. Fresh BF-2 cells in 80 cm^2 tissue culture flasks (Nunc) were infected with the plaque-purified virus, and the growth medium and cells were collected when the CPE was complete. The virus was released from cells by homogenising with a high speed mixing probe (4 × 5 s) on ice. The virus was cleared by low-speed centrifugation and the supernatant was kept on ice for 4 d until used for inoculation of fish. (Whittington & Reddacliff 1995). The preparation to be inoculated was titrated before and after inoculation. Preparations from non-infected BF-2 cell cultures were used for inoculation of control fish.

Infection of rainbow trout. Four 300 l tanks containing 40 rainbow trout were used in the experiment. The 1-yr-old fish (average weight 40 g) were obtained from a state fish farm (Saimaa Fisheries Research and Aquaculture) monitored for virological and bacteriological disease. They were acclimatised for 1 wk before infection and during this period the viscera of 5 fish were collected for virological examination. The fish were tranquillized with benzocain for 2 min before infection. Altogether 80 rainbow trout in 2 tanks were injected intraperitoneally (i.p.) with 0.1 ml of virus suspension containing 5×10^5 $TCID_{50}$ ml^{-1}. As a negative control, 40 fingerlings in 1 tank were infected i.p. with 0.1 ml of medium from non-infected cell cultures. The 40 fish in the 4th tank were infected by immersion for 30 min in a 20 l aerated tank containing 2.5×10^3 $TCID_{50}$ ml^{-1} of virus.

The well-being and motility of fish as well as the temperature of water were monitored twice daily. The temperature varied from 15.5 to 19.5°C. The oxygen content was monitored every second day and varied from 6.4 to 8.4 mg l^{-1}. Throughout the experiment the fish were maintained in static aerated water, one third of which was changed approximately every second day; all outflowing water was heated to 95°C.

The fish from one tank of i.p.-infected fish were examined virologically during the course of infection.

Seven i.p.-injected fish were killed on Days 2, 4, 9 and 16, respectively, and tissue material (liver, spleen, kidney) was collected for individual virological examination. The liver, spleen, and kidney from each fish were pooled and homogenised with pestle and mortar. A 10% suspension was prepared in growth medium and pre-cleared by low speed centrifugation. The supernatant was inoculated in BF-2 cell cultures in 10^{-1} and 10^{-2} dilutions. All fish from 3 other tanks (1 control, 1 bath-infected, 1 i.p.-infected) were killed after 24 d and examined virologically. Tissue material (liver, spleen, kidney) from 5 fish were pooled into 1 sample and processed as above. The presence of virus in the water from each tank was analysed at the end of the experiment. A 1 ml sample from each tank was taken with the help of a syringe, and the sample was filtered through a 0.45 µM filter and inoculated as 10^{-1} dilution in BF-2 cell cultures. The inoculated cell cultures were examined daily. A sample was considered negative if no CPE was observed after 3 passages (7 d between each passage) in BF-2 cells.

RESULTS

The iridovirus was isolated from pooled organ material of apparently healthy pike-perch fingerlings. The material was inoculated simultaneously onto BF-2 and EPC cells. CPE was first observed after 1 subcultivation in BF-2 cells. The virus generated small foci in the cell carpet with rounding of cells on the edges of the foci (Fig. 1a). When CPE was complete in 3 to 4 d, many of the rounded cells contained an intracytoplasmic vacuole or vacuoles (Fig. 1b) and the cells began to detach from the bottom of the flask. The vacuoles contained DNA, which was detected by staining with Hoechst stain. In addition to the nuclei, smaller intracytoplasmic inclusion bodies could be detected in the virus-infected cells 2 to 3 d postinoculation (results not shown). The staining pattern suggested packaging of DNA-containing viruses in these intracytoplasmic assembly sites.

The pike-perch virus was cytopathogenic in all the fish cell lines examined (BF-2, EPC, FHM, RTG-2, CHSE). The BF-2 and RTG-2 cells, however, were found to be the most sensitive to infection; in both cell lines CPE was detected 48 h post-inoculation. Titration of the virus in a growth medium containing 50 µM IUDR reduced the infectivity of the virus 1000-fold, indicating that the viral genome consisted of DNA. The same concentration of IUDR had no effect on the replication of 2 RNA viruses (IPNV and the European lake trout rhabdovirus; Koski et al. 1992, Björklund et al. 1994) analysed simultaneously with the pike-perch virus.

Treatment of virus with diethyl ether reduced the titre of the virus from $10^{5.6}$ to $10^{2.5}$ $TCID_{50}$ ml^{-1}, suggesting that infectivity of the virus is largely dependent on the lipid envelope surrounding the nucleocapsid. The pike-perch virus was sensitive to both heat and low pH; treatment of the virus for 30 min at 50°C abolished the infectivity of the virus in BF-2 cells. Similarly, infectivity was lost by maintaining the virus at low pH (2.9) for 4 h at room temperature.

In thin sections, the intracellular virus was detected as individual particles or in intracytoplasmic assembly sites as paracrystalline arrays of icosahedral virus particles (Fig. 2a). The mean diameter of 14 intracellular nucleocapsids was 127 nm with a standard deviation of 3 nm. The virus particles acquired an envelope by budding through plasma membranes (Fig. 2b, c). The size of the negatively stained enveloped viruses varied between 147 and 187 nm. The mean diameter of 10 particles was 167 nm with a standard deviation of 11 nm). The structure of the newly forming envelope was 3-layered with an inner electron-dense layer surrounded by a lighter area. The lightly pre-fixed and negatively stained virus particles retained some features of hexagonal symmetry (Fig. 2d). A regular array of subunits was visible on the surface of the virus (Fig. 2e). Without pre-fixing the integrity of the virus particle was lost, such that it could be visualised as a fuzzy, snowball-like particle, sometimes surrounded by a unit membrane (results not shown).

The fluorescence pattern of the iridovirus-infected FHM cells stained with various rabbit antisera is given in Table 1. Positive staining was clearly observed using rabbit antisera against EHNV, sheatfish iridovirus and cod iridovirus, respectively (Fig. 3). Staining was mainly observed as cytoplasmatic inclusion bodies and small perinuclear granula. Cell membranes were only stained in a few cells. No staining was observed with rabbit antisera against IPNV and lake trout rhabdovirus, respectively.

The pathogenicity of the virus was examined by infecting rainbow trout (10 to 15 cm) either by i.p. injection or immersion. For this, 80 fish in 2 separate tanks (40 fish $tank^{-1}$) were infected by i.p. inoculation (0.1 ml of solution containing 5×10^5 $TCID_{50}$ ml^{-1}) and 40 fish by immersion in 2.5×10^3 $TCID_{50}$ ml^{-1}. There was no mortality in the control tank with non-infected fish, or in tanks infected either by i.p. injection or immersion.

No signs of disease or abnormal behaviour were detected during the 24 d. The i.p.-injected rainbow trout in one tank were examined virologically during the course of the experiment. Seven fish were killed on Days 2, 4, 9 and 16 after infection. The internal organs were inspected and collected for virological examination. No gross abnormalities in the internal organs of infected fish could be detected. Furthermore, no signs

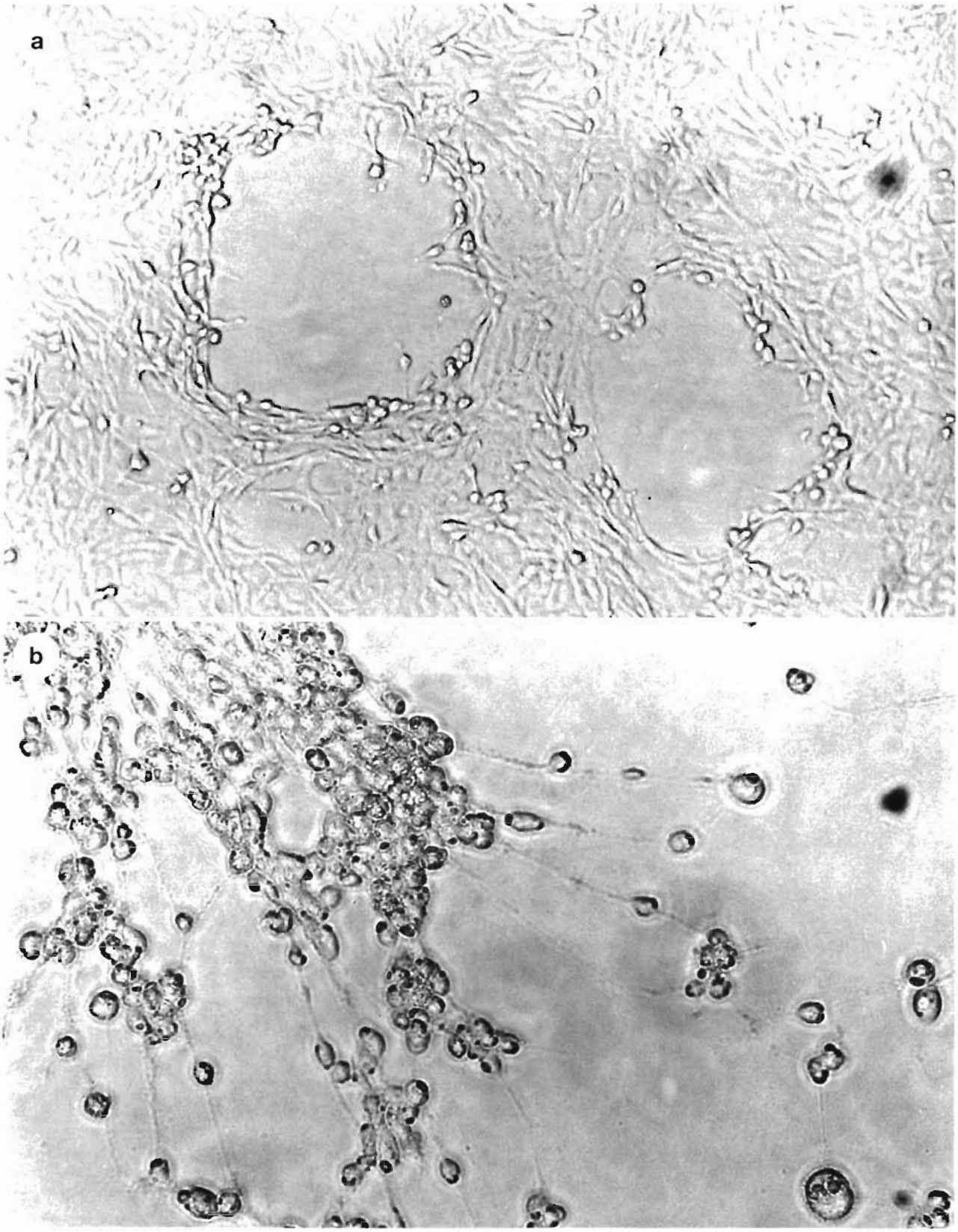

Fig. 1. BF-2 cells infected with the pike-perch virus. The CPE starts as discrete foci of infection with rounding of cells on the edges of the foci (a; ×100). Later in the course of infection the virus is assembled in intracytoplasmic light reflecting vacuoles (b; ×200)

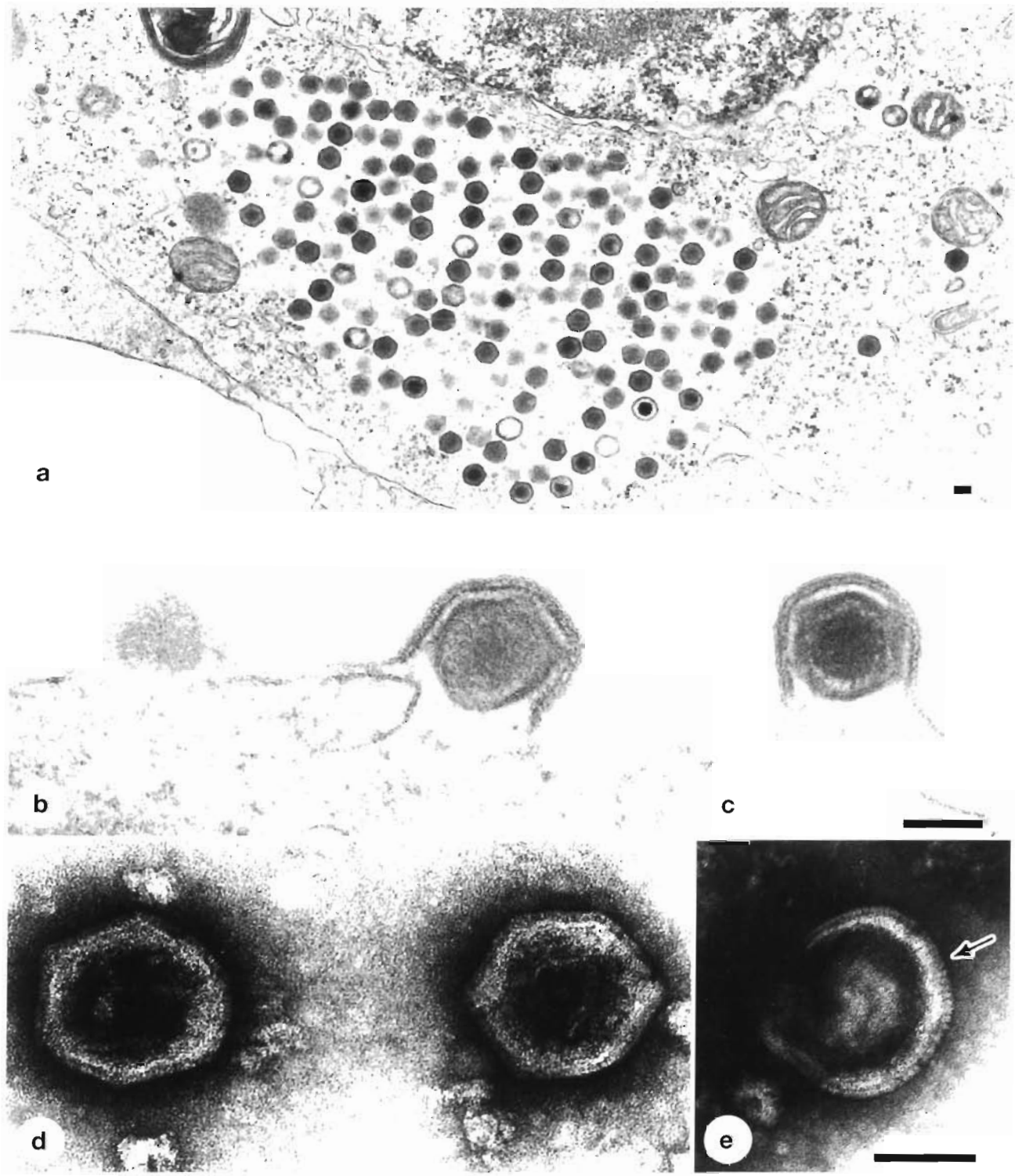

Fig. 2. EPC cells infected with the pike-perch virus, processed for EM as described in 'Materials and methods' (a–c) Thin sections of EPC cells infected with the pike-perch iridovirus. Viral nucleocapsids were mainly localised in intracytoplasmic assembly sites (a). The virus particles acquired the viral envelope by budding through plasma membrane (b, c). (d, e) Negative staining of the pike-perch virus. Scale bars = 100 nm. Bar for both (b) and (c) is shown in (c); bar for both (d) and (e) is shown in (e). Arrow shows the regular array of subunits on the surface of the virus particle

Table 1. Immunofluorescence results with pike-perch iridovirus, cod iridovirus, and 2 other fish viruses using homologous and heterologous antisera. Reactions: –, no reaction; +, weak; ++, moderate; +++: strong; nd, not done

Antiserum	Virus			
	Pike-perch iridovirus	Cod iridovirus	IPN virus	Lake trout rhabdovirus
Rabbit anti cod iridovirus	++	+++	nd	nd
Rabbit anti EHN virus	++	++	nd	nd
Rabbit anti sheatfish iridovirus	++	++	nd	nd
Rabbit anti IPN virus	–	–	+++	–
Rabbit anti lake trout rhabdovirus	–	–	–	+++

of disease or changes in the behaviour of fish were observed during the experiment, even though the virus could be recovered from the pooled internal organs (liver, spleen, kidney) of the infected fish. The iridovirus could be isolated from the viscera of all i.p.-infected fish examined (14/14) on Days 2 and 4 after infection, indicating a successful infection. Towards the end of the experiment the proportion of virus-positive fish was reduced. On Day 9, 3 out of 7 fish (43%) were virus-positive. When the experiment was terminated after 24 d, 2 out of 12 infected fish (17%) still carried the virus. The unconcentrated tank water was found to be negative for infective virus at the end of the experiment.

No virus was isolated from samples taken before infection. All fish in the remaining 3 tanks (1 control, 1 bath-infected and 1 i.p.-infected, respectively) were killed on Day 24 after infection. All fish were examined virologically in pools of 5 fish. Virus was re-isolated from 3 out of 8 pools of i.p.-infected fish. No virus could be isolated from fish infected by immersion or from the non-infected fish.

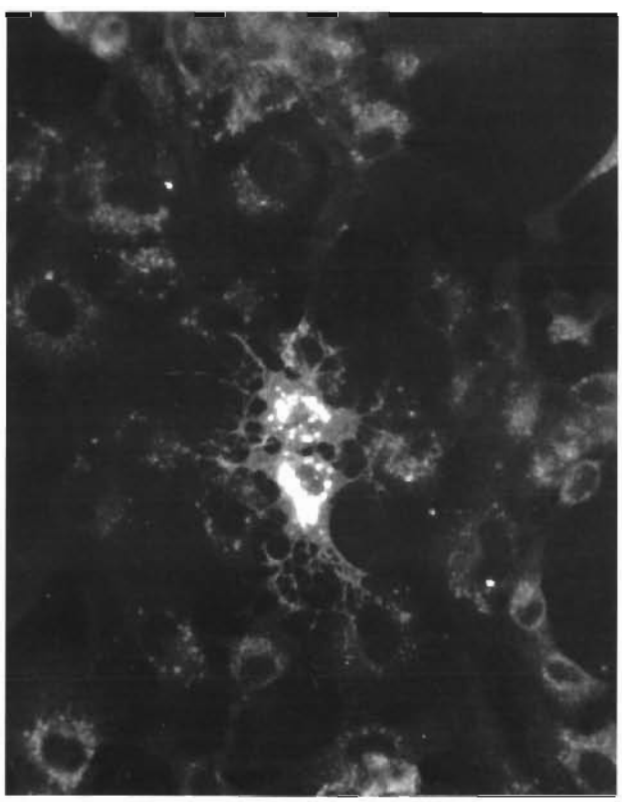

Fig. 3. Immunofluorescence micrograph showing the cross-reaction staining of pike-perch iridovirus infected FHM cells with rabbit antiserum against sheatfish iridovirus as the primary antibody and rhodamine-conjugated swine antibody against rabbit IgG as the secondary antibody (×400)

DISCUSSION

The immunofluorescence results in combination with the EM data indicate that the pike-perch virus should be classified in the *Iridoviridae* family. The large size, DNA genome, lipid-containing envelope and thermosensitivity are all properties typical of members of the *Iridoviridae*. Furthermore, we could detect DNA-containing inclusion bodies typical of the *Iridoviridae* family in the cytoplasm of infected EPC cells. The infectivity of the virus was reduced by treatment with ether indicating that the lipid envelope enhanced infectivity of the virus. Fish iridoviruses isolated thus far belong either to the genera *Lymphocystivirus* or *Ranavirus*. This and the fact that members of the genera *Iridovirus* and *Chloriridovirus* are resistant to ether (Francki et al. 1991) indicate that the pike-perch virus is a member of either the *Ranavirus* or *Lymphocystivirus* genera.

The ultrastructure of this virus resembled that of the previously studied fish iridoviruses (Klump et al. 1983, Berthiaume et al. 1984, Heppell & Berthiaume 1992). The ultrathin sections of the maturing virus showed an icosahedral particle surrounded by a 2 or 3 layered envelope. The negatively stained particles were surrounded by membrane with a regular array of subunits on the outer surface.

The growth properties and wide range of susceptible fish cell lines resemble those reported for EHNV and the iridovirus isolated from catfish (Langdon et al. 1986, Pozet et al. 1992, Hengstberger et al. 1993).

The Australian and European iridoviruses share common epitopes as they cross-react serologically, making the use of polyclonal rabbit antisera unsuitable for differentiation of the viruses by immunofluorescence. The results of the present study indicate that the 2 iridoviruses from pike-perch and cod, respectively, fall into this serologically similar group of iridoviruses. Immunoblotting and PCR (polymerase chain reaction) of the major viral coat protein combined with sequencing of the obtained PCR product have been successfully used to differentiate between fish iridoviruses (Hengstberger et al. 1993, Mao et al. 1997) and this approach will be used in the future to further characterize the iridovirus isolated from pike-perch. However, there is a strong need for the development of quicker and specific diagnostic tools especially for EHNV, but also for sheatfish iridovirus and other European pathogenic iridoviruses.

Apparently the present Finnish iridovirus isolate from pike-perch is not pathogenic for rainbow trout in these experimental conditions. Rainbow trout was chosen as the first fish species to be analyzed for susceptibility to the pike-perch iridovirus infection, because of the economical impact on the rainbow trout farming in Finland. Although the rainbow trout succumbs in natural infections caused by EHNV, it is known that it can be resistant to infection depending on experimental conditions. In our experiments we used conditions which would favour pathogenicity of EHNV for rainbow trout (Whittington & Reddacliff 1995). However, it is possible that the conditions we chose were not optimal for the pathogenicity of the pike-perch iridovirus. A small fraction of the i.p.-injected fish were found to carry the iridovirus 24 d after infection, suggesting that a true carrier status for this virus could exist in fish. A carrier state of several weeks has been reported in rainbow trout experimentally infected with EHNV (Whittington & Reddacliff 1995). Therefore, our data on the pathogenicity of the pike-perch iridovirus for rainbow trout and for other fish species is still inconclusive. The cod iridovirus from Denmark has been reported to cause some mortality in cod (Jensen & Larsen 1982).

The original isolations of this virus were from apparently healthy pike-perch from 2 separate locations in Finland during the summer 1995. It seems likely that pike-perch fingerlings originating from the first positive pond carried the iridovirus to the second location as the second owner had obtained his pike-perch fingerlings from the first owner.

The pike-perch iridovirus does not appear to be widespread in Finland since pike-perch fingerlings originating from several locations in Finland have been examined virologically for several years, and the virus has been detected only in 2 separate earth ponds. The source of this virus is unknown.

All our data suggest that the virus isolated from Finnish pike-perch is a member of the genus *Ranavirus* in the *Iridoviridae* family. Further comparison between the biochemical properties of EHNV, cod iridovirus and the pike-perch virus isolate is presently being undertaken.

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