Isolation and partial characterization of a novel paramyxovirus from the gills of diseased seawater-reared Atlantic salmon (Salmo salar L.)

Agnar Kvellestad,1,2 Birgit H. Dannevig2 and Knut Falk2

1Department of Morphology, Genetics and Aquatic Biology, Norwegian School of Veterinary Science, Oslo, Norway
2National Veterinary Institute, PO Box 8156 Dep., 0033 Oslo, Norway

A formerly undescribed virus has been isolated from the gills of farmed Atlantic salmon post-smolts in Norway suffering from gill disease. Cytopathic effects appeared in RTgill-W1 cells 9 weeks post-inoculation with gill tissue material. Virus production continued for an extended period thereafter. Light and electron microscopic examination revealed inclusions and replication in the cytoplasm. The viral nucleocapsid consisted of approximately 17 nm thick filaments in a herringbone pattern. Certain areas of the plasma membrane were thickened by the alignment of nucleocapsids on the internal surface and projections of 10 nm long viral glycoprotein spikes on the external surface. Virus assembly and release was achieved by budding through the modified plasma membrane. Negatively stained virions were spherical and partly pleomorphic with a diameter of 150–300 nm as seen by electron microscopy. The virus was sensitive to chloroform, heat and low and high pH, and replication was not inhibited by Br-dU or IdU indicating an RNA genome. Both haemagglutination and receptor-destroying enzyme activity were associated with the virions and the formation of syncytia in infected cultures indicated fusion activity. The receptor-destroying enzyme was identified as neuraminidase. The virus contained five major structural polypeptides with estimated molecular masses of 70, 62, 60, 48 and 37 kDa. Its buoyant density was 1.18–1.19 g ml⁻¹ in CsCl gradients. From the observed properties we conclude that this new virus belongs to the Paramyxoviridae and suggest the name Atlantic salmon paramyxovirus (ASPV). Furthermore, replication occurred at 6–21 °C, suggesting a host range confined to cold-blooded animals.

INTRODUCTION

Respiratory diseases are an important cause of losses in farmed Atlantic salmon (Salmo salar L.) in Norway, especially during the first months following seawater transfer. Disease outbreaks may last for weeks. The aetiology is apparently multifactorial and known causes include infection with protozoan ectoparasites such as trichodinids and Ichthyobodo necator and rickettsia- or chlamydia-like organisms causing epitheliocystis. Virus aetiology may also be suspected, as viruses primarily affecting the gills have been shown to cause mortality in fish. These include herpesvirus infection in Koi carp (Cyprinus carpio L.) (Hedrick et al., 2000), farmed Japanese eels (Anguilla japonica Temminck & Schlegel) (Lee et al., 1999a) and farmed European eels (Anguilla anguilla L.) (Haenen et al., 2002), and birnavirus infection in Japanese eels (Lee et al., 1999b). A herpes-like virus has apparently also caused massive mortalities among wild Australian pilchards (Sardinops sagax neopilchardus) following infection of the gill epithelium (Hyatt et al., 1997). Viruses of uncertain significance in relation to disease have been cultivated from gills, including an enveloped virus of size 70–80 nm from farmed bluegill (Lepomis macrochirus Rafinesque) (Hoffman et al., 1969) and an iridovirus (Shchelkunov & Shchelkunova, 1990) and a paramyxo-like virus (Body et al., 2000) from farmed common carp (C. carpio L.) with gill necrosis. To our knowledge, the latter is the first report of paramyxovirus as a possible aetiological agent of gill disease.

Members of the family Paramyxoviridae are responsible for a variety of diseases affecting humans and animals. Many of these diseases affect the respiratory organs. Over the past decades, several novel paramyxoviruses have been discovered in aquatic and terrestrial animals as a result of surveillance and disease investigations (Lamb et al., 2000; Lamb & Kolakofsky, 2001; Wang & Eaton, 2001). These include a paramyxovirus in diseased snakes (Clark et al., 1979; Ahne et al., 1999) and paramyx-like viruses in carp (Body et al., 2000) and in apparently healthy wild chinook salmon (Oncorhynchus tshawytscha) (Winton et al., 1985).
Paramyxoviruses are large (150–300 nm), enveloped, pleomorphic viruses with a non-segmented, single-stranded, negative-sense RNA genome of 15–16 kb. They replicate entirely in the cytoplasm. The virion has a nucleocapsid core containing the RNA genome and three nucleocapsid-associated proteins: an RNA-binding protein, a phosphoprotein, and a large protein. The matrix protein resides between the core and the envelope. The envelope is covered with spikes consisting of one glycoprotein involved in cell attachment (haemagglutinin–neuraminidase, haemagglutinin or protein G) and another glycoprotein involved in the fusion of the viral envelope with the plasma membrane of cells (Lamb et al., 2000; Lamb & Kolakofsky, 2001).

During attempts to culture the causative agents of epitheliocystis in Atlantic salmon, we isolated a hitherto-unknown paramyxovirus from a single fish. In this report we describe the isolation and partial characterization of this new virus, with special emphasis on morphology, replication and properties of the surface molecules.

**METHODS**

### Cell cultures and viruses.

Monolayer cultures of rainbow trout gill (RTgill-W1) cells (Bols et al., 1994) and CHSE-214 cells (Lannan et al., 1984) were maintained at 14 °C in Leibovitz L-15 medium (L15) supplemented with 5 or 10 % foetal bovine serum (L15-5; L15-10), 2 mM l-glutamine and either 24 µg gentamicin ml⁻¹ or 100 IU benzylpenicillin ml⁻¹ and 100 µg streptomycin ml⁻¹. SHK-1 cells were grown as previously described (Dannevig et al., 1995). BF-2 cells (Wolf et al., 1966) and EPC cells (Fijan et al., 1983) were grown at 20 °C in Eagle’s minimal essential medium, pH 7.6-7, supplemented with 10 % foetal bovine serum, 16-4 mM Tris, 4 mM l-glutamine and 50 µg gentamicin ml⁻¹.

Unless otherwise stated, RTgill-W1 cells were inoculated for virus propagation, incubated at 14 °C and inspected for cytopathic effects (CPE) by phase-contrast light microscopy. For primary virus isolation, cells in 24-well tissue culture plates were inoculated with tissue suspensions for 14 h, followed by washing and addition of fresh L15-10. The cultures were supplied with fresh L15-10 at intervals of 1–2 weeks. For higher passages of virus, supernatants from cultures with CPE were diluted in L15-5 and inoculated into new cultures. The characterization experiments were performed with virus from the third to fifth passage.

To test for susceptibility to this new virus, cultures of CHSE-214, BF-2, EPC and SHK-1 cells were inoculated with supernatants from parallel non-infected and infected RTgill-W1 cells with CPE and incubated at 15 °C.

Infectivity titre was determined by endpoint titration in 96-well culture plates with RTgill-W1 cells. CPE was read between 6 and 8 weeks post-inoculation (p.i.). The 50 % tissue culture infective dose (TCID₅₀) was estimated by the method of Spearman and Kärber (Kärber, 1931).

### Clinical samples and sample processing.

Gills were sampled in September 1995 from 20 Atlantic salmon previously transferred to seawater netpens in May. From the beginning of August the fish displayed signs of respiratory distress. Cumulative mortality reached 40 % during the next 3 months. Mean water temperature decreased from 17 °C in August to 12 °C in September. Histological examination prior to sampling revealed extensive gill changes, with thrombosis of lamellar blood vessels, necrosis of epithelial cells, proliferation of apparently poorly differentiated epithelial cells, infiltration of epithelium with inflammatory cells and many inclusions indicating epitheliocyctis. Areas of necrosis were also detected in the liver.

The gills of the fish, from which the described virus was isolated, were washed in buffers and disinfected with hydrogen peroxide and glutaraldehyde, essentially as described by Kvellestad et al. (2002), to prevent contamination of the cell cultures by normal surface microflora. The soft tissues were scraped from the gills and suspended in 10 ml L15-10 using a whirl mixer. Since infectious pancreatic necrosis virus (IPNV) is ubiquitous in Norwegian salmon farms (Melby et al., 1991), rabbit antisera against Ab and Sp serotypes of IPNV were added to the tissue suspension in appropriate dilutions to inhibit replication of this virus in the cell culture.

### Haemagglutination and haemadsorption assays.

Haemagglutination titrations were performed in microtitre plates by mixing 50 µl of virus supernatant diluted twofold in PBS (10 mM, pH 7.4) with 50 µl 0-5 % (v/v) washed erythrocytes. The endpoints were read after 1 h of incubation at room temperature except for influenza C virus, which was titrated at 4 °C. Erythrocytes from mammals, birds and fish were tested, including human (O), guinea pig, green monkey, rabbit, sheep, cow, horse, chicken, turkey, Atlantic salmon, brown trout (Salmo trutta L.), rainbow trout (Oncorhynchus mykiss Walbaum), Atlantic cod (Gadus morhua L.), crucian carp (Carassius carassius L.) and wolfish (Anarhichas lupus Olafsen). The haemagglutinating activity (HA units) was expressed as the reciprocal value of the highest dilution showing complete agglutination of the erythrocytes.

For haemadsorption, the cell cultures were first washed once with Hank’s balanced salt solution followed by incubation with 0-5 % (v/v) washed chicken erythrocytes in L15 for 30 min at room temperature. The cultures were then washed with L-15 to remove unattached erythrocytes and immediately examined by microscopy for adsorption.

Receptor-destructing enzyme activity was assessed by prolonged observation of the haemagglutination reaction at room temperature for up to 24 h. The elution of virus from the erythrocytes as shown by the conversion of positive haemagglutination patterns to a negative pattern indicated the presence of receptor-destructing enzyme activity. Following elution, fresh virus (4 HA units per well) was added to see whether the cells could be re-agglutinated.

### Assays of neuraminidase and acetylesterase activity.

Cell culture supernatant or pelleted virus was used in both assays. Neuraminidase activity was assayed by incubating virus with either fetuin (Sigma) or N-acetylneuraminyl-lactose (Sigma) in phosphate buffer (0-5 M, pH 6-0) at 15, 25 and 34 °C. Following incubation for 18 h, samples were assayed for free N-acetylneuraminic acid using the thiobarbituric acid method as described by the Centers for Disease Control (1982). The acetylesterase activity was determined by incubating 10 µl of virus sample with 300 µl 1 mM p-nitrophenyl-

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acetate (Sigma) in PBS. The release of acetate was monitored by determining the optical density at 405 nm (Vlasak et al., 1987).

**Metabolic inhibitors.** The nucleic acid type of the virus was presumptively determined by growing it in the presence of the thymidine analogues 5-bromo-2-deoxyuridine (Br-DU) or 5-iodo-2-deoxyuridine (IDU). RTgill-W1 cells in 25 cm² flasks were infected with the virus and incubated with or without 100 µg Br-DU or IDU ml⁻¹. After incubation for 9 and 22 days, the cells were examined by phase-contrast microscopy and the haemagglutination and infectivity titres were determined.

**Inactivation studies.** Lipid solvent sensitivity was tested by adding 1-5 ml chloroform to 3 ml cell culture supernatant, vortexing for 10 min at room temperature, centrifugation (2000 g, 10 min) and determination of the virus titre. The stability of the virus at different pH values was assayed by adding 0-5 ml cell culture supernatant to 4-5 ml aliquots of medium adjusted using HCl or NaOH. After incubation for 30 min at room temperature, an equal volume of medium containing NaOH or HCl was added to give a final pH of 7-4 and the virus titre determined. The temperature stability of the virus was tested by incubating aliquots of cell culture supernatant at 5, 14, 37 and 56°C, followed by determination of virus titre. The effect of freezing and thawing on virus infectivity was determined by repeated freezing (~70°C) and thawing in a water bath at 20°C.

**Isopycnic density.** Virus culture supernatant was clarified by centrifugation (67000 g, 20 min) and then precipitated by addition of polyethylene glycol (M, 8000) and NaCl to final concentrations of 70 and 23-2 g l⁻¹, respectively. The mixture was gently stirred at room temperature for 15 min, followed by further gentle stirring for 3 h at 4°C. The precipitate was collected by centrifugation (67000 g, 1 h) and resuspended in TNE buffer (10 mM Tris/HCl, 100 mM NaCl, 1 mM EDTA, pH 7-2). One ml of virus concentrate was overlaid on either a preformed 5-65% (w/v) sucrose gradient in TNE buffer or a discontinuous gradient composed of equal parts of 20, 30 and 40% CaCl₂ in TNE buffer. The gradients were centrifuged at 150000 g for 18–20 h (Beckman SW-41 rotor). Fractions of 0-25 or 0-5 ml were collected by upward displacement and tested for haemagglutinating activity. The buoyant densities of the collected fractions were calculated from the refractive index.

**Gel electrophoresis of viral polypeptides and Western blotting.** Virus was purified by sucrose gradient centrifugation as described above. The virus band was collected by side puncture of the tube wall, diluted in TNE buffer, pelleted at 100000 g for 1 h and resuspended in dissociation buffer (50 mM Tris/HCl, pH 6-8, 1% SDS, 50 mM dithiothreitol, 8 mM EDTA, 0-01% bromophenol blue). After heating for 5 min at 95°C, the proteins were separated by SDS-PAGE using the discontinuous system devised by Laemmli (1970) with 0-5 mm thin pre-cast 12.5% polyacrylamide gradient gels (ExcelGel SDS; Amersham) followed by staining with Coomassie blue. Gels were scanned in a desktop scanner (Image Scanner; Amersham) and subsequently analysed and printed out using Gel-Pro gel scanning software (Media Cybernetics). The molecular mass markers used were in the range 14-4–940 kDa (Amersham).

**Electron microscopy of cell cultures.** Infected and non-infected cells on Thermostax plastic coverslips (Nunc) and in 25 cm² flasks were fixed in glutaraldehyde (3% in 0-1 M sodium cacodylate buffer, pH 7-4), post-fixed in osmium tetroxide (2% in 0-1 M sodium cacodylate buffer, pH 7-4) for 2 h, dehydrated through a series of graded ethanol and embedded in Lx-112 embedding medium (Ladd Research Industries). Ultrathin sections were contrasted for 20 min in 5% uranyl acetate and for 2 min in Coggshall’s 0-2% lead citrate in 0-1 M sodium hydroxide and examined in a JEOL JEM-100S electron microscope operated at 60 keV.

**Electron microscopy of purified virus preparations.** Virus was purified by CsCl gradient centrifugation as described above. To reveal the nucleocapsid of the virions, the virus was lysed by mixing equal parts of purified virus and 0-4% Triton X-100 solution (pH 7-2). The mixture was kept at room temperature for 1–5 min before grid preparation. Negative staining was performed by applying droplets (5 µl) of lysed or non-lysed virus suspensions on carbon-filmed grids. After 1 min, the grids were washed in distilled water and stained with 0-5% sodium phosphotungstic acid, pH 7-0, for 1 min. The grids had previously been glow discharged in air to facilitate spreading of both virus and stain. The specimens were examined in a JEOL JEM-1010 electron microscope operated at 100 keV.

**RESULTS**

**Isolation of virus**

In one well with RTgill-W1 cells inoculated with tissue suspension, CPE began to appear 9 weeks p.i. In many foci of the monolayer, a low number of cells rounded up, shrank and lost contact with each other. The cells contained numerous small cytoplasm vacuoles and appeared either refractile (shining yellow) or darkened. These foci expanded over the next 3 weeks to involve most of the monolayer and cells displayed this type of CPE over long periods, prior to detachment. Transfer of supernatant 11, 14, 18 and 27 weeks p.i. from the primary well to new cultures at dilutions of 1:3–5 or more resulted in CPE in all second-passage cultures (Fig. 1b). All cells either detached or the number of cells with CPE decreased and the cells again started to proliferate.

**Observation of CPE in living cells and in cells following staining**

In a sequential study (7, 14, 21 and 28 days incubation), CPE appeared 14 days p.i. in a few cells as seen by phase-contrast light microscopy. Staining of these cultures revealed CPE during all observations at 7–28 days characterized by a few vacuolated cells (Fig. 1d) and many cells with purple-stained, round cytoplasmic inclusions up to 10 µm in size (Fig. 1e–g). After 21 days the number of mitotic figures was reduced and the majority of cells with CPE were shrunken, with a condensed and strongly basophilic nucleus and a strongly eosinophilic or in parts purple cytoplasm (Fig. 1g). Many of these cells were detached and some cells were disintegrated. Furthermore, a few large syncytia were also observed in infected cell cultures (Fig. 1h).

**Electron microscopy of cell cultures**

Structural changes were observed in the cytoplasm and plasma membrane of infected cells. The cytoplasmic inclusions in infected cells consisted of tightly packed coiled filaments approximately 17 nm in diameter (Fig. 2b, c). Small inclusions with loosely packed filaments of apparently greater diameter were seen in the cytoplasm of a very few cells (Fig. 2d). The plasma membrane displayed many small electron-dense areas with outer surface projections
Novel paramyxovirus from Atlantic salmon

a N
b C

c d

e f
g h
extending approximately 10 nm from the outer surface, while on the inside more or less parallel aligned filaments of the type previously described with a diameter of approximately 25 nm were observed (Fig. 2e). Highly pleomorphic virions were released by budding through these areas of altered plasma membrane (Fig. 2f). Elongated virions in the budding phase measured at least 200–600 nm in diameter and 400–2100 nm in length, with prominent filaments beneath the envelope (Fig. 2g). A few spherical particles with diameter 400–800 nm were observed. In those that were fully released, many 17 nm thick filaments had apparently been released from the envelope and coiled up in the interior (Fig. 2h). Rounded and shrunken cells contained a condensed nucleus and cytoplasm, inclusions and altered plasma membrane, but no budding was observed.

Haemagglutination and receptor-destroying activity

Virus supernatants with an infectivity titre of approximately \(10^6\) TCID\(_{50}\) ml\(^{-1}\) produced agglutination using erythrocytes from all species tested including fish, birds and mammals with the exception of sheep and monkey. Titres ranged from 40 to 1280 HA units ml\(^{-1}\), with guinea pigs giving the highest titres although more difficult to evaluate. Different incubation temperatures did not significantly influence these results. Furthermore, haemadsorption using chicken erythrocytes was also demonstrated in a few cells as early as 2 days p.i. in 24-well plate cultures inoculated with \(10^3\) TCID\(_{50}\). On appearance of CPE at 14 days p.i., haemadsorption was prominent.

Complete elution of virus from the agglutinated erythrocytes was observed after incubation at room temperature for 2–3 h indicating the presence of receptor-destroying enzyme activity. Erythrocytes previously agglutinated with the paramyxovirus could not be re-agglutinated with homologous virus or with Newcastle disease virus, indicating a relationship with this virus. In contrast, infectious salmon anaemia virus, influenza C virus and to a degree influenza A virus were able to re-agglutinate the paramyxovirus-treated erythrocytes.

Neuraminidase and acetyesterase activity

Significant neuraminidase activity was detected in the paramyxovirus preparation using both fetuin and N-acetylneuraminyl-lactose as substrates. In comparison with influenza A virus the magnitude of the reaction was similar at 34°C but twice as strong at 15 and 25°C, indicating that this new virus is adapted to cold-blooded animals. Acetyesterase activity was not detected in the paramyxovirus preparation.

Metabolic inhibition

No inhibition of virus replication was observed with either Br-dU or IdU.
Replication

To examine virus replication at different temperatures, RTgill-W1 cells in 162 cm² flasks were inoculated with a virus dose of 10⁵ TCID₅₀ for 24 h, incubated at 6, 10, 16 and 21 °C and assayed for infectivity titre over a period of several weeks. The results are presented in Fig. 3 and show that the lag phase in this experiment lasted for approximately 20–41 days depending on temperature. The highest titres were obtained at 10 °C, indicating a maximum rate of replication at around that temperature. Infected CHSE-214 cells showed CPE similar to that in RTgill-W1 cells, and the virus replicated at a slower rate, but the final titre was higher (data not shown). No CPE was observed in BF-2, EPC or SHK-1 cells inoculated with virus and incubated for 1 week.

Inactivation studies

Treatment with chloroform reduced the infectivity titre from 10⁴–⁴⁴ TCID₅₀ ml⁻¹ to below the detection limit of 10⁻⁷ TCID₅₀ ml⁻¹. The effect of temperature on virus stability revealed complete inactivation after 5 min at 56 °C or 12 h at 37 °C. The titre was reduced by approximately 1 and 3 log units after 77 days at 5 and 14 °C, respectively. Furthermore, the virus was stable in the pH range 5–11, but was partially inactivated at pH 4 and completely inactivated at pH 3–5 and 11–6. No reduced infectivity was observed after 10 freeze/thaw cycles (−70°C/20 °C).

Isopycnic density

A weak opalescent band was observed after centrifugation in the CsCl gradient, corresponding to peak haemagglutinating activity at a density of 1.18–1.19 g ml⁻¹ (Fig. 4). In later centrifugations, the band was frequently divided into two bands separated by approximately 1 mm. The sucrose gradient centrifugation gave essentially the same results (data not shown) although the virus band was less distinct.

Gel electrophoresis of viral polypeptides

SDS-PAGE analysis of virus purified by sucrose gradient centrifugation revealed five major structural polypeptides with estimated molecular masses of 70, 62, 60, 48 and 37 kDa (Fig. 5). For verification of viral origin, the purified...
virus preparations were examined in the electron microscope. Only virus particles were seen and no contaminating cellular material was found.

**Electron microscopy of negatively stained virus preparations**

Examination revealed spherical or pleomorphic particles covered with fine surface projections, about 10 nm in length, representing viral glycoprotein spikes (Fig. 6a). The diameter of the majority of the virions was within a range of 150–300 nm although some as large as 1200 nm were observed. In Fig. 6(b) the upper right portion of the virion has burst, allowing the escape of nucleocapsids exhibiting a typical herringbone pattern with a diameter of approximately 17 nm. These typical nucleocapsids were also seen in Triton X-100-lysed virus preparations (Fig. 6c).

**DISCUSSION**

In the present study a previously unknown virus was isolated in RTgill-W1 cells inoculated with gill tissue material from Atlantic salmon showing respiratory distress. The virus meets the criteria for inclusion in the family Paramyxoviridae (Berkaloff, 1963; Compans et al., 1966; Howe et al., 1967; Nakai et al., 1969; Darlington et al., 1970; Norrby et al., 1970; Lamb et al., 2000; Lamb & Kolakofsky, 2001). These include the morphology of the virion, virion surface spikes, nucleocapsids with a typical herringbone pattern, inclusions, altered plasma membrane and viral budding, as seen by electron microscopy. This was further supported by functional and biochemical properties, including lack of inhibition by Br-dU and IDU indicating an RNA genome, haemagglutinating and neuraminidase activity and the syncytium formation in cell cultures suggesting the presence of fusion activity.
Structural changes were observed in the cytoplasm and plasma membrane, but not in the nucleus, indicating replication in the cytoplasm only. Nucleocapsid diameter of approximately 17 nm in virions studied by negative staining and in thin sections is within the size range (13–18 nm) for members of the Paramyxoviridae (Lamb et al., 2000; Lamb & Kolakofsky, 2001). Furthermore, the typical herringbone structure of the nucleocapsid of paramyxoviruses was also demonstrated by negative staining. The varying diameter of filaments in inclusions and beneath the plasma membrane may reflect different developmental states of the nucleocapsid. The inclusions formed within 7 days. Their purple colour was due to binding of both haemalum and eosin and demonstrated the presence of anionic and cationic groups, respectively, the former being stained with haemalum and eosin and demonstrating the presence of anionic and cationic groups, respectively, the former including phosphates (Prentø et al., 2000) and the latter occurring in proteins (Prentø et al., 1985). This is consistent with the presence of RNA and proteins, among them phosphoprotein, in the nucleocapsid (Lamb et al., 2000; Lamb & Kolakofsky, 2001). Inclusions may indicate an imbalance between assembly of the nucleocapsid and budding of the virions (Compans et al., 1966). The nucleocapsid of inclusions with loose and tight packaging had similarities to the granular and smooth forms, described in cells persistently infected with Newcastle disease virus (McNulty et al., 1977).

The virus acquires an envelope when it is assembled and released by budding through areas of the plasma membrane that have been modified by alignment of nucleocapsids on the cytosolic side and insertion of macromolecules visible as spikes on the outside. The spike length of approximately 10 nm was within the 8–12 nm size range of paramyxovirus outer projections and the attachment to erythrocytes was consistent with the presence of haemagglutinins among the envelope glycoproteins (Lamb et al., 2000; Lamb & Kolakofsky, 2001). The detection of haemadsorption long before CPE showed that incorporation of haemagglutinin glycoproteins into the plasma membrane is an early event, which has also been observed after infection with parainfluenza virus (Fedova & Zelenkova, 1969). The receptor-destroying enzyme activity, demonstrated by the elution of erythrocytes in the haemagglutination and the haemadsorption assays, was identified as a neuraminidase, which is consistent with the presence of this enzyme in most paramyxoviruses (Lamb et al., 2000; Lamb & Kolakofsky, 2001). Failure of Newcastle disease virus to re-agglutinate erythrocytes eluted for virus further substantiated the relationship with the paramyxoviruses. Finally, the detection of syncytia in infected cell cultures strongly indicated the presence of fusion activity in the virion. Hence, this new virus has all three surface activities typical of most paramyxoviruses and orthomyxoviruses including haemagglutinating, receptor-destroying (neuraminidase) and fusion activities.

The apparent loosening of the nucleocapsid from the inner envelope of released virions has also been observed in other viruses of this family and has been interpreted as part of the maturation process (Kim et al., 1979; Bächli, 1980; Markwell & Fox, 1980). Whilst the data on virion size in cell cultures are presumably not representative, due to loss of released particles during processing for electron microscopy, in negatively stained preparations the majority of virions were pleomorphic or spherical with a diameter of 150–300 nm, which is in agreement with the size described for paramyxoviruses in general (Lamb et al., 2000; Lamb & Kolakofsky, 2001). The slow replication rate of this virus compared with several other fish viruses, for example infectious salmon anaemia virus in the same temperature range (Dannevig et al., 1995), is consistent with the slow replication rate of at least some paramyxoviruses (Choppin, 1964).

The paramyxovirus-like viruses isolated from chinook salmon (Winton et al., 1985) and carp (Body et al., 2000) have optimal temperatures of approximately 18 and 21 °C, respectively, for in vitro replication, while the results presented here indicated a lower optimal temperature for the Atlantic salmon paramyxovirus. The optimal replication temperatures of all these viruses suggest that their host ranges are confined to cold-blooded animals. The majority of viruses accepted as members of the Paramyxoviridae infect warm-blooded animals or man (Lamb et al., 2000; Lamb & Kolakofsky, 2001), but a virus infecting snakes has also been assigned to the family (Clark et al., 1979; Ahne et al., 1999; Lamb et al., 2000). The emergence of these new viruses from fish and reptiles provides opportunities to expand our knowledge of the evolution, epidemiology and pathogenesis of paramyxoviruses.

We do not know how long the Atlantic salmon paramyxovirus has been present in Norwegian aquaculture, as the extended length of time required to produce CPE on initial isolation may have allowed the virus to go undetected until now. However, gill diseases have for years been a problem in Norwegian aquaculture and the importance of this new virus as a possible aetiological agent should be further elucidated.

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