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INFECTIOUS SALMON ANAEMIA IN SALT WATER ATLANTIC SALMON (*SALMO SALAR* L.) IN NEW BRUNSWICK, CANADA

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Abstract

Infectious Salmon Anaemia was reported in New Brunswick in September 1997. The virus was isolated from several sites by culture on SHK-1 cells and confirmed by monoclonal indirect fluorescent antibody technique. Gross examination revealed branchial pallor, exophthalmos, external ventral petechial haemorrhage, ascites and congested pyloric caecae, lower intestines and spleens. Microscopic examination was characterised by renal interstitial haemorrhage and tubular necrosis, branchial lamellar and filamental congestion and congestion of the intestine and pyloric caecae. Perivascular inflammation was a common finding in the livers examined. The lesions in the kidney were consistent with Haemorrhagic Kidney Syndrome and have been reproduced experimentally. This is the first case report of infectious salmon anaemia in New Brunswick.

Introduction

Infectious salmon anaemia (ISA) is an infectious viral disease of salt water farmed Atlantic Salmon (*Salmo salar*) first identified in Norway in the autumn of 1984 (Thorud and Djupvik, 1988). It is characterised externally by exophthalmia and pale gills. Internal findings may include ascites, congestion and enlargement of the liver and spleen, congestion of the foregut and petechiae in the visceral and subperitoneal fat (Thorud and Djupvik, 1988). Microscopic examination generally reveals haemorrhagic liver necrosis and congestion of the spleen, kidney and foregut (Evensen *et al.*, 1991).

The infectious nature of the disease was demonstrated in 1988 by intraperitoneal injection of liver homogenate from diseased fish and also by cohabitation experiments (Thorud and Djupvik, 1988). The etiologic agent of ISA was shown to be an enveloped virus, 100 to 130 nm in diameter. This virus replicates in endothelial cells (Hovland *et al.*, 1994; Nylund *et al.*, 1995), endocardium and leucocytes (Nylund *et al.*, 1996). A long term cell line (SHK-1) supporting propagation of ISA virus was established in 1995 (Dannevig *et al.*, 1995) and monoclonal antibodies against the ISA virus were developed for diagnostic purposes (Falk and Dannevig, 1995).

This report describes the clinical and histopathological features of ISA virus infection in salt water farmed Atlantic salmon in the Bay of Fundy, New Brunswick, Canada in the late summer of 1997.

Materials and Methods

Samples of moribund fish from a salt water cage site were submitted to the New Brunswick Department of Fisheries and

Legend to figures (opposite)

Fig 1. Gill filaments of a post-smolt affected with HKS/ISAV. (VC) - arrow indicates vascular congestion of the central filamental sinus. Bar = 96 µm. **Fig 2**. Liver of a post-smolt affected with HKS/ISAV. (H) - arrow indicates haemorrhage within (N) the necrotic parenchyma. (SC) - arrow indicates areas of sinusoidal congestion and peliosis. Bar = 96 µm. **Fig 3**. Pyloric caecae and exocrine pancreas of a post-smolt affected with HKS/ISAV. (VC) - arrow indicates congestion of lamina propria and mesenteric vasculature. Bar = 96 microns. **Fig 4**. Trunk kidney of post-smolt affected with HKS/ISAV. (H) - arrow indicates region of interstitial haemorrhage. (TN) - indicates necrotic renal tubules with eosinophilic casts. Bar = 96µm

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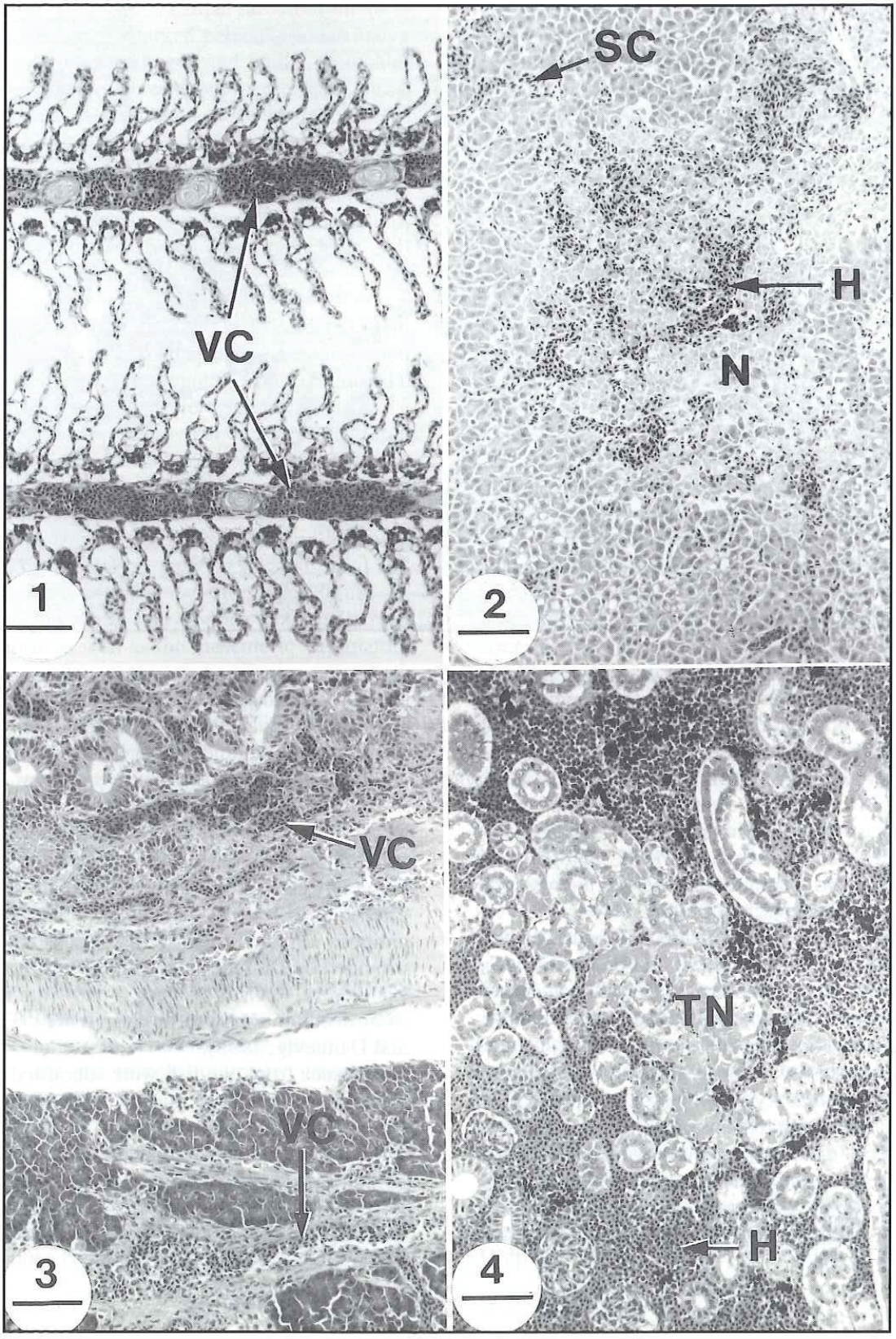
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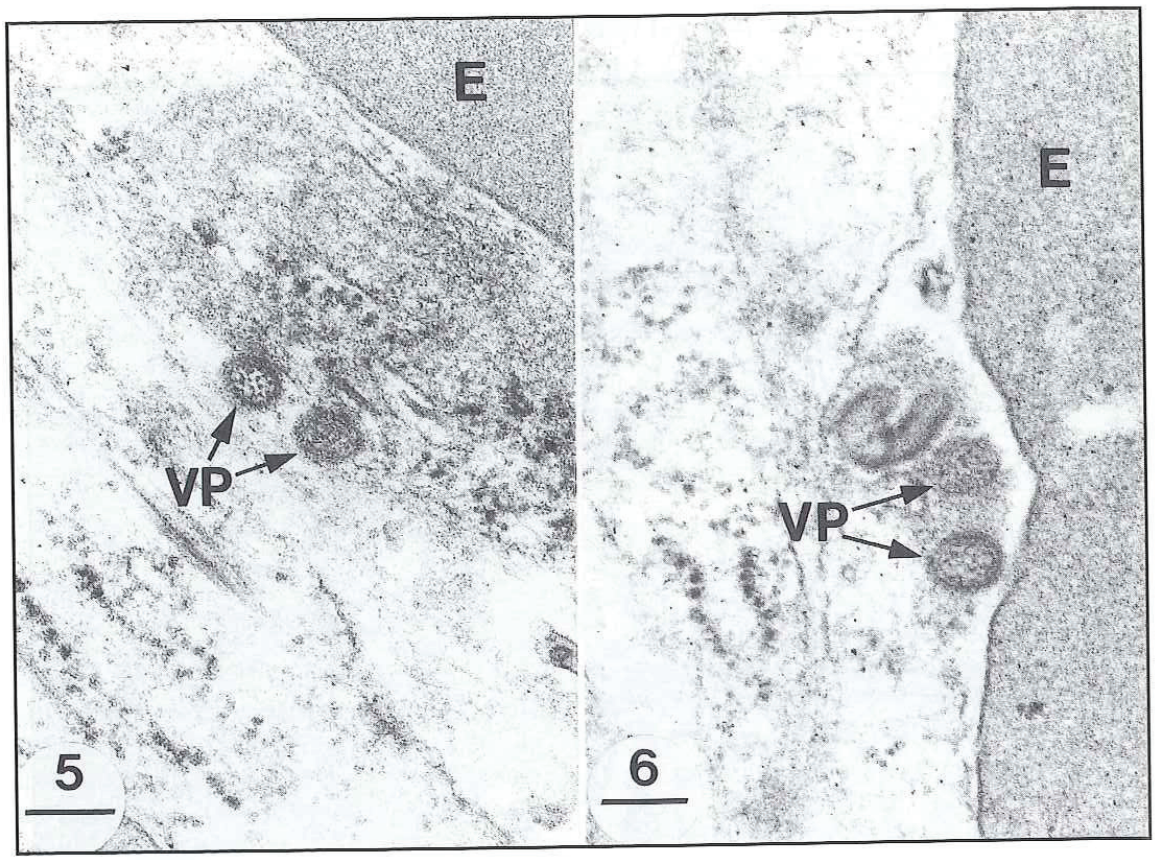

Fig 5. Transmission electron micrograph of lamellar endothelial cell and adjacent (E) erythrocyte. (VP) - arrows indicate ISA virus particles within cytoplasm. Bar = 0.156 µm.
Fig 6. Transmission electron micrograph of lamellar endothelial cell and (E) erythrocyte. (VP) - arrows indicate ISA virus particles located within lamellar capillary lumen. Bar = 0.156 µm.

Aquaculture Fish Health Laboratory, for examination on July 29, 1997. A total of 10 fish were examined. These fish entered salt water in the spring of 1997.

Portions of liver, kidney, gill, heart, spleen, intestine, pyloric caecae and body wall were placed in 10% neutral buffered formalin for 24-36 h. Tissue samples were dehydrated in ethanol, embedded in paraffin wax and sectioned at 5 µm. Sections were stained with haematoxylin and eosin. Eight fish were examined microscopically.

Pieces of kidney, spleen, pyloric caecae and gill were submitted on ice to the Atlantic Veterinary College for culture on SHK-1 cell lines following the protocol of Dannevig *et al.* (1995). These tissues were frozen at -80°C for approximately 12 weeks prior to culture. Cell cultures exhibiting cytopathic effects (CPE) were stained by monoclonal antibody directed against ISA virus, kindly provided by Dr. Nut Falk, Central Veterinary Laboratory, Oslo, using an indirect fluorescent antibody technique (IFAT) (Falk and Dannevig, 1995).

Gill tissues from one fish were submitted for transmission electron microscopy. Tissues were fixed in gluteraldehyde and 1 % osmium tetroxide, embedded in Epon Araldite and sectioned at 80 nm. Sections were stained with uranyl acetate and lead Sato. Grids were examined on a Hitachi 600 TEM.

Results

Gross findings: Unilateral to bilateral exophthalmos, marked petechial to suffusive ventral haemorrhage and mild branchial pallor were prominent external signs. Internally, the fish had moderate amounts of sero-sanguinous to haemorrhagic peritoneal fluid, congested lower intestines, pyloric caecae and spleens. Liver congestion was rarely observed, as were intra muscular, perivisceral and peritoneal petechiae. All fish were off feed.

Histopathology: Gills of affected fish showed, in most instances, severe congestion of the central filamental sinus (Figure 1). Hepatic lesions were characterised by a variety of morphological changes, including one or a combination of the following; vascular inflammation and cuffing by a mixed leucocyte population, multifocal to diffuse sinusoidal congestion and peliosis, and multifocal coagulative necrosis with and without associated haemorrhage (Figure 2). The intestinal and pyloric caecal lamina propria as well as the mesenteric vasculature were often congested (Figure 3), with degeneration and sloughing at the apical region of mucosal villi evident in severely affected fish. The spleen in all fish exhibited moderate to severe sinusoidal congestion with erythrophagia. Trunk kidney lesions were characterised by either or both acute tubular epithelial necrosis with eosinophilic casting and moderate, regional sinusoidal congestion and interstitial haemorrhage (Figure 4).

Cell culture: Cytopathic effects were noted on SHK-1 cell lines at day 4 post inoculation. Infectious salmon anaemia virus was detected in cell culture by monoclonal IFAT.

Electron Microscopy: Examination of thin sections from gill lamellae revealed the presence of virions, located both intracellularly within respiratory endothelium (Figure 5) and extracellularly in the lamellar capillaries (Figure 6). Virus particles measured approximately 95-125 nm in diameter, and showed morphological details consistent with an enveloped virus having a segmented genome. These findings were consistent to those previously reported for the orthomyxovirus identified as the causative agent of Infectious Salmon Anaemia in Norway (Nylund *et al.*, 1996, Koren and Nylund, 1997).

Discussion

Infectious salmon anaemia was first reported in marine farmed Atlantic salmon in the Bay of Fundy, New Brunswick in September, 1997 (Griffiths pers. commun). This was the first documented occurrence of ISA outside Norway. Since then, cell lysates from tissue samples collected from numerous sites have been confirmed positive for ISA virus by several techniques at different laboratories.

Gross examination of fish infected with ISA in New Brunswick in the summer of 1997 appeared somewhat similar to reports from Norway (Thorud and Djupvik, 1988; Evensen *et al.*, 1991). Gill pallor, ascites and congestion of the pyloric caecae and lower intestine were fairly consistent findings in both countries. However, in the New Brunswick fish, gross liver congestion was a rare finding, not a common finding as described in the literature (Thorud and Djupvik, 1988; Evensen *et al.*, 1991). Interestingly, over the past few months gross hepatic congestion of ISA virus infected fish has become a more common finding in the New Brunswick Department of Fisheries and Aquaculture laboratory. Similarly, histological liver lesions in the New Brunswick fish differed to those described for fish infected with ISA virus in Norway. Liver lesions in the New Brunswick fish were quite variable and ranged from vascular inflammation or sinusoidal congestion to multifocal coagulative necrosis with or without interstitial haemorrhage whereas, the most characteristic microscopic lesion of ISA virus infection in Norway is hepatic zonal haemorrhagic necrosis (Thorud and Djupvik, 1988; Evensen *et al.*, 1991).

Microscopically, ISA virus infection in New Brunswick is characterised by a variety of lesions in different organs. The histological findings of congestion of pyloric caecae, gills and intestine are consistent with Norwegian studies (Thorud and Djupvik, 1988; Evensen *et al.*, 1991) and are supported by the recent ultra structural findings of ISA virus replication in endothelial cells, endocardium and leucocytes (Hovland *et al.*, 1994; Nylund *et al.*, 1995, Nylund *et al.* 1996). Trunk kidney lesions are consistent with those described for Haemorrhagic Kidney Syndrome (HKS) (Byrne *et al.*, 1998) and with renal lesions described in an earlier Norwegian experimental study of ISA virus infection (Hovland, 1995). This contradicts the earlier assumption that HKS is a separate disease from ISA. Additionally, recent archival reviews of Norwegian case submissions support the presence of renal lesions similar to those described in New Brunswick (T. Poppe, pers. commun) and most importantly, the histological features of HKS can be reproduced using either tissue homogenates from New Brunswick salmon infected with ISAV or ISAV laden tissue culture filtrates alone (Jones, S. and MacKinnon, A-M, pers. comm.). Similar work to confirm this finding is currently on-going at both federal and university laboratories in Atlantic Canada.

It is clear from results of 18 months of intensive disease survey work in the Bay of Fundy, that the clinical and pathological presentation of the disease has changed since the initial outbreak in the summer of 1996. Most significant has been the development of hepatic, branchial and enteric lesions typical of ISA, in addition to the pathognomonic kidney lesions first described for HKS (Byrne *et al.*, 1998). The differences in gross and microscopic presentation of ISA virus in New Brunswick compared to Norway may be a result of the interplay of many factors such as; Atlantic salmon Stock, viral strain, a concomitant infection, water temperature, infectious dose, age and/or immune status of the fish. At this juncture only the answer to this question would be speculative. In this regard it is imperative that additional research be undertaken to establish the exact nature of the ISA outbreak in New Brunswick. It would also be interesting to compare the genomic sequences of Canadian, Norwegian and Scottish ISA viruses.

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