Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01005 **Date** 16-Mar-2006 **Report** 27-Mar-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16070 #5617, Marine Harvest PO CL9210
Farm:
Vet Clinic:
Attending Dr. Diane Morrison

Specimen: Tissue - Fresh **Count** **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Log No. 5617. Date: March 15/06 PO #CL9210.
Sample size: one sample for virology PCR
Sex: regular
Saltwater
One fish from Pen 8 showing signs of VHS. Please run PCR for VHS and IHN, and tissue culture. Sample previously frozen.

Virology

Viral Hemorrhagic Septicemia virus positive.
VHS-like CPE on EPC and SSN cell cultures.

Molecular Diagnostics/PCR

Sample 5617 - 1 org: VHSV Positive by PCR and IHNV Negative by PCR.

* Results faxed on Mar 27/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01006 **Date** 16-Mar-2006 **Report** 27-Mar-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16071 #5624, Marine Harvest PO CL9211,
Farm:
Vet Clinic:
Attending Dr. Diane Morrison

Specimen: Tissue - Fresh **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Log No. 5624. Date: March 15/06. PO #CL9211.
Sample size: 1 sample for virology
Sex: regular
Saltwater
One fish with petechial hem of the swim bladder and pyloric ceca. Please run PCR for IHNV and VHSV and tissue culture. Sample previously frozen.

Virology

Fish viruses negative by tissue culture.

* Results faxed Apr. 7/06.

Molecular Diagnostics/PCR

Sample: 5624 -1 org - IHNV and VHSV Negative by PCR.

* Results faxed on Mar 27/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01007 **Date** 16-Mar-2006 **Report** 27-Mar-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16072 #5626, PO #CL9212
Farm:
Vet Clinic:
Attending Dr. Diane Morrison

Specimen: Tissue - Fresh **Count** 3 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Log No. 5626. PO #CL9212
Date: March 15/06
Sample size: 3 samples
Sex: regular
Saltwater entry: 2004
Saltwater
Three samples for PCR for IHNV and VHS and tissue culture. 2/3 fish with severe hem of liver and/or swim bladder and/or pyloric caeca. Sample previously frozen.

Virology

3 samples inoculated onto tissue culture - all negative.

* Results faxed Apr. 7/06.

Molecular Diagnostics/PCR

Samples 5626-2 org; 5626-3 org; 5626-4 org: IHNV and VHSV Negative by PCR.

* Results faxed on Mar 27/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01148 **Date** 24-Mar-2006 **Report** 31-Mar-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16161 #5626, Marine Harvest PO CL9223
Farm:
Vet Clinic:
Attending Dr. Dianne Morrison

Specimen: Blood **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

PO #CL9223. Atlantic salmon. Sex: regular. Saltwater. Once vacutainer with blood removed from swim bladder of moribund fish. Smear revealed rickettsia (piscirickettsia) like organisms. Please run PCR for Piscirickettsia if possible.

Molecular Diagnostics/PCR

Whole blood, plasma, and buffy coat: Piscirickettsia salmonis Negative by PCR.

* Results faxed on Mar 31/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01149 **Date** 24-Mar-2006 **Report** 27-Mar-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16163 #5635, Marine Harvest PO #CL9222
Farm:
Vet Clinic:
Attending Dr. Diane Morrison

Specimen: Tissue-Fresh f Forma **Count** **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-01149 **Case Report**

History/Symptoms

PO #CL9222. Atlantic salmon. Sex: regular. Saltwater entry: 2006 S1. Sample size: 2 histo and 1 virology.

Two moribund Atl fish for histology. Fish #2 with bilateral exophthalmos and hemorrhage of swim bladder. Tissues collected for virology and PCR for IHNV and VHSv. Histo collected on Mar 22/06. Shipped with water. Viral collected on March 22/06 and shipped frozen. No growth on blood agar nor TSA.

Histopathology

Two cassettes of tissues were submitted for histopathology and processed routinely into paraffin.

Slide 1 (5635-1): liver, spleen, heart, head kidney, trunk kidney, intestinal ceca and mesenteric fat.
Slide 2 (5635-2): liver, spleen, heart, head kidney, trunk kidney, stomach, intestinal ceca and mesenteric fat.

All major organs on each slide were examined. Organs not listed below have no significant lesions, or the organ was not present for analysis.
Quality control: Tissue preservation is excellent for all organs. The margin of the liver in slide 1 has 3 foci, each 1 - 1.5 mm long, that are slightly compressed and stain poorly (i.e., hepatocyte cytoplasm stains pale blue instead of red, and erythrocyte cytoplasm stains yellow instead of red); features are about the same as postfixation dehydration, but the foci are about the size of the ends of forceps and not as diffuse as would be expected with postfixation dehydration. Other tissues (e.g., spleen, heart, and kidney) have small foci of postfixation dehydration. The sections have no acid hematin deposits.

Measures of physiologic condition:
1. Hepatocellular glycogen: none (slides 1, 2)
2. Mesenteric fat: moderate (slide 1), abundant (slide 2)

These patterns in the measures of physiologic condition are consistent with fish that were growing but stopped feeding normally in the past few (slide 2) to many (slide 1) days.

Virology

1 sample inoculated onto tissue culture - viruses negative.

Molecular Diagnostics/PCR

VHSV and IHNV Negative by PCR.

* Results faxed on Mar 31/06.

Submission 2006-01149 **Case Report**

Diagnosis

1. Spleen, intestine, and mesenteric fat: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles 50 - 300 µm in diameter, mild (slide 2), severe (slide 1)
2a. Trunk kidney: renal tubular epithelial necrosis, multifocal, with interstitial fibrosis and tubular regeneration, subacute, mild (slide 2)
2b. Trunk kidney: renal tubular mineralization, focal, mild (slide 2)
3a. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild (slide 2)
3b. Heart: endocarditis, diffuse, with endothelial cell hypertrophy, moderate (slide 2)
4a. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 2)
4b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 2)

Final Comments

Peritonitis is the most severe lesion in the fish in slide 1. By comparison, the fish in slide 2 has several systemic changes. Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared. Fibrosis is evidence of more extensive damage and efforts at repair. Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin). I have also seen this change in salmon exposed to high concentrations of chromium (Farag et al. 2006).

Renal mineralization is common in cultured fish species. The pathogenesis is not fully understood, but renal mineralization has been experimentally reproduced through high carbon dioxide levels, magnesium deficiency, selenium toxicity, and a diet low in minerals (source, "Systemic Pathology of Fish", 1989, by H. Ferguson). Clinically, renal mineralization is most commonly associated with high carbon dioxide levels.

In slide 2, epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. The pattern of endocardial inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Hypertrophic endothelial cells are basophilic and up to 10 µm thick.

Lymphocytic inflammation around bile ductules (liver) is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system. Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other salmon it might be related to increased protein needed as part of an inflammatory response.

Literature cited:
Farag, A. M., T. May, G. D. Marty, M. Easton, D. D. Harper, E. E. Little, and L. Cleveland. 2006. The effect of chronic chromium exposure on the health of juvenile Chinook salmon (Oncorhynchus tshawytscha). Aquatic Toxicology. In Press.

/sr/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01214 **Date** 29-Mar-2006 **Report** 03-Apr-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16201 #5644, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue-Fresh f Forma **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-01214

Case Report

History/Symptoms

4 samples for routine virology (tissue culture only - no PCR needed) and 2 histo labelled 28A and 27A.

Routine follow up to a report of one fish with mild internal hemorrhage. These fish bore no visible lesions upon gross examination.

Atlantic/Regular/R27, R28

Histopathology

Two cassettes of formalin-fixed tissues were submitted for histopathology.

Slide 1 (5644-28A) - liver, trunk kidney, heart, spleen, intestine, swimbladder, intestinal ceca, mesenteric fat, and exocrine pancreas
Slide 2 (5644-R27A) - gill, liver, trunk kidney, head kidney, heart, spleen, intestine
All organs were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is excellent for all organs. Organs have no postfixation dehydration and no significant deposits of acid hematin.

Measures of physiologic condition
Hepatocellular glycogen: none (slide 1), moderate (slide 2)
Mesenteric adipose tissue: abundant (slides 1, 2)
These patterns in the measures of physiologic condition are consistent with healthy growing fish, although the fish in slide 1 might not have eaten as recently as the fish in slide 2 (i.e., inferred from differences in hepatocellular glycogen).

Virology

4 samples inoculated onto tissue culture - all viruses negative.

* Results faxed on Apr 26/06.

Molecular Diagnostics/PCR

Infectious Hematopoietic Necrosis Virus negative by PCR.
Viral Hemorrhagic Septicemia Virus negative by PCR.

Diagnosis

1. Liver: pericholangitis, lymphocytic, focal, mild (slide 1)
2. Spleen, intestine, and mesenteric fat: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate (slide 1) and 2 granulomas 150-500 µm in diameter (slide 2)

Final Comments

Other than granulomatous inflammation associated with a vaccine reaction, the microscopic findings in these fish are minor; consistent with the gross findings.

Lymphocytic inflammation around a single bile ductule (liver) is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system.

/bb/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01329 **Date** 06-Apr-2006 **Report** 13-Apr-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16263 #5648, Marine Harvest (PO CL9235)
Farm:
Vet Clinic:
Attending Dr. Diane Morrison

Specimen: Tissue - Fresh **Count** 2 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Collected March 29/06
Species: Atlantic
Saltwater entry: 2005
Saltwater
Sample size: 2 virology
Two samples for virology. PCR for IHNV and VHSV. Samples are labelled 5648-1 and 5648-2.

Virology

2 samples inoculated onto tissue culture - both viruses negative.

* Results faxed on May 1/06.

Molecular Diagnostics/PCR

Samples 1 and 2: IHNV and VHSV Negative by PCR.

* Results faxed on Apr 12/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01330 **Date** 06-Apr-2006 **Report** 10-Apr-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16264 #5652, Marine Harvest (PO CL9232)
Farm:
Vet Clinic:
Attending Dr. Diane Morrison

Specimen: Tissue-Fresh f Forma **Count** 8 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-01330

Case Report

History/Symptoms

Date: April 5/06
Species: Atlantic
Saltwater entry: 2006
Netpen/tank ID: all
Saltwater
Sample size: 4 virology and 4 histology
Four samples for virology, PCR for VHSV and IHNV. Samples are pooled fish and are labelled as 5652 (1-2); 5662 (3-5); 5652 (6-7) and 5652 (8-9). Four histo samples collected - labelled 1 through 4. No growth on bacteriology. Mortality rate on the increase. Fish #2 with hem. of viscera.

Histopathology

Four cassettes of formalin-fixed tissues were submitted for histopathology.

Slide 1 (5652-1) - liver, trunk kidney, head kidney, heart, spleen, skeletal muscle, 1.1mm diameter nerve (optic nerve?), stomach, intestinal ceca, mesenteric fat, and exocrine pancreas
Slide 2 (5652-2) - liver, trunk kidney, head kidney, heart, spleen, swimbladder(?), stomach, intestinal ceca, mesenteric fat, and exocrine pancreas
Slide 3 (5652-3) - liver, trunk kidney, head kidney, heart, spleen, skeletal muscle, eye, optic nerve, stomach, intestinal ceca, mesenteric fat, and exocrine pancreas
Slide 4 (5652-4) - liver, trunk kidney, head kidney, heart, spleen, skeletal muscle, intestinal ceca, mesenteric fat, and exocrine pancreas
Organs not listed below have no significant lesions.

Quality control: Tissue preservation is excellent for all organs except some sections of intestinal ceca, which have mild autolysis on the tips of the villi (this is common for immersion-fixed intestines). Organs have no postfixation dehydration and no significant deposits of acid hematin.

Measures of physiologic condition
Hepatocellular glycogen: none (slides 1, 2, 3, 4)
Mesenteric adipose tissue: abundant (slides 1, 2, 3, 4)
These patterns in the measures of physiologic condition are consistent with healthy growing fish (mesenteric fat) that recently stopped feeding normally (lack of hepatocellular glycogen).

Virology

4 samples inoculated onto tissue culture - all viruses negative.

* Results faxed on May 1/06.

Submission 2006-01330 **Case Report**

Molecular Diagnostics/PCR

Samples 1-2, 3-5, 6-7, and 8-9 IHNV and VHSV Negative by PCR.

* Results faxed on Apr 13/06.

/mm

Diagnosis

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slides 3, 4)
2. Spleen, intestinal ceca, and mesenteric fat: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, moderate (slides 1, 2, 3, 4)
3. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 1)
4. Heart: endocarditis, diffuse, with endothelial cell hypertrophy, mild (slide 3)

Final Comments

These fish have a number of changes that are fairly common in fish dying in net pens; the only findings specific for a single cause is the peritoneal granulomatous inflammation-probably a reaction to a vaccine. Fish #2 has small foci of hemorrhage in the mesenteries, but I cannot differentiate these foci from hemorrhage associated with sampling.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Renal tubular epithelial protein droplets are normal in some species, or they may be an indication of glomerular disease. Ferguson ("Systemic Pathology of Fish," 1989) reports an association of renal protein droplets and high ammonia levels in salmonids. Roberts ("Fish Pathology, Third Edition," 2001) describes the change, but offers no cause.

The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Hypertrophic endothelial cells are basophilic and up to 10 µm thick.

/sr/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01331 **Date** 06-Apr-2006 **Report** 10-Apr-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16265 #5653, Marine Harvest (PO CL9236)
Farm:
Vet Clinic:
Attending Dr. Diane Morrison

Specimen: Tissue - Formalized **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-01331

Case Report

History/Symptoms

Date: April 5, 2006.
Species: Atlantic
Sex: regular
Saltwater entry: 2006 SI
Netpen/Tank ID: 1,5
Saltwater
Sample size: one histology cassette
On histo cassette from one larger silver with swollen kidney and no other signs of disease.

Histopathology

One cassette of formalin-fixed tissues was submitted for histopathology.

Slide 1 (5653) - liver, trunk kidney, head kidney, heart, spleen, stomach, intestinal ceca, mesenteric fat, and exocrine pancreas.
Organs not listed below have no significant lesions.

Quality control: Tissue preservation is good to excellent for all organs. Organs have no postfixation dehydration and no significant deposits of acid hematin.

Measures of physiologic condition
Hepatocellular glycogen: none
Mesenteric adipose tissue: abundant
These patterns in the measures of physiologic condition are consistent with a healthy growing fish (mesenteric fat) that recently stopped feeding normally (lack of hepatocellular glycogen).

Diagnosis

1. Trunk kidney: renal tubular epithelial necrosis, tubular regeneration, and tubular dilation, multifocal to diffuse, chronic, moderate
2. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
3. Spleen and intestinal mesenteries: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, moderate

Final Comments

The microscopic kidney changes are consistent with the gross findings. The combination of renal tubular necrosis and regeneration is evidence that this fish had ongoing exposure to nephrotoxins. These toxins could be endogenous (e.g., from viral hemorrhagic septicemia virus - VHSV - or a bacterial infection elsewhere in the body) or exogenous (e.g., from the water or the feed). Cellular inflammation is not part of the lesion in this fish.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Granulomatous peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

/sr

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01600 **Date** 26-Apr-2006 **Report** 05-May-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16422 #5659, Marine Harvest PO# CL 9245.
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 2 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted fresh tissue for virology and PCR. IHN and VHSV. Samples labeled 5659-61 and 5659-29.

Fish #29 with focal hemorrhage on liver and swim bladder.
Fish #61 was moribund with heart lesions.

PO# CL 9245.

Virology

2 samples inoculated onto tissue culture - both negative.

* Results faxed May 19/06.

Molecular Diagnostics/PCR

Samples 29 and 61: IHN and VHS Virus negative by PCR.

* Results faxed May 5/06.

/bb

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01601 **Date** 26-Apr-2006 **Report** 05-May-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16423 #5672, Marine Harvest (PO CL 9246)
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 3 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted fresh tissue for virology and PCR for IHNV and VHSV.

Fish showing lethargic behaviour. Losing 200 fish per day prior to this sample. Mortality started to decline the next day.

PO # CL 9246.

Virology

3 samples inoculated onto tissue culture - all negative.

* Results faxed May 19/06.

Molecular Diagnostics/PCR

Samples A, B and C - IHN and VHS Virus negative by PCR.

* Results faxed May 5/06.

/bb

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01683 **Date** 04-May-2006 **Report** 10-May-2006

Report Copies

Submitter: 11036 Mainstream Canada (M)
Owner 16474 Mainstream 06-12 PO 7512124 Cypress Harbour
Farm:
Vet Clinic: 15092 McKenzie, Peter (Fax: 250-286-0042)
Attending

Specimen: Tissue - Formalized **Count** **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-01683 **Case Report**

History/Symptoms

Case 06-12. Attention: Jeanine Sumner fax:250-286-0042.

Samples from Brood Site. Hem. and Internal swelling seen in mortalities.
PM lesions: Hemorrhaging, swollen kidney.
Submitted 4 histology cassettes (fixed in formalin and shipped in Ethanol).
Tests required: prepare for histo exam by Dr. Gary Marty.

Histopathology

Four full cassettes of formalin-fixed tissues were submitted in ethanol for histopathology. After the tissues were processed into paraffin, tissues from some cassettes were separated into two blocks for sectioning (A and B). Slide 3 was also stained with Twort's Gram stain.

Slide 1A (CH01) - trunk kidney (4 pieces), liver, skin and skeletal muscle, intestinal ceca and mesenteric fat
Slide 1B - gill
Slide 2A (CH02) - trunk kidney (2 pieces), liver, spleen, skin and skeletal muscle, intestinal ceca and mesenteric fat
Slide 2B - gill (3 pieces)
Slide 3 (CH03) - gill, liver (2 pieces), skin and skeletal muscle, intestine
Slide 4A (CH04) - intestine, gill, spleen, trunk kidney
Slide 4B - skin and skeletal muscle
All organs were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is fair to excellent for all tissues. The intestinal epithelium has mild autolysis (this is normal for immersion fixed tissues). Tissues have good differential staining. Organs have no postfixation dehydration and no deposits of acid hematin.

Measures of physiologic condition
Hepatocellular glycogen: none (slides 1A, 2A, 3)
Mesenteric fat: abundant (slides 1A, 4A), moderate amounts (slide 2A)

This pattern in the measures of physiologic condition is consistent with healthy growing fish (moderate to abundant mesenteric fat) that recently stopped feeding (severe glycogen depletion).

Submission 2006-01683 **Case Report**

Diagnosis

1a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slides 1A, 3)
1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 1A, 2A), moderate (slide 3)
1c. Liver: peritonitis, granulomatous, focal, with small numbers of intralesional Gram-positive bacterial rods consistent with Renibacterium salmoninarum, severe (slide 3)
1d. Liver: hepatitis, granulomatous, multifocal, with scattered multinucleate giant cells and small numbers of intralesional Gram-positive bacterial rods consistent with Renibacterium salmoninarum, severe (slide 3)
1e. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slide 3)
2. Skin and skeletal muscle: myositis and dermatitis, histiocytic, fibrinous, with plump fibroblasts, focal, mild (slide 2A), moderate (slide 1A)
3. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 2A)
4. Mesenteric fat: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, mild (slide 2A)
5a. Gill: lamellar subepithelial edema, multifocal, mild (slide 2B)
5b. Gill: branchitis, granulomatous, with multinucleate giant cells and small numbers of intralesional Gram-positive bacterial rods consistent with Renibacterium salmoninarum, multifocal, moderate (slide 3)
6. Intestine: enteritis, granulomatous, multifocal, moderate (slide 3)
7. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (slide 4A)
8. Skeletal muscle: myonecrosis, peracute, focal, mild (slide 4B)

Final Comments

These fish have several lesions that could have contributed to morbidity. The most severe is the disseminated granulomatous inflammation in fish 3 and foci of dermatitis/myositis in fish 1.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other salmon it might be related to increased protein needed as part of an inflammatory response.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

The most common cause of disseminated granulomatous inflammation (e.g., liver and intestine in slide 3) is Renibacterium salmoninarum, the cause of bacterial kidney disease. Because of the presence of multinucleate giant cells in the liver and gill, consider as a differential the co-occurrence of a vaccine reaction.

Pigment in the liver could be lipofuscin, hemosiderin, or both. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. Hemosiderin accumulation in the liver is evidence of increased turnover of red blood cells.

Inflammation in the dermis (slide 1A) is consistent with a bacterial infection and a nearby ulcer, although neither bacteria nor an ulcer are included in the 4 x 2 mm piece of skin and skeletal muscle examined. If this is a sample from a more widespread lesion, it probably is the cause of death.

Renal tubular epithelial protein droplets are normal in some species, or they may be an indication of glomerular disease. Ferguson ("Systemic Pathology of Fish," 1989) reports an association of renal protein droplets and high ammonia levels in salmonids. Roberts ("Fish Pathology, Third Edition," 2001) describes the change, but offers no cause.

Separation of gill lamellar epithelium from underlying pillar cells can be a result of edema, and it also is a common postmortem artefact. In this case, accumulation of proteinaceous fluid deep to the separated epithelium supports the diagnosis of edema over artefact. Lamellar edema is commonly associated with exposure to toxicants, including formalin and hydrogen peroxide overdose. Lamellar edema is reversible if the inciting cause is removed. The significance of the change here depends on whether the fish was alive or dead when sampled. If the fish was alive, the change is probably real; if the fish was dead, chances are better that the change is a postmortem artefact.

Granulomatous branchitis in slide 3 is evidence of chronic immune stimulation. The presence of short Gram-positive rods, including within multinucleate giant cells, is consistent with Renibacterium salmoninarum infections; the primary differential is a vaccine reaction.

Interstitial cell hyperplasia in the kidney results from increased demand for erythrocytes or white blood cells somewhere in the body (renal interstitial cells are the blood-forming or hematopoietic cells in the kidney). In Chinook salmon, this lesion is often associated with the clinical diagnosis of "Marine anemia."

Myonecrosis in this fish is limited to scattered individual fibers with flocculent cytoplasm and loss of cross striations. This pattern is consistent with capture myopathy or agonal contractions. It might also be evidence of underlying deficiency of vitamin E or selenium (reference: Fish Pathology, 3rd Edition. 2001. R.J.

Roberts).

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01701 **Date** 04-May-2006 **Report** 11-May-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16487 #5685, Marine Harvest (PO 9250)
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 2 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

Submitted two fresh tissue samples for PCR for IHN and VHS.

Samples collected on April 27, 2006 and held in freezer.

Atlantic, regular, 2006, S1, all pens, saltwater.

Virology

Fish Viruses Negative.
Tissue culture = negative.

* Results faxed on May 29/06.

Molecular Diagnostics/PCR

Samples: org 5685-1: IHN and VHS Virus negative by PCR.
Samples: org 5685-2: IHN and VHS Virus negative by PCR.

* Results faxed May 11/06.

/bb/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01758 **Date** 10-May-2006 **Report** 17-May-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16522 #5703 Marine Harvest (PO #CL9252)
Farm:
Vet Clinic:
Attending Dr. Diane Morrison

Specimen: Tissue-Fresh f Forma **Count** **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: Atlantic **Sex:** Regular

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-01758

Case Report

History/Symptoms

Species: Atlantic
Sex: Regular
Saltwater entry: 2004
Netpen/Tank ID: 4, 7
Saltwater
Sample size: 3 viral for PCR also request histology.
Comments: 3 frozen samples collected on May 6/06 for PCR-IHNV and PCR VHSV. Six histo cassettes, three with tissues and three with corresponding gills labelled W1-W3.
Three moribund fish collected during plankton bloom. No visible lesions internally. Gill smear revealed chaetoceros sp. and Dichyocha sp. Gill tissues in separate cassettes labelled W1 through W3.

Histopathology

Six cassettes of formalin-fixed tissues were submitted for histopathology in a moist wrapping. The cassettes were placed in EDTA solution overnight for mild decalcification, and then processed routinely into paraffin.

Slide 1 (W-1 5/6/06) - multiple pieces of gill
Slide 2 (W-3 5/6/06) - liver, head kidney, trunk kidney, heart, spleen, intestine (2 pieces)
Slide 3 (W-3 5/6/06) - multiple pieces of gill
Slide 4 (W-2 5/6/06) - liver, head kidney, trunk kidney, heart, spleen, intestine (3 pieces), mesenteric fat
Slide 5 (W-2 5/6/06) - multiple pieces of gill
Slide 6 (W-1 5/6/06) - liver, head kidney, trunk kidney, heart, spleen, intestinal ceca, mesenteric fat
All organs were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is good excellent for all organs, and bones in the gills are fully decalcified. The gill in slide 5 has mild autolysis. Organs have no postfixation dehydration and no significant deposits of acid hematin.

Measures of physiologic condition
Hepatocellular glycogen: none (slide 2, 4, 6)
Mesenteric adipose tissue: abundant (slides 4, 6)
This pattern in the measures of physiologic condition is consistent with healthy growing fish (abundant mesenteric fat) that recently stopped eating normally (no hepatocellular glycogen).

Virology

3 samples inoculated onto tissue culture - all negative.

* Results faxed on Jun 6/06.

Molecular Diagnostics/PCR

Specimens 1, 2, 3:
Infectious Hematopoetitic Necrosis virus Negative
Viral Haemorrhagic Septicemia virus Negative

Diagnosis

1a. Gill: lamellar hyperplasia and fusion, multifocal, moderate (slides 1, 3), with intralesional diatoms, moderate (slide 5)
1b. Gill: lamellar telangiectasis, multifocal, with intralesional thrombosis, mild (slide 3)
1c. Gill: filament fibrocartilage-bony callus, multifocal, mild to moderate (slide 3)
2a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 2), moderate (slide 6), severe (slide 4)
2b. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, (slide 2)
2c. Liver: sinusoidal fibrin, multifocal, acute, mild (slide 2)
2d. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 4, 6)
2e. Liver: hepatic necrosis, acute, multifocal, moderate (slide 6)
3a. Spleen: reticular eosinophilic deposits, with congestion, moderate (slide 4)
3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 6)

Final Comments

These fish have lesions consistent with exposure to diatoms followed by secondary bacterial infection.

Gill lamellar fusion with lamellar hypertrophy may be a result of physical damage from exposure to a parasite or diatoms; lack of organisms in slides 1 and 3 might be a result of superficial organisms being lost during processing of the tissues to a slide. [Organisms in slide 5 are not common, but can be found using the coordinates 37.5 × 113.5 on the stage of the Nikon Eclipse 50i microscope in my office (Animal Health Centre, room 106); the coordinates will work when the slide is mounted on the stage with the white part of the slide on the left.] In slide 1, hyperplasia is mostly associated with lamellae fused at the tips; this provides evidence that the fusion occurred first, followed by hyperplasia. In slides 3 and 5, fusion occurs at various parts of the lamellae (base and tip). Telangiectasis in the gill most commonly results from trauma (e.g., handling). The irregular focus of bony fibrocartilage (callus) is evidence a healing microfracture in the affected filament. In slide 3, only one filament is moderately affected, but several other filaments are mildly affected.

The diatoms Chaetoceros concavicornis, C. convolutus, and a Corethron sp. have been associated with mortality of salmon reared in seawater netpens. Mortality due to Chaetoceros can occur within a few days of exposure, as a result of physical damage to the gills and the resultant inflammatory response. Larger fish tend to be more susceptible than small fish. Characteristic gill lesions include mucous cell hyperplasia, lamellar epithelial cell hyperplasia and necrosis, and variable numbers of neutrophils. Diatoms and their processes are on the surface of the gill epithelium and sometimes entrapped by multinucleate giant cells. [Source: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.]

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Pigment in the liver could be lipofuscin, hemosiderin, or both. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. Hemosiderin accumulation in the liver is evidence of increased turnover of red blood cells.

Multifocal fibrin deposits in the liver are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin. I have also seen this response in fish that are PCR positive for VHSV. Lack of remodelling of the fibrin is consistent with these deposits forming just before death.

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins) or come from outside the fish (e.g., from the water or the feed).

Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, Gram negative bacteria). This case has no obvious organisms. Consider bacteriology if not already done. Proliferative lesions in the biliary system are evidence for a chronic toxic cause for the hepatic necrosis.

Reticular eosinophilic deposits in the spleen (slide 4) probably are plasma that has separated from erythrocytes. The pale staining of the deposits differentiates it from the primary differential, sinusoidal fibrin, which would be more intensely eosinophilic. The deposits might be part of an acute inflammatory response.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been

vaccinated.

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01759 **Date** 10-May-2006 **Report** 16-May-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16523 #5702, Marine Harvest (PO #CL9253)
Farm:
Vet Clinic:
Attending Dr. Diane Morrison

Specimen: Tissue - Fresh **Count** **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: Atlantic **Sex:** Regular

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Species: Atlantic
Saltwater entry: 2005 S1
Sample size: 2 virology
Two fish sampled for virology - PCR for IHNV and VHSV.

Virology

2 samples inoculated onto tissue culture - both negative.

* Results faxed on June 6/06.

Molecular Diagnostics/PCR

Specimens: Pen 4 and Brood - IHNV and VHSV Negative by PCR.

* Results faxed on May 16/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01760 **Date** 10-May-2006 **Report** 16-May-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16524 #5697, Marine Harvest (PO #CL9254)
Farm:
Vet Clinic:
Attending Dr. Diane Morrison

Specimen: Tissue - Fresh **Count** 8 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: Atlantic **Sex:** Regular

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Species: Atlantic
Saltwater entry: 2006
Freshwater
Sample size: 8 frozen fish for virology
Comments: 8 pooled samples (of 3 fish each) were collected on May 3rd and frozen for virology - PCR for IHNV and VHSV prior to shipment.

Virology

8 samples inoculated onto tissue culture - all negative.

* Results faxed on Jun 6/06.

Molecular Diagnostics/PCR

Specimens 1, 2, 3, 4, 5, 6, 7, 8 IHNV and VHSV Negative by PCR.

* Results faxed on May 16/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01840 **Date** 16-May-2006 **Report** 26-May-2006

Report Copies

Submitter: 11036 Mainstream Canada (M)
Owner 16583 #6778, Mainstream PO #6778
Farm:
Vet Clinic:
Attending

Specimen: Multiple Specimens **Count** 6 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

Submitted cassettes and 6 whirl paks. Please do histology on preserved tissues: green - 04/05 yc, yellow -03 yc. Please PCR tissues in Whirl paks for Piscirickettsia salmonis - 6 samples total.

PO# 6778.

Histopathology

Nine cassettes of formalin-fixed liver and trunk kidney were submitted for histopathology. Slides 1-3 were derived from green cassettes (= 04/05 year class); slide 4-9, yellow cassettes (= 03 year class).
All organs were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation for liver is poor (i.e., severe autolysis); autolysis in the kidney varies from moderate to severe. Organs have no postfixation dehydration and no significant deposits of acid hematin. Differential staining is good.

Molecular Diagnostics/PCR

Samples: Cypress 04yc-1,2,3 and samples Cypress 05yc-1,2,3: Piscirickettsia salmonis Negative by PCR.

Diagnosis

1a. Liver: hepatitis, granulomatous, focal, moderate (probable Renibacterium salmoninarum; slides 4, 6); multifocal, severe, with central necrosis (probable Renibacterium salmoninarum; slides 5, 7, 8)
1b. Liver: sinusoidal fibrin, multifocal, acute, mild (slides 1, 4, 5), moderate (slide 6)
1c. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slides 1, 2, 3, 6)
2a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, moderate (slide 5), severe (slides 6, 7, 8, 9)
2b. Trunk Kidney: glomerulonephritis, membranous, diffuse, moderate (slide 3)
2c. Trunk kidney: renal tubular casts of yellow-brown pigment, multifocal, mild (slides 3, 4, 6, 8), moderate (slide 9)
3a. Spleen: splenitis, granulomatous, multifocal, moderate ((probable Renibacterium salmoninarum; slide 9)
3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 9)

Submission 2006-01840 **Case Report**

Final Comments

Granulomatous inflammation in the liver, kidney, and spleen is consistent with a chronic bacterial infection, and Renibacterium salmoninarum is the most common organism associated with these lesions. Although gross liver lesions associated with Renibacterium salmoninarum and Piscirickettsia salmonis can be similar, the microscopic lesions are distinct. Renibacterium salmoninarum infection results in a range of lesions from granulomatous to necrotic, whereas Piscirickettsia salmonis results in more of an acute inflammatory response that usually includes neutrophils and variable amounts of necrosis. Fish that die of Piscirickettsia salmonis infection always have organisms associated with the inflammatory response; this case has no such organisms. In this case, all of the 2003 year class have lesions consistent with Bacterial Kidney Disease, but none of the 2004/2005 year class has evidence of Bacterial Kidney Disease.

Multifocal fibrin deposits in the liver are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin. I have also seen this response in fish that are PCR positive for VHSV. Lack of remodeling of the fibrin is consistent with these deposits forming just before death.

Pigment in the liver could be lipofuscin, hemosiderin, or both. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. Hemosiderin accumulation in the liver is evidence of increased turnover of red blood cells.

Membranous glomerulonephritis is fairly common in older salmonids, particularly Chinook salmon; it is often associated with infections in other parts of the fish, but a link to immune complex deposition has not been demonstrated. Membranous glomerulonephritis has been associated with cardiomyopathy syndrome (in Atlantic salmon), nephrocalcinosis, and infections with a number of bacteria and parasitic species.

Pigments in the renal tubules probably include lipofuscin. Accumulation of lipofuscin is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. Variation in size of epithelial nuclei and cytoplasm is evidence of cellular degeneration and regeneration and consistent with persistent damage to the tubules.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

/sr

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00186 **Date** 16-Jan-2006 **Report** 23-Jan-2006

Report Copies

Submitter: 11036 Mainstream Canada (M)
Owner 16251 Mainstream 06-01
Farm:
Vet Clinic:
Attending

Specimen: Other **Count** 3 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-00186

Case Report

History/Symptoms

Submitted 3 agar plates for culture and sensitivity.

History of BKD (pen specific). Suspect Rickettsia.

PM: internally - sm-large white granulomas in K, L, S. Internal swelling.

Please use case 06-01 on report. Please report to Jeanine Sumner.

Bacteriology

See attached sheets for results.

* Results faxed Jan 23/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01900 **Date** 18-May-2006 **Report** 26-May-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16617 #5711, Marine Harvest PO#CL9259
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

Submitted fresh tissue for VHSV and IHNV PCR. One fish with suspect VHSV.

Atlantic, regular 2005, ID: 917, saltwater.

PO# CL9259

Virology

1 sample inoculated onto tissue culture - negative.

* Results faxed on June 14/06.

Molecular Diagnostics/PCR

IHN Virus and VHS Virus negative by PCR.

* Results faxed May 26/06.

/bb/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01901 **Date** 18-May-2006 **Report** 26-May-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16618 #5709, Marine Harvest PO CL9258
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 2 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

Submitted fresh tissue for IHNV and VHSV PCR testing.

Routine fish health visit on May 15, 2006. Some fish showing visceral hemorrhage and external lesions. No growth on bacteriology.

PO# CL9258

Virology

2 samples inoculated onto tissue culture - both negative.

* Results faxed on June 14/06.

Molecular Diagnostics/PCR

Samples 1-2, 3-4: IHN and VHS negative by PCR.

* Results faxed May 26/06.

/bb/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-01971 **Date** 25-May-2006 **Report** 05-Jun-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16656 Log 5715, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 7 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

Submitted 7 viral (pools of 2) for PCR IHNV and VHSV.

Atlantic, regular, saltwater entry 06 (S0),

PO# BB3701

Virology

7 samples inoculated onto tissue culture - all negative for viruses.

* Results faxed on Jun 21/06.

Molecular Diagnostics/PCR

Samples 1-2, 3-4, 5-6, 7-8, 9-10, 11-12, 13-14: IHN and VHS Virus Negative by PCR.

* Results faxed on Jun 5/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-02030 **Date** 31-May-2006 **Report** 05-Jun-2006

			Report	Copies
Submitter:	11036	Mainstream Canada (M)		
Owner	16696	#06-14, Raza Is. PO6797		
Farm:				
Vet Clinic:	11036	Mainstream Canada (M)		
Attending		Dr. P. McKenzie		

Specimen: Tissue - Formalized **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-02030

Case Report

History/Symptoms

Submitted 4 histology cassettes fixed and shipped in formalin. Please prepare for histology exam.

Site has history of Net Pen Liver Disease. Low levels of harmful plankton seen in water. Increased mortality.

Shrunken liver, pale green in colour. All mortality seen with this are "pin heads".

Please use case 06-14 on report and send report to Jeanine Sumner.

Histopathology

Four cassettes of formalin-fixed tissues were submitted for histopathology.

Slide 1 (fish 3-1) - liver, trunk kidney, heart, spleen, intestinal ceca, and gill
Slide 2 (fish 3-2) - liver, trunk kidney, spleen, intestinal ceca, and gill
Slide 3 (fish 3-3) - liver, trunk kidney, heart, spleen, intestinal ceca, and gill
Slide 4 (fish 3-4) - liver, trunk kidney, spleen, intestinal ceca, and gill
All organs were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is fair to poor for all organs. Organs have no postfixation dehydration and no significant deposits of acid hematin.

Measures of physiologic condition
Hepatocellular glycogen: none (all slides)
Mesenteric adipose tissue: not present for analysis
These patterns in the measures of physiologic condition are consistent with fish that have not been eating normally for the past few days.

Diagnosis

1a. Liver: hepatocellular single cell necrosis, diffuse, mild (slides 1, 2)
1b. Liver: disseminated yellow-brown pigment (probably lipofuscin), mild (slides 1, 2, 4), severe (slide 3)
1c. Liver: hepatitis, lymphoplasmacytic, multifocal, mild (slides 2, 3), moderate (slide 1)
1d Liver: peritonitis, lymphoplasmacytic, diffuse, mild (slide 3)
2. Trunk kidney: renal tubular mineralization, multifocal, mild (slide 3)

Final Comments

These livers have evidence of chronic exposure to toxins. Differentials include algal toxins or other toxins in the water or feed (e.g., aflatoxins). The livers lack hepatocellular karyomegaly/megalocytosis often associated with net pet liver disease, but other features are consistent with netpen liver disease: accumulation of lipofuscin and lymphoplasmacytic hepatitis. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies.

Causes of hepatocellular single cell necrosis have not been well defined in fish. Possible differentials include exposure to toxins (endogenous or exogenous), viral infection (VHSV), or remodelling of the liver in rapidly growing fish that suddenly go off feed about 24 hours before death.

Renal mineralization is common in cultured fish species; when severe (not here), the condition is termed nephrocalcinosis. The lesion is not considered fatal, although feed conversion may be adversely affected. The pathogenesis is not fully understood, but renal mineralization has been experimentally reproduced through high carbon dioxide levels, magnesium deficiency, selenium toxicity, and a diet low in minerals (source, "Systemic Pathology of Fish", 1989, by H. Ferguson). Clinically, renal mineralization is most commonly associated with high carbon dioxide levels.

/sr

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-02184 **Date** 09-Jun-2006 **Report** 07-Jul-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16799 Log 5729, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

One sample for virology and PCR for IHNV and VHS.

PO: CL 9269.

Virology

1 sample inoculated onto tissue culture - negative for viruses.

* Results faxed on Jul 6/06.

Molecular Diagnostics/PCR

Sample 1 - IHN and VHS Virus Negative by PCR.

* Results faxed on Jun 16/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00219 **Date** 18-Jan-2006 **Report** 24-Jan-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 15690 #5522, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Other **Count** 2 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-00219

Case Report

History/Symptoms

Submitted 2 slides.

One moribund, pale Atlantic. One fresh dead Atlantic with fluid filled. Mortality low at site.

PO#CL5385.

Atlantic, Regular, 2005, Saltwater.

Histopathology

Two cassettes of preserved tissues were submitted for histopathology in formalin. Cassettes were arbitrarily assigned slide numbers as follows:

Slide 1 (5522-2) - heart, gill, liver, head kidney, trunk kidney, spleen, intestinal ceca, and mesenteric fat
Slide 2 (5522-2) - heart, gill, liver, head kidney, trunk kidney, spleen, intestinal ceca, gall bladder, and mesenteric fat
All major organs on each slide were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is excellent for all organs. Organs have no deposits of acid hematin and no postfixation dehydration.

Measures of physiologic condition:
Hepatocellular glycogen depletion, severe (slides 1, 2)
Mesenteric adipose tissue depletion: none (slides 1, 2)

These patterns in the measure of physiologic condition are consistent with fish that were eating and growing (no depletion of mesenteric fat) but stopped feeding normally in the past few days (severe hepatocellular glycogen depletion).

Heart (slide 2) - The central lumen of the ventricle is about 90% occluded by a plug of degenerating inflammatory cells. Nuclear morphology varies. Some cells are relatively normal neutrophils and histiocytes, other cells have nuclear streaming (a common "squish artefact" that in this case could be real if this plug represents an embolus). About 10% of the nuclei in the plug are undergoing pyknosis or karyorrhexis.

Submission 2006-00219 **Case Report**

Diagnosis

1. Intestinal ceca and spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1), moderate (slide 2)
2a. Heart: cellular thromboembolus, intraventricular, 1 mm in diameter, acute, moderate (slide 2)
2b. Heart: endocarditis and epicarditis, multifocal, lymphohistiocytic, mild (slide 1)
3a. Liver: biliary preductular cell hyperplasia, diffuse, moderate (slide 1)
3b. Liver: peritonitis, diffuse (150 µm thick), with activated fibroblasts, fibrin, and fibrocellular fronds, moderate (slide 1)
3c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 2)
4a. Trunk kidney: casts of protein and yellow-brown pigment in tubules and urinary space, multifocal, mild (slide 1)
4b. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild

Final Comments

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Peritonitis on the liver is not a common vaccine reaction (this probably is the fish with abundant abdominal fluid); consider an active bacterial infection as a differential.

The 1-mm-diameter cellular thromboembolus in the ventricle of the fish in slide 2 is an unusual finding. However, it might have occluded enough blood flow to have suddenly killed the fish. It is possible that this fish was swimming along normally one minute, but unconscious and dying the next minute. Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins) or come from outside the fish (e.g., from the water or the feed). Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Pigments in the renal tubules and urinary space probably include lipofuscin. Accumulation of lipofuscin is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants.

Renal tubular epithelial protein droplets are normal in some species, or they may be an indication of glomerular disease. Ferguson ("Systemic Pathology of Fish," 1989) reports an association of renal protein droplets and high ammonia levels in salmonids. Roberts ("Fish Pathology, Third Edition," 2001) describes the change, but offers no cause.

/sr

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-02444 **Date** 26-Jun-2006 **Report** 29-Jun-2006

Report Copies

Submitter: 11036 Mainstream Canada (M)
Owner 16920 #06-17 Simmonds Point, PO#6811
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Formalized **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-02444

Case Report

History/Symptoms

Submitted 5 histo cassettes; 2 pools K, L, S virology. Histo - please prepare for histo exam. Viro - screen for viruses.

See attached sheet.

Case# 06-17.
PO# 6811.

Histopathology

Five cassettes of formalin-fixed tissues were submitted for histopathology.
Slide 1 (pen 1) - liver, trunk kidney, spleen
Slide 2 (pen 2) - liver, trunk kidney, spleen
Slide 3 (pen 8) - liver, trunk kidney, spleen
Slide 4 (pen 6) - liver, trunk kidney, spleen
Slide 5 (pen 14) - liver, trunk kidney, spleen
All organs were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is good to excellent for most organs. Some gill sections have moderate to severe postfixation dehydration (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cell types stains poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes (e.g., during shipment). Large foci of erythrocytes (e.g., spleen in slide 1, liver in slide 2) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. Tissues have no postfixation dehydration.

Measures of physiologic condition:
Hepatocellular glycogen: none (all slides)
Mesenteric adipose tissue: not included on the slides

Diagnosis

1a. Liver: hepatitis, granulomatous, multifocal, consistent with Renibacterium salmoninarum infection, severe (slide 4)
1b. Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate (slide 2)
1c. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 1)
1d. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 1, 2, 3), moderate (slide 5)
1e. Liver: pericholangitis, lymphocytic, multifocal, moderate (slide 1)
2a. Spleen: splenitis, granulomatous, multifocal, coalescing, consistent with Renibacterium salmoninarum infection, severe (slide 4)
2b. Spleen: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, mild (slide 2)
3a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, consistent with Renibacterium salmoninarum infection, severe (slide 4)
3b. Trunk kidney: abundant intracytoplasmic eosinophilic granules in endothelial cells, diffuse (slide 3)

Final Comments

The most common organism associated with disseminated granulomas in salmon is Renibacterium salmoninarum, the cause of bacterial kidney disease. These findings in slide 4 are consistent with clinical findings from this fish. The lack of microscopic lesions in fish 5 is also consistent with clinical findings. Other changes follow.

Hepatic sinusoidal congestion (sometimes called "peliosis") is evidence of sinusoidal damage. In BC Atlantic salmon, I have seen it associated with viral hemorrhagic septicemia virus and Listonella anguillarum. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes; this type of inclusion has not been described with any salmon virus. Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins) or come from outside the fish (e.g., from the water or the feed). Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Lymphocytic inflammation around bile ductules (liver) is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Accumulation of eosinophilic granules in the cytoplasm of cells lining small vessels in the kidney is a distinctive finding in Atlantic salmon. These granules probably accumulate in response to systemic immune stimulation. The granules might be part of eosinophilic granular cells or endothelial cells; granule size seems too large and variable for eosinophilic granular cells. Increased numbers of eosinophilic granular cells have been associated with experimental infection with Renibacterium salmoninarum, the cause of bacterial kidney disease (Flaño et al. 1996), but they are not described as a common finding in clinical cases. Slide 3 has no granulomatous inflammation, but slide 4 does.

Literature cited:

Flaño, E., López-fierro, P., Razquin, B. E., and A. Villena. 1996. In vitro differentiation of eosinophilic granular cells in Renibacterium salmoninarum-infected gill cultures from rainbow trout. J. Fish & Shellfish Immunology 3: 173-184.

/sr

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00246 **Date** 23-Jan-2006 **Report** 24-Jan-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 15701 #5545, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue-Fresh f Forma **Count** 2 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-00246

Case Report

History/Symptoms

Submitted two samples for tissue culture and PCR for IHN and VHS. Also submitted two samples for histology.

Fish showing internal hemorrhage. Typical of viral infection.

Please quote PO# CL5387.

Regular, saltwater.

Histopathology

Two cassettes of preserved tissues and were submitted for histopathology.

Slide 1 (5545-1) - heart, stomach, intestine, spleen, liver, trunk kidney, head kidney
Slide 2 (5545-2) - heart, spleen, liver, head kidney, trunk kidney, stomach, intestinal ceca and mesenteric fat.
All major organs on each slide were examined. Organs not listed below have no significant lesions.

Quality control: Autolysis for the liver varies from moderate (slide 2) to severe (slide 1). Large foci of erythrocytes (e.g., liver in slide 2) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. Organs have no postfixation dehydration.

Measures of physiologic condition:
Hepatocellular glycogen depletion, severe (slides 1 and 2)
Mesenteric adipose tissue depletion: none (slides 1 and 2)

These patterns in the measure of physiologic condition are consistent with fish that were growing and eating fairly normally (abundant mesenteric fat), but stopped eating normally a few days before they died (severe hepatocellular glycogen depletion).

Virology

2 samples inoculated onto tissue culture - both negative.

* Results faxed Feb. 24/06.

Molecular Diagnostics/PCR

Infectious Hematopoetic Necrosis virus Negative by PCR.
Viral Hemorrhagic Septicemia virus Negative by PCR.

Submission 2006-00246 **Case Report**

Diagnosis

1. Stomach: peritoneal granulation tissue and peritonitis, diffuse, severe (2 mm thick, slide 1)
2. Mesenteric fat: interstitial congestion and hemorrhage, diffuse, moderate (slide 1)
3. Stomach and mesenteric fat: peritonitis, granulomatous, multifocal, with intralesional vacuoles about 50 µm in diameter, moderate (slide 2)
4a. Spleen: Spleen: capillary fibrin, diffuse, acute, moderate (slide 1)
4b. Spleen: leukocytic karyorrhexis, disseminated, mild (slide 1)
4c. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 2)
5. Heart: endocarditis, diffuse, with endothelial cell hypertrophy, mild (slide 1)

Final Comments

The red colour of the viscera (noted grossly) probably is a result of congestion and hemorrhage in the mesenteric fat and abundant immature blood vessels in the granulation tissue on the surface of the stomach. These changes are consistent with chronic irritation of the serosal surface of the stomach; consider a severe vaccine reaction as a likely differential.

The head and trunk kidney have several pale foci, each about 100 µm in diameter, that often include small numbers of rod-shaped bacteria. These foci probably are a result of postmortem change; the differential diagnosis is interstitial necrosis, characteristic of infection with Infectious Hematopoietic necrosis virus.

The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Hypertrophic endothelial cells are basophilic and up to 10 µm thick.

The presence of degenerating nuclei (karyorrhexis) in the spleen (slide 1) is evidence of increased cell turnover, possibly as part on an active inflammatory response. Diffuse fibrin deposits in the spleen are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin. I have also seen this response in salmon that are PCR positive for VHSV.

Gastric and splenic peritonitis (slide 2) is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

/sr

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-02463 **Date** 27-Jun-2006 **Report** 30-Jun-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16932 #5752, Marine Harvest, PO CL1505
Farm:
Vet Clinic:
Attending

Specimen: Tissue-Fresh f Forma **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-02463 **Case Report**

History/Symptoms

Submitted 8 histo cassettes, 4 samples for PCR. PCR for IHNV and VHS.

Histology and virology collected from 8 slow swimmers at the surface of net pen. All fish with varying degrees of gill damage and worn tails. No growth on blood agar or 2 1/2 TSA.

Atlantic/Regular/2005 S1/Saltwater

Histopathology

Eight cassettes of formalin-fixed tissues were submitted for histopathology. After the tissues were processed into paraffin, organs were split into 2 blocks for embedding and sectioning: gills (block A) and other organs (block B).

Slide 1A (5752-1) - gill
Slide 1B - liver, trunk kidney, spleen, heart, brain, intestinal ceca, and mesenteric fat
Slide 2A (5752-2) - gill
Slide 2B - liver, trunk kidney, head kidney, spleen, heart, brain, intestinal ceca, and mesenteric fat
Slide 3A (5752-1) - gill
Slide 3B - liver, trunk kidney, spleen, heart, brain, intestinal ceca, and mesenteric fat
Slide 4A (5752-2) - gill
Slide 4B - liver, trunk kidney, spleen, heart, brain, intestinal ceca, and mesenteric fat
Slide 5A (5752-1) - gill
Slide 5B - liver, head kidney, spleen, heart, brain, intestinal ceca, and mesenteric fat
Slide 6A (5752-2) - gill
Slide 6B - liver, trunk kidney, head kidney, spleen, heart, brain, intestinal ceca, and mesenteric fat
Slide 7A (5752-1) - gill
Slide 7B - liver, trunk kidney, spleen, heart, brain, intestinal ceca, and mesenteric fat
Slide 8A (5752-2) - gill
Slide 8B - liver, trunk kidney, head kidney, spleen, heart, brain, intestinal ceca, and mesenteric fat
All organs were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is good to excellent for most organs; tips of intestinal villi sometimes have mild autolysis. Large foci of erythrocytes (e.g., spleen in slides 3B and 5B) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. Organs have no postfixation dehydration.

Measures of physiologic condition:
Hepatocellular glycogen: none (slides 1B, 2B, 3B, 4B, 5B, 8B), small amounts (slide 6B), moderate amounts (slide 7B)
Mesenteric adipose tissue: abundant (slide 1B, 2B, 3B, 4B, 5B, 6B, 7B, 8B)
These patterns in the measures of physiologic condition are mostly consistent with fish that have not been eating normally for the past few days. The only exception is the fish in slide 6, which probably was eating until the time it was sampled (based on hepatocellular glycogen).

Virology

Fish viruses Negative
Tissue culture = negative.

* Results faxed on Aug 1/06.

Molecular Diagnostics/PCR

Viral Hemorrhagic Septicemia Virus Negative by PCR.
Infectious Hematopoetic Necrosis Virus Negative by PCR.

Diagnosis

1a. Gill: lamellar epithelial hyperplasia, multifocal, with scattered multinucleate giant cells, mild (slide 4A), moderate (slide 8A), severe (slides 1A, 3A, 5A)
1b. Gill: eosinophilic granular cells in the loose connective tissue of filaments, moderate numbers (slides 5A, 6A, 7A), abundant (slide 2A)
2a. Heart: epicarditis and endocarditis, multifocal, lymphohistiocytic, mild (slide 1B)
2b. Heart: endocarditis, multifocal, lymphohistiocytic, mild (slides 5B, 6B)
3a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 4B, 5B)
3b. Liver: hepatocellular cytoplasmic lipid, diffuse, small amounts (slide 2B)
3c. Liver: pericholangitis, lymphocytic, focal, mild (slide 8B)
4. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (slide 3B)
5. Spleen: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, mild (slides 4B, 6B)

Submission 2006-02463 **Case Report**

Final Comments

The most severe lesions in these fish are in the gills. Hyperplasia of the epithelium lining gill lamellae is a nonspecific response to irritation. Inciting causes include parasites, bacteria, viruses, and toxins. In this case, hyperplasia has resulted in several foci where adjacent lamellae are fused. The presence of multinucleate giant cells is evidence on indigestible foreign material. Two sections each contain a multinucleate giant cell with a cytoplasmic focus of basophilic material that is about 5 x 15 µm (coordinates on my Nikon 50i microscope are slides 1A, 26.7 x 113.3; 5A, 24.6 x 111.5). Consider physical irritation from diatoms (e.g., Chaetoceros or Corethron) as a differential.

Increased numbers of eosinophilic granular cells in the gill have been associated with chronic disease. Increased numbers of eosinophilic granular cells are sometimes associated with chronic parasitic infections (e.g., parasitic copepods), but the inciting cause is not included in the sections examined.

Epicarditis and endocarditis are evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins) or come from outside the fish (e.g., from the water or the feed). Some degree of lipid accumulation in the cytoplasm of hepatocytes might be normal. Abnormal accumulation of hepatocellular lipid (lipidosis) occurs when fish are not feeding and in cases of inadequate nutrition. Lymphocytic inflammation around bile ductules (liver) is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system.

Interstitial cell hyperplasia in the kidney results from increased demand for erythrocytes or white blood cells somewhere in the body (renal interstitial cells are the blood-forming or hematopoietic cells in the kidney). For the fish in slide 3, branchial hyperplasia might be the inciting cause.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

/sr/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-02465 **Date** 27-Jun-2006 **Report** 05-Jul-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16934 #5747, Marine Harvest, PO CL 1504
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted one sample for virology and PCR for VHS and IHN.

Atlantic/2006/5

Histopathology

N/A

Virology

Fish Viruses Negative
Tissue culture = negative.

* Results faxed on Aug 1/06.

Molecular Diagnostics/PCR

Infectious Hematopoietic Necrosis Virus negative by PCR.
Viral Hemorrhagic Septicemia Virus negative by PCR.

* Results faxed July 5/06.

/bb/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-02569 **Date** 06-Jul-2006 **Report** 18-Jul-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16996 #5762. PO CL1514, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 7 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

Submitted fresh tissue.

Samples labeled pen 1, pen 2, pen 2B, pen 3, pen 4, pen 5, and pen 6 for virology. Please do PCR for IHNV and VHSV.

Virology

7 samples inoculated onto tissue culture - all negative.

* Results faxed on Aug 2/06.

Molecular Diagnostics/PCR

Samples A (Pen 1), B (Pen 2), C (Pen 2B), D (Pen 3), E (Pen 4), F (Pen 5), and G (Pen 6): VHS and IHN Negative by PCR.

* Results faxed on July 18/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-02570 **Date** 06-Jul-2006 **Report** 18-Jul-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16997 #5764, PO CL 1513
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 4 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted 4 samples for routine virology. Please do PCR for IHNV and VHSV.

Virology

4 samples inoculated onto tissue culture - all negative.

* Results faxed on Aug 2/06.

Molecular Diagnostics/PCR

Samples A (1), B (2), C (3), D (4): VHS and IHN Negative by PCR.

* Results faxed on July 18/06.

Toxicology

N/A

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-02575 **Date** 07-Jul-2006 **Report** 18-Jul-2006

Report Copies

Submitter: 11036 Mainstream Canada (M)
Owner 16999 06-16, PO 6820, Cypress Harbour
Farm:
Vet Clinic: 11036 Mainstream Canada (M)
Attending Dr. P. McKenzie

Specimen: Tissue - Fresh **Count** 3 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-02575

Case Report

History/Symptoms

Submitted 3 pools for virology. Please screen for viruses.

Brood site. History of BKD.

Please use case #06-16 on report.
PO# 6820.

Report to Jeanine Sumner.

Virology

3 samples inoculated onto tissue culture - all negative.

* Results faxed on Aug 2/06.

Molecular Diagnostics/PCR

Samples A (Pen 113), B (Pen 115), and C (Pen 116): IHN and VHS Negative by PCR.

* Results faxed on July 18/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-02826 **Date** 26-Jul-2006 **Report** 02-Aug-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17159 #5793, Marine Harvest PO #CL1527
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 3 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

S.A. Raverty, D.V.M.,
Veterinary Pathologist

Case Report

History/Symptoms

Species: Atlantic Salmon. Sex: Regular. Saltwater. Sample size: 3 samples for PCR, IHN and VHSV. Pooled Virology - 3 samples labelled 1-2, 3-4, 5-6, for PCR for IHNV and VHSV. Samples submitted for "rule-out" Fish appear to have been handled poorly. No gross lesions internally to suggest viral etiology.

PO #CL1527.

Virology

3 samples inoculated onto tissue culture - all negative.

* Results faxed Aug. 30/06.

Molecular Diagnostics/PCR

Samples A)1-2, B)3-4, C)5-6: Infectious Hematopoietic Necrosis virus and Viral Hemorrhagic Septicemia Negative by PCR.

** Results faxed on August 2, 2006.

/mb/bb

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-02847 **Date** 28-Jul-2006 **Report** 08-Aug-2006

Report Copies

Submitter: 11036 Mainstream Canada (M)
Owner 17173 #06-19, Brent Island, PO#7713
Farm:
Vet Clinic:
Attending

Specimen: Multiple Specimens **Count** 4 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: Atlantic Salmon **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

1 Peritonitis
2 Dermatitis, non-specific

S.A. Raverty, D.V.M.,
Veterinary Pathologist

Submission 2006-02847

Case Report

History/Symptoms

PO#7713, Brent Island.
Please fax results to Jeanine Sumner at (250) 286-0042.

Fish have history of BKD.
Fair amount of lice seen on fish, may be causing red abrasions seen on belly of fish sampled.
Treatments: Oxytetracycline for BKD - November, 2005. Tribrissen for mouth rot - January, 2006 (select pens); SLICE - April, 2006.

Post mortem lesions: no internal signs. Red abrasions observed on bell of fish.

Specimens: 5 histology cassettes, 2 pools Virology.
Tests: Histo - please prepare for histo. exam. by Dr. Gary Marty.
Virology - please screen for viruses; PCR for Furunc. And Rickettsia.

Virology

Fish Viruses Negative
Tissue culture = negative

* Results faxed Aug. 26/06.

Molecular Diagnostics/PCR

Aeromonas salmonicida negative by PCR.
Piscirickettsia salmonis negative by PCR.

* Results faxed Aug. 2nd and 3/06.

Submission 2006-02847 **Case Report**

Diagnosis

MORPHOLOGIC DIAGNOSES:

Slide 1, 11-5, July 25, 2006
1). Kidney: Granuloma, moderate, multifocal to coalescing, with variably myelopoiesis
2). Spleen: Splenitis, capsular, moderate, nodular to diffuse, granulomatous with reactive mesothelia and sinusoidal histiocytosis
3). Adipose tissue: Peritonitis, moderate, multifocal, granulomatous, with mesothelial papillary hyperplasia and hypertrophy
4). Heart: Epicarditis, mild, fibrinogranulomatous, chronic
5). Skin, lateral line: Erosion and ulceration, moderate, diffuse
6). Skeletal muscle: Degeneration and necrosis, myocellular, mild to moderate, multifocal, acute

There are no significant lesions within the liver, pancreas, small intestine, gills, peripheral vasculature, or peripheral nerves.

Slide 2, 11-4, July 25, 2006:
1). Adipose tissue and pancreatic lobules: Peritonitis, moderate, nodular to diffuse, granulomatous with scattered melanomacrophages, coalescing epithelioid macrophage and histiocytes around clear vacuoles (vaccine induced periontitis)
2). Spleen: Splenitis, capsular, moderate, nodular to diffuse, granulomatous with reactive mesothelia, lymphoid depletion and scattered extracellular coccobacilli
3). Skeletal muscle: Degeneration, myocellular, moderate, laminar, acute
4). Skin: Ulceration, moderate, diffuse with scattered folliculitis
5). Liver: Degeneration, hepatocellular, mild, multifocal, random, acute
6). Heart: Epicarditis, mild, multifocal, lymphohistiocytic, chronic with scattered melanomacrophages and fibroplasias
7). Heart, compact layer: Myocarditis, perivascular, mild, multifocal, lymphohistiocytic
8). Heart, spongy layer: Myocarditis, minimal, focal, necrotising, granulomatous

There are no significant lesions within the gills, peripheral vasculature or peripheral nerves.

Slide 3, 11-3, July 25, 2006:
1). Skin: Erosion and ulcerate, moderate, focally extensive, subacute with superficial subacute furunculosis and scattered re-epithelialization
2). Heart: Epicarditis, myocarditis and endocarditis, mild, multifocal, random, granulomatous
3). Skeletal muscle: Edema, mild to moderate, multifocal, acute

There are no overt lesions within the spleen, peripheral nerves, peripheral vasculature, pylorus, pancreas or liver.

Slide 4, 11-2, July 25, 2006:
1). Skin: Folliculitis, mild, multifocal, subacute with variably extensive erosion and ulceration
2). Heart: Epicarditis, mild, multifocal, lymphohistiocytic, chronic

There are no overt lesions within the small intestine, peripheral nerves, peripheral vasculature, adipose tissue, pancreas, kidney, spleen, or liver.

Slide 5, 11-1, July 25, 2006:
1). Skin: Dermatitis, moderate, multifocal, erosive and ulcerative, with spongiosis, lymphocytic exocytosis, and subacute folliculitis
2). Liver: Hepatitis, portal, mild, multifocal, subacute

There are no overt lesions within the skeletal muscle, peripheral nerves, peripheral vasculature, gills, adipose tissue, small intestine, kidney, heart or spleen.

Final Comments

With the exception of slide 1, 11-5, July 15, 2006, the multisystemic inflammatory infiltrate noted within the examined tissues is considered low to intermediate grade and presumably would not have contributed significantly to antemortem morbidity; the granulomatous peritonitis in multiple slides features individual to coalescing clear vacuoles bound by macrophages and dispersed melanomacrophages, morphologically consistent with a vaccine induced peritonitis. If on gross examination, this process involved a sufficient volume of the ceolomic viscera, there is a possibility of impaired growth and productivity. The epicarditis, capsular splenitis and portal hepatitis are likely sequelae to this process, although an underlying past bacterial infection cannot be discounted. In slide 1, the multifocal renal granulomas are consistent with long past bacterial kidney disease. Close examination of the skin did not reveal any discernible pathogens; within the scale beds of select fish, there are variable accumulations of inflammatory infiltrate occasionally admixed with small amounts of eosinophilic necrotic debris. There was no conclusive evidence of a vasculitis or infarction as may be appreciated in spawning rash (bacterial kidney disease) and the history of ectoparasitism suggests erosions and ulcerations may be secondary to physical excoriation associated with the sea lice.

/mb

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00285 **Date** 24-Jan-2006 **Report** 02-Feb-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 15723 #5501, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Other **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

Submitted one isolate for identification.

Atlantic, regular.

Billing purposes, please quote PO# 167213.

Bacteriology

Bacteriology plate/slope: Iodobacter fluviatilis.

Iodobacter fluviatilis is a Gram-negative rod that is one of the spreading chromobacteria. It has not been associated with disease in fish. Until 1989, this bacterium was called Chromobacterium fluviatile.

/sr

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00286 **Date** 24-Jan-2006 **Report** 01-Feb-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 15724 #5545, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Other **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted one isolate for identification.

Atlantic, regular, 2006, Tank ID: 3, saltwater.

For billing purpose, please quote PO# 167212.

Bacteriology

Bact plate/slope - Tank 3: identified as Yersinia ruckeri.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-02987 **Date** 09-Aug-2006 **Report** 15-Aug-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17232 #5797, Marine Harvest, PO CL 1539
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 4 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

Submitted 4 samples (liver) collected on August 2, 2006 and frozen. Rickettsia like organism on liver tissue imprints. Please confirm by PCR.

Atlantic, regular.

PO CL 1539.

Molecular Diagnostics/PCR

Samples #1, 2, 3, and 4 Piscirickettsia salmonis positive by PCR.

* Results faxed on Aug 15/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00305 **Date** 26-Jan-2006 **Report** 30-Jan-2006

Report Copies

Submitter: 11036 Mainstream Canada (M)
Owner 15735 Mainstream Burdwood
Farm:
Vet Clinic:
Attending

Specimen: Tissue-Fresh f Forma **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-00305 **Case Report**

History/Symptoms

Submitted 5 histo cassettes - fixed in formalin, shipped in Ethanol and 2 pools virology - PCR for Rickettsia.

History of BKD. Suspect Rickettsia.

Pen spec. OTC Sept 05.

Int. - sm- lg white granulomas (K, L, S). Kidney swollen.

Please use case # 06-01 on report. Report to Jeanine Sumner.

Histopathology

Five cassettes of preserved tissues were submitted for histopathology in formalin. Each slide contains liver, trunk kidney, and spleen. Cassettes were arbitrarily assigned slide numbers as follows:

Slide 1 - Mainstream label 12-1
Slide 2 - Mainstream label 10-1
Slide 3 - Mainstream label 5-1
Slide 4 - Mainstream label 7-1
Slide 5 - Mainstream label 7-2
All major organs on each slide were examined. Organs not listed below have no significant lesions.

Quality control: Autolysis for the liver varies from none (slide 1), to mild (slide 2) to severe (slide 3). The periphery of some organs has almost complete loss of eosinophilic staining; I only see this change in tissues that have been transferred from 10% formalin to ethanol. Organs have no deposits of acid hematin and no postfixation dehydration.

Molecular Diagnostics/PCR

Piscirickettsia salmonis Negative by PCR.

Diagnosis

1a. Liver: hepatitis, granulomatous, multifocal, moderate (slides 1, 4)
1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 2)
1c. Liver: sinusoidal fibrin, multifocal, acute, mild (slide 2)
2a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, mild (slide 1)
2b. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 4)

Final Comments

The most common organism associated with disseminated granulomas in salmon is Renibacterium salmoninarum, the cause of bacterial kidney disease. However, chronic cases of Yersinia ruckeri are also associated with granulomatous inflammation. The tissues have no evidence of infection with Piscirickettsia salmonis. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Multifocal fibrin deposits in the liver are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin. I have also seen this response in fish that are PCR positive for VHSV. Lack of remodelling of the fibrin is consistent with these deposits forming just before death. Consider bacteriology and virology, if not already done.

Renal tubular epithelial protein droplets are normal in some species, or they may be an indication of glomerular disease. Ferguson ("Systemic Pathology of Fish," 1989) reports an association of renal protein droplets and high ammonia levels in salmonids. Roberts ("Fish Pathology, Third Edition," 2001) describes the change, but offers no cause.

/sr

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-03094 **Date** 15-Aug-2006 **Report** 22-Aug-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17281 #5802, Marine Harvest, PO#BB1658
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-03178 **Date** 22-Aug-2006 **Report** 28-Aug-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17329 #5804, Marine Harvest, PO BB1662
Farm:
Vet Clinic:
Attending

Specimen: Tissue-Fresh f Forma **Count** 6 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

Submitted 2 samples (viral/histo), please do PCR INHV and VHSV.

Saltwater entry 05, S1.

PO# BB1662.

Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology and processed into 4 slides at the Animal Health Centre:

Slide 1 - brain, liver, heart, spleen, head kidney, trunk kidney
Slide 2 - skin, skeletal muscle (2 pieces)
Slide 3 - brain, liver, heart, spleen, head kidney, trunk kidney
Slide 4 - skin, skeletal muscle (2 pieces), gill
All organs were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is excellent for all organs. Organs have no postfixation dehydration and no deposits of acid hematin.

Measure of physiologic condition:
Hepatocellular glycogen: none (slides 1, 3)

Virology

2 samples inoculated onto tissue culture - both negative.

Molecular Diagnostics/PCR

Samples A (#1) and B (#2) - Infectious Hematopoietic Necrosis Virus negative by PCR.
Samples A (#1) and B (#2) - Viral Hemorrhagic Septicemia Virus negative by PCR.

Diagnosis

1a. Skin: dermatitis, ulcerative, focal (1 ulcer in each piece of skin), granulomatous, neutrophilic, severe (slide 2)
1b. Skin: dermatitis, ulcerative, focal (1 ulcer in each piece of skin), granulomatous, lymphocytic, severe (slide 4)

Final Comments

Although ulcers in fish are commonly related to filamentous bacteria (e.g., Tenacibaculum maritimum), the ulcers in this case have no obvious filamentous bacteria; either the bacteria were lost during processing (this is common), or bacteria were not involved in the ulcer. In both slides 2 and 4, inflammation is mostly limited to the dermis and hypodermis; the underlying skeletal muscle is only minimally affected. Enlargement of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection). Identification of the bacteria requires culture or PCR.

Lymphoplasmacytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

/bb/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-03180 **Date** 22-Aug-2006 **Report** 28-Aug-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17330 #5803, Marine Harvest, PO BB1551.
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 7 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-03180

Case Report

History/Symptoms

Submitted fish tissue for PCR for IHNV, and VHSV.

Bags 1-3, 4-6, 7-9 are pen 3
Bags 10-12, 13-15, 16-18 are pen 6
Bags 69 fresh mort pen 11.

PO# BB1661.

Virology

7 samples inoculated onto tissue culture - all negative.

* Results faxed on Sept 14/06.

Molecular Diagnostics/PCR

Samples
A) Pen 3 (#1-3), B) Pen 3 (#4-6), C) Pen 3 (#7-9), D) Pen 6 (#10-12), E) Pen 6 (#13-15), F) Pen 6 (#16-18), G) Pen 11 (#69) - Infectious Hematopoietic necrosis Virus negative by PCR and Viral Hemorrhagic Septicemia Virus negative by PCR.

* Results faxed Aug. 28/06.

/bb/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-03313 **Date** 31-Aug-2006 **Report** 07-Sep-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17401 #5820, Marine Harves, PO#CL1544
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 5 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted 5 pools for virology - PCR for IHN, and VHS please. Samples labeled 21-22, 23-24, 25-26, 27-28, 29-30.

Atlantic, Regular.

Virology

5 pooled samples inoculated onto tissue culture - all negative.

Molecular Diagnostics/PCR

IHNV and VHSV Negative by PCR.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-03380 **Date** 06-Sep-2006 **Report** 11-Sep-2006

			Report	Copies
Submitter:	9439	Marine Harvest Canada (M)		
Owner	17426	#5828, Marine Harvest CL 1548		
Farm:				
Vet Clinic:				
Attending				

Specimen: Tissue - Fresh **Count** 2 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted two samples from pen 7 and pen 10 for virology and PCR for IHN and VHS.

Mortality spike in two pens. Fish with fungus. No growth on bacteriology.

Log 5828.
PO CL 1548.

Virology

2 samples inoculated onto tissue culture - both negative.

* Results faxed on Oct 2/06.

Molecular Diagnostics/PCR

Samples A (Pen 7) and B (Pen 10) are IHNV and VHSV Negative by PCR.

* Results faxed on Sept 11/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-03419 **Date** 08-Sep-2006 **Report** 12-Sep-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17437 #5831, Marine Harvest, PO #CL 1550
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Formalized **Count** 7 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-03419

Case Report

History/Symptoms

Submitted 7 histo cassettes.

Recent spike in mortality with loss of 30% of pen population. Mortality is returning back to minimal. No growth on bacteriology i.e.: TSA and TSA with Blood Agar.

Pens 2 and 4 histo from affected fish and 2 cassettes from pen 11- these are unaffected fish.

TDX: Fungus

Log No: 5831 PO# CL1550

Gross Pathology

N/A

Histopathology

Seven cassettes of juvenile salmon tissues fixed in formalin were submitted for histopathology. Most slides contain liver, trunk kidney, head kidney, heart, spleen, gill, intestinal ceca, and mesenteric fat; some sections contain skin and/or eye. Cassettes were converted to slides as follows:
Slides 1, 2, and 5 (Vic Lk Pen 2 09/05/06)
Slides 3, 4, and 6 (Vic Lk Pen 4 09/05/06)
Slide 7 (Vic Lk Pen 11 09/05/06)

All major organs on each slide were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is excellent for most organs, but the tips of intestinal villi are slightly autolyzed in some sections. Differential staining is good. Organs have no postfixation dehydration and no acid hematin deposits.

Measures of physiologic condition:
Hepatocellular glycogen: none (slides 1, 5, 6), small amounts (slides 2, 4, 7), abundant (slide 3)
Mesenteric adipose tissue: abundant (slides 1, 2, 5, 3, 4, 6, 7)
These patterns in the measures of physiologic condition provide a variable pattern; the fish in slide 3 is relatively healthy and feeding, while most of the other fish have evidence that they have not been eating normally for a few days.

Submission 2006-03419 **Case Report**

Diagnosis

1a. Liver: hepatocellular hydropic degeneration, disseminated, acute, severe (slide 5)
1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 1, 4, 6)
2a. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (slide 2)
2b. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 2), moderate (slide 7)
2c. Trunk kidney: renal tubular epithelial necrosis, focal, acute, mild (slide 7)
3a. Spleen and liver: peritonitis, lymphoplasmacytic, granulomatous, regionally diffuse, with fine fibrocellular fronds, mild (slides 5, 4), moderate (slides 1, 2, 7)
3b. Spleen and intestinal mesenteries: peritonitis, lymphoplasmacytic, granulomatous, regionally diffuse, with fine fibrocellular fronds, mild (slide 3, spleen only), moderate (slide 7)

Final Comments

Morphologic lesions in only one fish clearly separate the affected fish in pens 2 and 4 from the unaffected fish in pen 11. In slide 5 (pen 2), hydropic degeneration among large numbers of hepatocytes (2 – 20 cells per 400× objective-lens field) provides evidence that the liver was being exposed to toxins. Potential sources of the inciting toxins include the water and a bacterial infection. In this case, cytoplasm of affected hepatocytes is expanded by fine to large foamy vacuoles. After hydropic degeneration can no longer be reversed, the changes are called single cell necrosis. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Interstitial cell hyperplasia in the kidney results from increased demand for erythrocytes or white blood cells somewhere in the body (renal interstitial cells are the blood-forming or hematopoietic cells in the kidney), often as a result of inflammation (e.g., in this case, peritonitis)

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease. Ferguson ("Systemic Pathology of Fish," 1989) reports an association of renal protein droplets and high ammonia levels in salmonids. Roberts ("Fish Pathology, Third Edition," 2001) describes the change, but offers no cause.

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). The presence in the fish from pen 11 is evidence that some fish from this pen might also be unhealthy. Causes of renal tubular epithelial necrosis in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxins (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin). Consider virology, if not already done.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. The presence of moderate peritonitis lining the liver in several fish is unusual (more commonly, the inflammation is limited to the spleen and surrounding intestinal mesenteries, as in slide 3). Did these fish have a different vaccination protocol from fish in other pens?

/mb

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road
Abbotsford BC V3G 2M3 Telephone: (604) 556-
Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-03461 **Date** 13-Sep-2006 **Report** 17-Oct-2006

Report Copies

Submitter: 11036 Mainstream Canada (M)
Owner 17451 #12080 Mainstream Canada
Farm:
Vet Clinic:
Attending

Specimen: Whole Animal **Count** 10 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-03461

Case Report

History/Symptoms

Submitted 10 Atlantic Salmon Smolts for Virus via cell culture.

Please pool 5 fish to equal one sample.

Water temp. 14 degrees C

Identification : A) SM/ pen 2/ 10

Purchase Requisition#: 12080
Case #: 12080

Virology

2 samples inoculated onto tissue culture - both negative.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-03525 **Date** 20-Sep-2006 **Report** 25-Sep-2006

			Report	Copies
Submitter:	11036	Mainstream Canada (M)		
Owner	17480	Brent Is., Case# 06-23, PO#:8270		
Farm:				
Vet Clinic:	11036	Mainstream Canada (M)		
Attending		Dr. Peter McKenzie		

Specimen: Tissue - Fresh **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-03525

Case Report

History/Symptoms

Submitted fresh fish tissue for PCR screening for Viruses, BKD and Rickettsia.

History of BKD
Fish sampled "should be eating", hang out in corners.

OTC treatment for BKD 05.

Specimens: 2 pools virology

Please use Case# 06-23 on report. Please send report Att: Jeanine Sumner
Fax:(250) 286-0042

Post mortem Lesions: See attached sheet.

Virology

2 pooled samples inoculated onto tissue culture - both negative.

* Results faxed on Oct 18/06.

Molecular Diagnostics/PCR

Sample A (Pen 2, 6, and 9) and Sample B (8, 10, and 12) are IHNV, VHSV, and Piscirickettsia salmonis Negative by PCR.

Sample A (Pen 2, 6, and 9) is Renibacterium salmoninarum Positive by PCR. Sample B (8, 10, and 12) is Renibacterium salmoninarum Negative by PCR.

* Results faxed on Sept 25/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-03624 **Date** 26-Sep-2006 **Report** 11-Oct-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17520 #5844, CL1557, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Other **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-03624

Case Report

History/Symptoms

Two pooled samples labelled 5844-1 and 5844-2-3 for virology. Please do PCR for IHNV and VHSV. One blood agar with bacterial isolate for identification.

Fish showing signs for furunculosis.

Atlantic, Regular, 2005, Saltwater S1.
Tank 9, 10.

Bacteriology

Aeromonas salmonicida ss masoucida.

See attached sheet for sensitivities.

* Results faxed on Oct 11/06.

Virology

3 samples inoculated onto tissue culture - all negative.

* Results faxed on Oct 24/06.

Molecular Diagnostics/PCR

Samples A (5844-1), B (5844-2-3), and C (5845-2): IHNV and VHSV Negative by PCR.

* Results faxed on Oct 11/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-03693 **Date** 28-Sep-2006 **Report** 29-Sep-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17560 #5849, PO CL 1561, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue-Fresh f Forma **Count** 4 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-03693

Case Report

History/Symptoms

Submitted 2 virology and 2 histology samples.

Two samples labeled 5849-1 and 5849-2 for PCR for IHNV and VHSV. Samples frozen for shipment.

Histology from fish #1 for interest - fish displayed, hard, small (0.5 - 1mm) goldish brown lumps on lining of kidney (this fish was a fresh mort.) Histology #2 had hemorrhage in liver. Increased moribunds in this pen.

Atlantic, Regular, 2005, Saltwater.

Histopathology

Two cassettes of formalin-fixed tissues were submitted for histopathology

Slide 1 (5849-1 9/26/06) - trunk kidney, head kidney, heart, liver, skeletal muscle, spleen, intestinal ceca, and mesenteric fat
Slide 2 (5849-2 9/26/06) - trunk kidney (3 pieces), each with a margin of dense irregular connective tissue that is ~180 µm thick (swimbladder? the tissues do not have an epithelial lining)
Pieces were labelled a, b, c, and a, b based on distance from the frost on the slide ("a" furthest from the frost)

Quality control: Autolysis varies from none (most organs) to mild (tips of intestinal villi). Tissues have no postfixation dehydration and no acid hematin deposits.

Measure of physiologic condition
Hepatocellular glycogen: none (slide 1)
Mesenteric fat - abundant (slide 1)
This pattern in the measures of physiologic condition is consistent with healthy growing fish (abundant mesenteric fat) that recently stopped feeding (severe glycogen depletion).

Virology

2 samples inoculated onto tissue culture - both negative.

* Results faxed on Oct 24/06.

Molecular Diagnostics/PCR

Samples A (5849-1) and B 95849-2) - Infectious Hematopoietic Necrosis Virus and Viral Hemorrhagic Septicemia Virus negative by PCR.

Submission 2006-03693 **Case Report**

Diagnosis

1. Liver: yellow-brown pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slide 1)
2. Trunk kidney: renal tubular casts of yellow-brown pigment, multifocal, mild (slide 1)
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild (slide 1)
4. Para-renal connective tissue: cartilaginous material, multifocal (slide 2, 1 kidney)

Final Comments

Pigment in the liver could be lipofuscin, hemosiderin, or both. Pigment in the renal tubules and spleen probably is lipofuscin. Accumulation of lipofuscin in the liver is a non-specific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. Hemosiderin accumulation in the liver is evidence of increased turnover of red blood cells.

Both sections of kidney in slide 1 include the ventral margin, but neither contains a distinct brown mass consistent with the gross description. However, the gross description is consistent with foci of concentrated lipofuscin pigment that might have simply been missed in the plane of section included on the slide. The gross description is also consistent with a focus of hemorrhage that has become oxidized to methemoglobin.

In slide 2, the connective tissue on the margin of one kidney has 3 foci of cartilage that are each about 22 mm wide and 200 µm thick. Based on the regular margin, I suspect they are normal. The kidneys have no changes that might explain the hemorrhage in the liver, described grossly.

/bb

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-03694 **Date** 28-Sep-2006 **Report** 04-Oct-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17561 #5848, PO CL 1562, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 3 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

Submitted 3 virology samples labeled 5848-1, 5848-2-3, 5848 - 4-5 for PCR for IHNV and VHSV. Some taken from fresh morts, suspect fish #1 with hem of heat, swim bladder and pyloric caeca.

Atlantic, Regular, 2006, Saltwater.

Virology

3 samples inoculated onto tissue culture - all negative.

* Results faxed on Oct 24/06.

Molecular Diagnostics/PCR

Samples A (5848-1), B (5848, 2-3), and C (5848, 4-5) are IHNV and VHSV Negative by PCR.

* Results faxed on Oct 4/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-03695 **Date** 28-Sep-2006 **Report** 04-Oct-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17562 #5846, PO CL 1563, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 3 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

Submitted 3 samples labeled 5846-41; 5846-42-43; 5846-44-45 for PCR for IHNV and VHS. Sampled fish were fresh morts with suspected external lesions, hem of liver, swim bladder and ?

Atlantic, Regular, 2006.

Virology

Fish Viruses Negative
Tissue culture for viruses = negative.

* Results faxed on Nov 20/06.

Molecular Diagnostics/PCR

Samples A (5846-41) and B (5846, 42-43) are VHSV Positive by PCR.

Samples A (5846-41), B (5846, 42-43), C (5846, 44-45) are IHNV Negative by PCR and samples C (5846, 44-45) is VHSV Negative by PCR.

* Results faxed on Oct 4/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-04059 **Date** 24-Oct-2006 **Report** 27-Oct-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17707 #5890, PO CL1575, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue-Fresh f Forma **Count** 3 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-04059

Case Report

History/Symptoms

Submitted 3 Histo Cassettes and 3 tissue samples for PCR IHN and VHS.

Sudden rise in mortality. 3 histo cassettes from fresh dead; gills were not fresh~3 Hrs old.

3 pooled samples for IHN and VHSV-PCR

Water sample shipped to HAMP for plankton check-results pending.

Histopathology

Formalin-fixed tissues were submitted for histopathology and distributed into 5 cassettes at the Animal Health Centre.

Slide 1A (5090-1A) - heart, liver, spleen, head kidney, trunk kidney
Slide 1B (5090-1B) - gill
Slide 2A (5890-2A) - heart, liver, spleen, head kidney, trunk kidney
Slide 2B (5890-2B) - gill
Slide 3 - gill (6 pieces)
All organs were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is fair to good for most organs; the gills have moderate autolysis. Organs have no postfixation dehydration and no significant deposits of acid hematin.

Measure of physiologic condition
Hepatocellular glycogen: none (slides 1A, 2A, 3A)
This pattern in the measure of physiologic condition is consistent with fish that have not been eating normally for the past few days.

Virology

Fish Viruses Negative
Tissue culture = negative.

* Results faxed on Nov 20/06.

Molecular Diagnostics/PCR

Samples A (#1-2), B (#3-4) and C (#5-6) are Infectious Hematopoetic Necrosis virus and Viral Hemorrhagic Septicemia virus Negative by PCR.

Submission 2006-04059 **Case Report**

Diagnosis

1a. Liver: sinusoidal congestion, acute, multifocal, mild (slide 3), moderate (slide 1A)
1b. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slides 1A, 2A)
1c. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 2A)
1d. Liver: hepatic necrosis, acute, focal, mild (slide 2A)
2. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1A)

Final Comments

Sinusoidal congestion in the liver is evidence of sinusoidal damage. In BC Atlantic salmon, sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and Listonella anguillarum. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. Sinusoidal congestion has also been described in wild fish (dab) surveyed in the North Atlantic (source: http://www.cefas.co.uk/publications/aquatic/aemr41.pdf), but the cause was not determined. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data).

Pigment in the liver could be lipofuscin, hemosiderin, or both. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. Hemosiderin accumulation in the liver is evidence of increased turnover of red blood cells.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, Piscirickettsia salmonis). This case has no obvious organisms. Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

/mb/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-04152 **Date** 31-Oct-2006 **Report** 06-Nov-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17753 #5896, PO# CL 1584, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Multiple Specimens **Count** 8 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-04152 **Case Report**

History/Symptoms

Submitted 4 histo and 4 virology samples.

Mortality has dropped to 20-30 fish/day. Do's have remained in 4 mg/L range. Fish are not feeding. Plankton counts are low. Fish #1 and #2 were fresh morts. Fish #3 from pen 106, fish #4 from pen 104. No growth on BA or blood agar. Please do PCR for IHNV and VHSV.

Atlantic, Regular, 2005 S1, Saltwater.

Mortality: recently high but has dropped to 20-30 fish/day.

Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology. After the cassettes were processed into paraffin, the tissues were embedded into 2 or 3 blocks for slide production (slides A, B, and C, if needed).
Slide 1A (5896-1) - liver, spleen, heart, head and trunk kidney, intestinal ceca and mesenteric fat
Slide 1B (5896-1) - gill
Slide 2A (5896-2) - liver, spleen, heart, head and trunk kidney, intestinal ceca and mesenteric fat; a Gram stain was done on a step section from block 2A
Slide 2B (5896-2) - gill
Slide 3A (5896-3) - liver, spleen, head and trunk kidney, intestinal ceca and mesenteric fat
Slide 3B (5896-3) - gill
Slide 3C (5896-3) - heart, liver, intestinal ceca and mesenteric fat
Slide 4A (5896-4) - liver, spleen, heart, head and trunk kidney, intestinal ceca and mesenteric fat
Slide 4B (5896-4) - gill
All organs were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is fair to good for most organs from fish 1 and 2 (the gills and tips of intestinal villi are autolyzed); excellent for all organs from fish 3 and 4. Organs have no postfixation dehydration and no significant deposits of acid hematin.

Measure of physiologic condition
Hepatocellular glycogen: none (slides 1A, 2A, 3A, 4A)
Mesenteric fat, abundant (slides 1A, 2A, 3C, 4A)
This pattern in the measure of physiologic condition is consistent with fish that were eating well, but have not been eating normally for the past few days.

Virology

4 samples inoculated onto tissue culture - all negative for viruses .

* Results faxed on Nov 23/06

Molecular Diagnostics/PCR

Samples A(#1), B(#2), C(#3), and D(#4) are Infectious Hematopoetic Necrosis virus and Viral Hemorrhagic Septicemia virus Negative by PCR.

Diagnosis

1a. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slides 1A, 3A/3C)
1b. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 1A)
1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 1A, 2A); multifocal, moderate (slide 3A/3C)
1d. Liver: sinusoidal fibrin, multifocal, acute, mild (slide 3A/3C)
1e. Liver: hepatocellular single cell necrosis (apoptosis), multifocal, acute, moderate (slide 3C)
2. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1A)
3. Trunk and head kidney: interstitial macrophages distended with homogenous protein, disseminated, moderate (slides 1A, 4A)
4. Heart: endocarditis, granulomatous, focal, moderate (slide 2A)
5a. Gill: mural lamellar thrombosis, focal, mild (slide 2A); multifocal, mild (slide 4B)
5b. Gill: lamellar hyperplasia and fusion, multifocal, mild (slide 4B)

Final Comments

These fish have a number of microscopic changes that provide evidence of chronic stress (lipofuscin pigment) and acute stress (inflammation and necrosis).

Pigment in the liver could be lipofuscin, hemosiderin, or both. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. Hemosiderin accumulation in the liver is evidence of increased turnover of red blood cells.

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins) or come from outside the fish (e.g., from the water or the feed). Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Multifocal fibrin deposits in the liver are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin. I have also seen this response in fish that are PCR positive for VHSV. Lack of remodelling of the fibrin is consistent with these deposits forming just before death.

Causes of hepatocellular single cell necrosis have not been well defined in fish. Possible differentials include exposure to toxins (endogenous or exogenous), viral infection (VHSV), or remodelling of the liver in rapidly growing fish that suddenly go off feed about 24 hours before death. Apoptosis is the normal way in which hepatocyte numbers are decreased (i.e., the hepatocytes are not needed when growing fish stop feeding because few to no nutrients are being absorbed into the blood and entering the liver for processing).

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Accumulation of homogenous protein within renal interstitial macrophages is probably a response to protein leaked from blood vessels, probably part of an inflammatory reaction. Most of the cytoplasmic protein droplets are 8 to 15 µm in diameter; some cells have a single droplet, whereas other cells have several droplets.

Differentials for the focus of granulomatous inflammation in the heart include a reaction to a vaccine, or chronic bacterial infection. The Gram stain reveals no organisms. Sometimes, very small numbers of Renibacterium salmoninarum cause granulomatous inflammation, so the Gram stain does not completely rule out bacterial kidney disease. Other differentials include a chronic infection with other bacteria like Yersinia ruckeri.

Thrombosis in the gill is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection; the slide (2B) has not obvious organisms.

Gill lamellar fusion with lamellar hypertrophy may be a result of physical damage from exposure to a parasite or diatoms, but this lesion contains no organisms (they might have been lost during processing). Gill lamellar fusion, without lamellar hypertrophy, has been associated with exposure to heavy metals and with hypoxia (thought to be from fish gasping for oxygen at the water-air interface). In slide 4B, fusion and hypertrophy are limited to foci of lamellae at the distal ends of the filaments.

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-04153 **Date** 31-Oct-2006 **Report** 07-Nov-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17754 #5912, PO CL 1580, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

Submitted one virology sample.

Anemic fish with hemorhage of swim bladder and p.c. Fish sampled on Oct 25. Sample frozen. Virology please - PCR for IHNV and VHSV.

Atlantic, Regular, 2006 S0, Netpen/Tank 2. Mortality minimal.

Virology

Fish viruses negative - by inoculation on tissue culture.

* Results faxed on Nov 23/06.

Molecular Diagnostics/PCR

Sample #1 is IHNV and VHSV Negative by PCR.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-04154 **Date** 31-Oct-2006 **Report** 07-Nov-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17755 #5895, PO# CL 1583, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue-Fresh f Forma **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-04154 **Case Report**

History/Symptoms

Histo and virology collected from 4 fish sampled on Oct 24/06. Chaetoceros (both species) has been present in water. C. Convolutus at 30 cells/ml outside pens, and 10-15 cells/ml inside pens. Do's have been in high 4's.

Fish #1 - thin fish with gill damage.
#2 dark/thin with gill damage.
#3 silver on feed with gill damage.
#4 gritse male with gill damage.

Please do PCR for IHNV and VHSV.

Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology. After the cassettes were processed into paraffin, the tissues were embedded into 2 or 3 blocks for slide production (slides A, B, and C, if needed).
Slide 1A (5895-1) -, spleen, head and trunk kidney; additional sections from this block were stained with Schmorl's lipofuscin, PAS, and Von Kossa mineral stains.
Slide 1B (5895-1) - gill
Slide 1C (5895-1) - liver, heart, intestinal ceca and mesenteric fat
Slide 2A (5895-2) - liver, spleen, heart, head and trunk kidney, intestinal ceca and mesenteric fat;
Slide 2B (5895-2) - gill
Slide 3A (5895-3) - liver, spleen, heart, trunk kidney (2 pieces), intestinal ceca and mesenteric fat
Slide 3B (5895-3) - gill
Slide 4A (5895-4) - liver, spleen, heart, trunk kidney (2 pieces), intestinal ceca and mesenteric fat
Slide 4B (5895-4) - gill
All organs were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is excellent for most organs; the tips of intestinal villi have mild autolysis, and there is slight separation of gill lamellar epithelium from supporting structures. Organs have no postfixation dehydration and no significant deposits of acid hematin.

Measure of physiologic condition
Hepatocellular glycogen: none (slides 1C, 2A, 3A, 4A)
Mesenteric fat, abundant (slides 1C, 2A, 3A, 4A)
This pattern in the measure of physiologic condition is consistent with fish that were eating well, but have not been eating normally for the past few days.

Virology

4 samples inoculated onto tissue culture - all negative for viruses.

* Results faxed on Nov 23/06.

Molecular Diagnostics/PCR

Samples A(#1), B(#2), C(#3) and D(#4) are Infectious Hematopoetic Necrosis virus and Viral Hemorrhagic Septicemia virus Negative by PCR.

Diagnosis

1a. Gill: lamellar epithelial hyperplasia and fusion, multifocal, with intralesional diatom spines/setae, severe (slides 1B, 3B, 4B)
1b. Gill: lamellar epithelial hyperplasia and fusion, multifocal, mild (slide 2B)
2. Trunk kidney: renal tubular casts of protein and yellow-brown pigment (lipofuscin), focal, mild (slide 2A); multifocal, moderate (slide 1A)
3a. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, moderate (slides 2A, 3A, 4A), severe (slide 1C)
3b. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 1C)
3c. Liver: hepatic necrosis, acute, multifocal, moderate (slides 3A, 4A)
3d. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 4A
4a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate (slides 1A, 4A)
4b. Spleen: parenchymal golden pigment (lipofuscin), disseminated, intracellular, mild (slides 1A, 2A), moderate (slides 3A, 4A)
5. Intestinal ceca: peritonitis, chronic, multifocal, with fibrocellular fronds, mild (slide 1C), and with multinucleate giant cells, moderate (slide 2A)
6. Heart: mural thrombosis, multifocal, moderate (slide 3A)

Final Comments

The lesions in these fish are consistent with harmful algal blooms as the primary cause of morbidity. The diatoms Chaetoceros concavicornis, C. convolutus, and a Corethron sp. have been associated with mortality of salmon reared in seawater net pens at numbers as low as 5/mL (Taylor and Harrison 2002). The space between the base of gill filaments often contain moderate numbers of unstained structures that are consistent with the diatom Chaetoceros. Chains of cell bodies are about 25 µm in diameter, and individual spines/setae are about 3.5 µm in diameter. Slide 4A contains deep-penetrating spines surrounded by multinucleate giant cells. Mortality due to Chaetoceros can occur within a few days of exposure, as a result of physical damage to the gills and the resultant inflammatory response. Affected fish are also more vulnerable to Vibrio infections. Larger fish tend to be more susceptible than small fish. Characteristic gill lesions include mucous cell hyperplasia, lamellar epithelial cell hyperplasia and necrosis, and variable numbers of neutrophils. Diatoms and their processes are on the surface of the gill epithelium and sometimes entrapped by multinucleate giant cells. [Source: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.] Lesions due to Corethron sp. are similar (Spear et al. 1989). The gills from fish 2 have similar morphologic changes, but no obvious diatoms in the section; the lesions are consistent with diatom exposure (the diatoms might have been lost in processing of the tissues to the section on the slide).

The yellow-brown to golden pigment in the renal tubules and splenic parenchyma is lipofuscin. Accumulation of lipofuscin is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Pigment in the liver could be lipofuscin, hemosiderin, or both. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. Hemosiderin accumulation in the liver is evidence of increased turnover of red blood cells. In this case, the liver from fish #1 has the most abundant hepatic pigment that I have seen in an Atlantic salmon cultured in British Columbia.

Lymphocytic inflammation around bile ductules (liver) is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system.

Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, Piscirickettsia salmonis). This case has no obvious organisms. Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Peritonitis (spleen and intestinal ceca) is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection.

Literature cited:

Speare DJ, Brackett J, Ferguson HW. 1989. Sequential pathology of the gills of coho salmon with a combined diatom and microsporidian gill infection. Canadian Veterinary Journal 30(7):571-575.

Taylor, F.J.R., and P.J. Harrison. 2002. Harmful algal blooms in western Canadian coastal waters. In Report #23 of the North Pacific Marine Science Organization, "Harmful algal blooms in the PICES region of the North Pacific."

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-04155 **Date** 31-Oct-2006 **Report** 07-Nov-2006

Report Copies

Submitter: 11036 Mainstream Canada (M)
Owner 17756 #06-24, Brent Is.
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Formalized **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-04155 **Case Report**

History/Symptoms

Submitted 3 histo cassettes of Atlantic Salmon.
Submitted by: Jeanine Sumner
History of BKD
TX: Oxytetracycline treatment Dec 2005

Post mortem lesions: Severe Popeye on all fish.

2-1: mild adhesions on P.C., spleen slightly swollen

2-2 : mild/mod adhesions on P.C., mild hemorrhaging on flesh around Pectoral fins.

2-3: No internal abnormalities.

ID#Case 06-24 Brent Is.

Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology. After the cassettes were processed into paraffin, the gills were embedded into a separate block for slide production (tissues = slide A; gill = slide B). Tissues on most A slides include liver, heart, brain, spleen, kidney, intestine, and mesenteric fat.
Slides 1A, 1B - Brent Is. Oct 24/06 (2-1)
Slides 2A, 2B - Brent Is. Oct 24/06 (2-2); a Twort's gram stain was done on a step section from block 2A
Slides 3A, 3B - Brent Is. Oct 24/06 (2-3)

All organs were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is excellent for all organs. Large foci of erythrocytes (e.g., spleen in slide 1A) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. Organs have no postfixation dehydration.

Measure of physiologic condition
Hepatocellular glycogen: none (slides 1A, 2A)
Mesenteric fat, small amounts (slide 1A), abundant (slides 2A, 3A)
This pattern in the measure of physiologic condition is consistent with fish that were eating well, but have not been eating normally for the past few days.

Submission 2006-04155 **Case Report**

Diagnosis

1a. Liver: hepatitis, granulomatous, focal, moderate (slide 1A); multifocal, moderate, with intralesional Gram-positive bacterial rods consistent with Renibacterium salmoninarum (slide 2A)
1b. Liver: hepatitis, perivascular, lymphohistiocytic, multifocal, mild (slide 1A)
1c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1A, 2A)
2a. Heart: endocarditis, multifocal, lymphohistiocytic, mild (slides 1A, 3A)
2b. Heart: endocarditis, multifocal, lymphohistiocytic, with endocardial hypertrophy and intralesional Gram-positive bacterial rods consistent with Renibacterium salmoninarum, moderate (slide 2A)
3a. Head and trunk kidney: nephritis, interstitial, granulomatous, multifocal, mild (slide 2A)
3b. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 1A)
4. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1A, 2A)
5. Intestinal ceca: peritonitis, granulomatous, multifocal, moderate (slide 1A)
6. Stomach: peritonitis, granulomatous, focal, with an intralesional vacuole 100 µm in diameter, severe (slide 1A)
7. Gill: branchitis, granulomatous, multifocal, moderate (slide 2B)

Final Comments

Two of the three fish examined have solid evidence of bacterial kidney disease, consistent with the clinical history. The most common organism associated with disseminated granulomas in salmon is Renibacterium salmoninarum (the cause of bacterial kidney disease). Differentials include other bacteria like Yersinia ruckeri.

Lymphocytic inflammation around vessels in the liver is evidence of chronic immune stimulation, e.g., from a bacterial infection.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Endocardial hypertrophy is a fairly common reaction in Atlantic salmon; it probably is a result of exposure to toxins in the circulation.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease. Among the 221 dead Atlantic salmon examined as part of the Ministry's Auditing and Surveillance Program in the first half of 2006, 15% had renal tubular intracytoplasmic protein droplets. Ferguson ("Systemic Pathology of Fish," 1989) reports an association of renal protein droplets and high ammonia levels in salmonids. Roberts ("Fish Pathology, Third Edition," 2001) describes the change, but offers no cause.

Peritonitis adjacent to the spleen, stomach, and intestinal ceca is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

/mb

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-04156 **Date** 31-Oct-2006 **Report** 07-Nov-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17759 #5894, PO CL 1581, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Formalized **Count** 2 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted two virology samples.

Two fresh silvers sampled. Both fish with mild petechiation of the liver +/- p.c. No bacterial growth on TSA or Blood agar. Samples collected on Oct 24/06 and frozen.

Please do PCR for IHNV and VHSV.

Atlantic, Regular, 2006 S1, Saltwater; no mortality.

Virology

2 samples inoculated onto tissue culture - both negative for viruses.

* Results faxed on Nov 23/06.

Molecular Diagnostics/PCR

Sample A (#1) and B (#2) are IHNV and VHSV Negative by PCR.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-04283 **Date** 09-Nov-2006 **Report** 16-Nov-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 17836 Log 5934, PO# BB1676, Marine
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 3 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted fresh tissue for PCR INH and VHS.

(1-2), (3-4), and (5-6).

Log Number 5934.
PO# 1676.

Virology

3 pooled samples inoculated onto tissue culture - all negative.

* Results faxed on Dec 6/06.

Molecular Diagnostics/PCR

Samples A (1-2), B (3-4), and C (5-6) are IHNV, and VHSV Negative by PCR.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-04359 **Date** 17-Nov-2006 **Report** 24-Nov-2006

Report Copies

Submitter: 2007 Microtek International
Owner 17871 # 6-2173, Microtek International
Farm:
Vet Clinic:
Attending

Specimen: Multiple Specimens **Count** 4 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-04359 **Case Report**

History/Symptoms

Submitted 3 Atlantic salmon liver samples for histology testing and one bacterial slant for Bacteriology.

ID: 6-2173-1, 6-2173-2, 6-2173-3

Sent in by Tim Hewison

Bacteriology

Bact plate/slope: Streptococcus agalactiae.

Identification was done by DNA sequencing. Streptococcus agalactiae is commonly isolated from cattle and human sources, and it is common enough in some warm water fish that vaccines have been developed to control associated disease (e.g., Pasnik et al. 2006).

Pasnik, DJ; Evans, JJ; Klesius, PH. 2006. Passive immunization of Nile tilapia (Oreochromis niloticus) provides significant protection against Streptococcus agalactiae. Fish & Shellfish Immunology [Fish Shellfish Immunol.]. 21(4):365-371.

Histopathology

Formalin-fixed livers were submitted in water for histopathology:
Slide 1 - 6-2173-1
Slide 2 - 6-2173-2
Slide 3 - 6-2173-3 (this slide also contains ovary); step sections were stained with GMS (for fungus) and Twort's Gram stain

Quality control: Tissue preservation is good, although some margins have severe autolysis (these areas were probably next to the gallbladder). Tissues have no post fixation dehydration and no deposits of acid hematin.

* Results e-mailed on Nov 21/06.

Diagnosis

1a. Liver: peritonitis, focal (2 × 0.7 mm, slide 1; 4 × 0.4 mm, slide 2), granulomatous, with intralesional vacuoles (20-30 µm in diameter), moderate (slide 1)
1b. Liver: peritonitis, multifocal, granulomatous, with scattered multinucleate giant cells, mild (slide 3)
1c. Liver: hepatitis, granulomatous, multifocal, with scattered multinucleate giant cells, moderate (slide 3)
2. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1, 2, 3).

Final Comments

Granulomatous peritonitis and hepatitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. I more commonly see the reaction on the spleen, but I occasionally see it on or in the liver. The GMS and Gram stains rule out organisms as the cause for the lesion.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-04612 **Date** 12-Dec-2006 **Report** 14-Dec-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 18013 #5974, PO BB1679
Farm:
Vet Clinic:
Attending

Specimen: Tissue-Fresh f Forma **Count** 4 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-04612

Case Report

History/Symptoms

Submitted two viral PCR IHNV and VHSV and two histo fresh mortalities.

Atlantic, Regular, 2005, S1, Saltwater.

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology. After the cassettes were processed into paraffin, the gill was embedded into a separate block for slide production.
Slide 1A (5973-1) - spleen, liver, heart, head kidney, trunk kidney, skeletal muscle, gill filament (1), intestine and mesenteric fat.
Slide 1B (5973-1) - gill
Slide 2A (5973-2) - spleen, liver, heart, head kidney, trunk kidney, skeletal muscle, intestine and mesenteric fat.
Slide 2B (5973-2) - gill
All organs were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is fair to good for most organs; gill autolysis is poor (slide 1B) to good (slide 2B); intestinal autolysis is moderate (slides 1A, 2A). Organs have no postfixation dehydration and no acid hematin deposits.

Measure of physiologic condition
Hepatocellular glycogen: none (slides 1A, 2A)
Mesenteric fat, abundant (slides 1A, 2A)
This pattern in the measure of physiologic condition is consistent with fish that were eating well, but have not been eating normally for the past few days.

Virology

2 samples inoculated onto tissue culture - both negative.

* Results faxed Jan. 11/07.

Molecular Diagnostics/PCR

Samples A (#1) and B (#2) are Infectious Hematopoetic Necrosis virus and Viral Hemorrhagic Septicemia virus Negative by PCR.

Submission 2006-04612 **Case Report**

Diagnosis

1a. Liver: hepatic necrosis, acute, multifocal, coalescing, severe (slide 2A)
1b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slides 1A, 2A)
1c. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slide 1A), moderate (slide 2A)
2. Trunk kidney: renal tubular epithelial necrosis and regeneration, multifocal, subacute, mild (slide 2A), moderate (slide 1A)
3a. Spleen: parenchymal golden pigment, disseminated, intracellular, mild (slide 1A)
3b. Spleen: peritonitis, chronic, regionally diffuse, with fibrocellular fronds, mild (slide 2A)

Final Comments

The liver in slide 2A has one of the most severe cases of hepatic necrosis that I have ever seen in Atlantic salmon. Hepatic necrosis is fairly common in Atlantic salmon that die in marine net pens, affecting 8% of the 450 fish examined in 2006 as part of the Province's Fish Farm Auditing and Surveillance Program. Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, Piscirickettsia salmonis); the cause in most cases is not determined. This case has no obvious organisms, and PCR results rule out VHSV. Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

Pigment in the liver could be lipofuscin, hemosiderin, or both; pigment in the spleen probably is lipofuscin. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. Hemosiderin accumulation in the liver is evidence of increased turnover of red blood cells.

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Regeneration is evidence of ongoing damage and repair. Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxins (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin).

The gill in slide 1B has a few small foci of filamentous bacteria. These probably are a result of postmortem growth, but they might be significant (need to correlate with clinical findings to determine significance).

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

/mb

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-04613 **Date** 12-Dec-2006 **Report** 19-Dec-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 18014 #5977, PO BB1679
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 4 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted 4 viral PCR IHNV and VHSV.

#1 Pen 1
#2/3 Pen 4
#4 Pen 6

Atlantic, 2006, S1, Saltwater.

Virology

4 samples inoculated onto tissue culture - all negative.

* Results faxed Jan. 11/07.

Molecular Diagnostics/PCR

Samples A, B, C, and D are IHNV and VHSV Negative by PCR.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-04614 **Date** 12-Dec-2006 **Report** 19-Dec-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 18016 #5976, PO BB 1679
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 3 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted 3 viral PCR IHNV and VHSV.

#1/2 Pen 9
#3 Pen 10.

Atlantic, 2005, S1, Saltwater

Virology

3 samples inoculated onto tissue culture - all negative.

* Results faxed Jan. 11/07.

Molecular Diagnostics/PCR

Samples A, B, and C are IHNV and VHSV Negative by PCR.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00473 **Date** 08-Feb-2006 **Report** 14-Feb-2006

			Report	Copies
Submitter:	11036	Mainstream Canada (M)		
Owner	15803	Maintstream 06-05 PO 7510476 Simmonds Pt		
Farm:				
Vet Clinic:				
Attending		Dr. P. McKenzie		

Specimen: Tissue - Fresh **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted fresh Atlantic salmon tissue for virology - screen for viruses.

Increased number of silvers from mortality collection. No internal signs.

Please use case # 06-05 on report. PO# 7510476. Report to Jeanine Sumner.

Virology

Fish viruses negative.

* Results faxed on Mar 7/06

Molecular Diagnostics/PCR

Infectious Hematopoetitic Necrosis Virus and Viral Haemorragic Septicemia Virus - Negative by PCR.

* Results faxed on Feb 14/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-04786 **Date** 27-Dec-2006 **Report** 04-Jan-2007

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 18093 PO# BB1681, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 10 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted fresh salmon tissue for PCR- IHNV& VHSV.

ID: 3 fish pools
PO# BB 1681

Virology

10 samples inoculated onto tissue culture - all negative.

* Results faxed on Jan 24/07.

Molecular Diagnostics/PCR

Samples #6000-1 - #6000-10 are Infectious Hematopoetic Necrosis Virus and Viral Hemorrhagic Septicemia Virus Negative by PCR.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00510 **Date** 10-Feb-2006 **Report** 14-Feb-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 15821 #5579, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue-Fresh f Forma **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-00510 **Case Report**

History/Symptoms

Submitted 5 samples for virology - PCR for IHN and for VHS. All fish had petechial hemorrhaging of the liver, swim bladder, and adipose tissue. Fish #2 had hemorrhage in the muscle, in addition to the above mentioned viscera. Mortality is currently low. Four cassettes for histology, transported in water.

Please quote PO# CL 5396 for billing purposes.

2004, S1, Saltwater.

Histopathology

Four overstuffed cassettes of formalin-fixed tissues were submitted for histopathology in water. Cassettes were processed into paraffin; tissues from each cassette were embedded into two blocks (slides A and B) for sectioning, and assigned slide numbers as follows:

Slide 1A (5579-1) - trunk kidney, liver, intestinal ceca and mesenteric fat
Slide 1B (5579-1) - head kidney, spleen, heart
Slide 2A (5579-3) - spleen, intestinal ceca and mesenteric fat
Slide 2B (5579-3) - trunk kidney, liver, head kidney, heart
Slide 3A (5579-4) - heart, liver, intestinal ceca and mesenteric fat
Slide 3B (5579-4) - trunk kidney, spleen, head kidney
Slide 4A (5579-5) - trunk kidney, head kidney, intestinal ceca and mesenteric fat
Slide 4B (5579-5) - heart, liver, spleen
All major organs on each slide were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is excellent for all organs. Some large foci of erythrocytes (e.g., spleen in slide 2A) have precipitates of acid hematin; however, most sections have none or minimal acid hematin deposits. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. Organs have no postfixation dehydration.

Measures of physiologic condition
Hepatocellular glycogen, none (slides 1A, 2B, 3A, 4B)
Mesenteric adipose tissue depletion, none (slides 1A, 2A, 3A, 4A)
This pattern in the measures of physiologic condition is consistent with healthy growing fish (abundant mesenteric fat) that recently stopped feeding (no glycogen deposits).

Virology

5 samples inoculated onto tissue culture - all negative.

* Results faxed on Mar 21/06.

Molecular Diagnostics/PCR

Samples A, B, C, D, and E - IHNV and VHSV Negative by PCR.

Submission 2006-00510 **Case Report**

Diagnosis

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1A, 3A, 4B), moderate (slide 2B)
1b. Liver: sinusoidal congestion, with hepatocellular intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, mild (slides 1A, 3A, 4B)
1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 2B, 3A), moderate (slide 4B)
1d. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 4B)
2. Trunk kidney, thin-walled vessels: endothelial cell hypertrophy, with granular eosinophilic cytoplasm, diffuse, mild (slides 3B, 4A), moderate (slides 1A, 2B)
3. Head kidney, thin-walled vessels: endothelial cell hypertrophy, with granular eosinophilic cytoplasm, diffuse, mild (slide 4A), moderate (slides 1B, 2B, 3B)
4a. Spleen, thin-walled vessels: endothelial cell hypertrophy, with granular eosinophilic cytoplasm, diffuse, mild (slides 1A, 2B, 3B, 4B)
4b. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, mild (slides 2A, 4B)
5. Heart: epicarditis, multifocal, with multinucleate giant cells surrounding central vacuoles, mild (slide 3A)

Final Comments

These fish have a number of changes that provide evidence of systemic toxins, but nothing that points to a specific cause. The source of the toxins could be inflammatory, bacterial, or exogenous.

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins) or come from outside the fish (e.g., from the water or the feed). Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Lymphocytic inflammation around bile ductules (liver) is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system.

Sinusoidal congestion (sometimes called "peliosis") in the liver is evidence of sinusoidal damage (slide 4B has the best example). In BC Atlantic salmon, sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and Listonella anguillarum. Consider bacteriology (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes; this type of inclusion has not been described with any salmon virus.

Endothelial cell hypertrophy with granular eosinophilic cytoplasm is a distinctive finding in the kidney and sometimes the spleen of Atlantic salmon. As a differential, these granules might be part of eosinophilic granular cells; however, granule size seems too large and variable for eosinophilic granular cells. Endothelial cell hypertrophy probably is related to systemic immune stimulation (e.g., a bacterial infection). I have seen these granules associated with a case of Piscirickettsia salmonis, but other cases have no known cause.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Granulomatous epicarditis is less common, but it might also be part of a vaccine reaction.

/sr/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00517 **Date** 13-Feb-2006 **Report** 14-Mar-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 15824 #5578, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 2 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

2 virology samples collected from poor performers with external erosions on the belly and flank. Some fish with stomachs filled with bloody mucous and congealed blood in the lower intestine towards the colon. Overall mortality at the site is low except for one pen which appears to have mostly poor performers showing similar to damage to those above. Samples submitted to monitor population for VHS and IHN.

Please run PCR for VHS and IHN.

For billing purposes please quote PO CL5398

** Note - samples are already in Virology (sent on Friday).

Virology

Fish viruses negative by culture.

Molecular Diagnostics/PCR

Infectious Hematopoietic Necrosis Virus negative by PCR.
Viral Hemorrhagic Septicemia Virus negative by PCR.

/bb

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00593 **Date** 16-Feb-2006 **Report** 27-Feb-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 15863 #5584, Marine Harvest (Brad Boyce)
Farm:
Vet Clinic:
Attending

Specimen: Multiple Specimens **Count** 5 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-00593 **Case Report**

History/Symptoms

7 bags of tissue. Five cassettes for histology. One culture for ID. PO #MP6108. PCR for IHNV and VHSV.

Bacteriology

Culture submitted for ID - Yersinia ruckeri.

Histopathology

Four full cassettes of formalin-fixed tissues were submitted for histopathology. Cassettes were processed into paraffin; after processing, the gill and heart were moved from cassettes 1, 2, and 4 and embedded in a separate block (slide B); and the remaining tissues were left together (slide A). Sides were labelled as follows:
Slide 1 (#5 5584, Feb 13/06) - liver, heart, trunk kidney, spleen, stomach, intestinal ceca, and mesenteric fat

Slide 2 (#2 5584, Feb 13/06) - liver, trunk kidney, intestinal ceca, and mesenteric fat; heart and gill in slide B

Slide 3 (#1 5584, Feb 13/06) - liver, spleen, trunk kidney/head kidney, intestinal ceca, skin/skeletal muscle, and mesenteric fat; heart and gill in slide B
Slide 4 (#4 5584, Feb 13/06) - liver, spleen, trunk kidney, intestinal ceca, and mesenteric fat; heart and gill in slide B
All major organs on each slide were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is fair to good for most organs; intestinal ceca sometimes have complete loss of villous epithelium due to autolysis. Liver autolysis varies from none (slide 2A), mild (slide 1), moderate (slide 4A), to severe (slide 3A). Some large foci of erythrocytes (e.g., liver vessels in slide 2A) have precipitates of acid hematin; however, most sections have none or minimal acid hematin deposits. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. Margins of some organs have minimal postfixation dehydration (e.g., kidney in slide 1).

Measures of physiologic condition
Hepatocellular glycogen, none (slides 1, 2A, 3A, 4A)
Mesenteric adipose tissue depletion, none (slides 1, 2A, 3A, 4A)
This pattern in the measures of physiologic condition is consistent with healthy growing fish (abundant mesenteric fat) that recently stopped feeding (no glycogen deposits).

Gill (slide 2B) - About 30% of the lamellar capillaries are plugged with occlusive thrombi. In the filaments on one margin of the gill (ventral or dorsal?), nearly every lamella contains thrombi; the other margin of the gill is mostly unaffected. About 10% of the thrombosed capillaries are also expanded, up to 40 µm in diameter. Most thrombi are concentrated in capillaries at the base of the lamellae, but some extend to capillaries in the

Virology

7 samples inoculated onto tissue culture - all negative.

Molecular Diagnostics/PCR

Samples A, B, C, D, E, F, G.

Infectious Hematopoetitic Necrosis virus Negative by PCR.
Viral Hemorrhagic Septicemia virus Negative by PCR.

Diagnosis

1. Gill: lamellar thrombosis, acute, regionally diffuse, severe (slide 2B)
2. Liver: sinusoidal congestion, acute, multifocal, moderate (slide 1)
3. Stomach and mesenteric fat: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate (slide 1)
4. Spleen, intestinal ceca and mesenteric fat: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, moderate (slides 3A)
5. Intestinal ceca and mesenteric fat: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, mild (slides 4A)
6. Skeletal muscle: myonecrosis, acute, multifocal, moderate (slide 3A)
7. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 3A)

Final Comments

These salmon have a number of lesions that are common in pen-reared Atlantic salmon in British Columbia. However, widespread capillary thrombosis in the gill of fish #2 (slide 2B) is a pattern that I have not previously seen. The lesion is significant and probably contributed significantly to morbidity. Our Auditing and Surveillance Program does not routinely sample gill, and gill autolysis occurs quickly; therefore, this lesion might be more common than it seems. Possible causes include focal trauma, systemic bacterial infection (e.g. Yersinia ruckeri), or exposure to endothelial toxins (endogenous or exogenous).

Sinusoidal congestion in the liver is evidence of sinusoidal damage. In BC Atlantic salmon, sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and Listonella anguillarum. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.

Granulomatous peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Skeletal muscle degeneration has been associated with feeding of rancid oils and dietary deficiency of vitamin E and selenium (reference: Fish Pathology, 3rd Edition. 2001. R.J. Roberts). It can also occur is fish that are not eating; muscle tissue is broken down to provide nutrients for critical organ survival. The affected area of skeletal muscle might have appeared pale when the tissue was fresh.

Renal tubular epithelial protein droplets are normal in some species, or they may be an indication of glomerular disease. Ferguson ("Systemic Pathology of Fish," 1989) reports an association of renal protein droplets and high ammonia levels in salmonids. Roberts ("Fish Pathology, Third Edition," 2001) describes the change, but offers no cause.

/bb/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00594 **Date** 16-Feb-2006 **Report** 27-Feb-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 15864 #5585, Marine Harvest (Brad Boyce)
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 7 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Seven bags of tissue - PCR IHNV and VHSV (2 fish pools). PO #MP6107.

Virology

7 samples inoculated onto tissue culture - all negative.

* Results faxed on Mar 21/06.

Molecular Diagnostics/PCR

Samples A, B, C, D, E, F, and G - IHNV and VHSV negative by PCR.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00595 **Date** 16-Feb-2006 **Report** 27-Feb-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 15865 #5586, Marine Harvest (Brad Boyce)
Farm:
Vet Clinic:
Attending

Specimen: Tissue-Fresh f Forma **Count** **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

Three bags tissue for PCR for IHNV and VHSV. Six cassettes for histology. PO #MP6109.

Additional History (21 Feb. 2006 e-mail from Diane Morrison to Gary Marty) - the swimbladder was full of blood (>10 mL) and in some cases mucoid type material.

Submission 2006-00595 **Case Report**

Histopathology

Six full cassettes of formalin-fixed tissues were submitted for histopathology. Cassettes were processed into paraffin; after processing, the gill and 1-3 other organs were embedded in a separate block (slide B); the remaining tissues were left together (slide A). Sides were labelled as follows:
Slide 1A (5586 (6)); also stained with Twort's Gram stain and GMS - liver, spleen, intestinal ceca, and mesenteric fat
Slide 1B - gill, heart
Slide 2A (5586 (4-3)) - liver, swimbladder, heart, intestinal ceca, and mesenteric fat
Slide 2B - gill, spleen, trunk kidney
Slide 3A (5586 (4-2)) - liver, trunk kidney, head kidney, spleen, skeletal muscle, intestine, intestinal ceca, and mesenteric fat
Slide 3B - gill, heart
Slide 4A (5586 (4-1)) - liver, swimbladder, heart, intestinal ceca, and mesenteric fat
Slide 4B - gill, spleen
Slide 5A (5586 (5)) - heart, spleen, swimbladder, intestinal ceca, and mesenteric fat
Slide 5B - gill, liver
Slide 6A (5586 (4)) - liver, heart, trunk kidney, spleen, intestinal ceca, and mesenteric fat
Slide 6B - gill, trunk kidney
All major organs on each slide were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is good to excellent for most organs; the tips of intestinal ceca sometimes have mild autolysis (this is common). Liver autolysis is none for most fish (slides 1A, 2A, 3A, 4A, 5A), and mild for one fish (slide 6A). Some large foci of erythrocytes (e.g., spleen in slide 6A) have precipitates of acid hematin; however, most sections have none or minimal acid hematin deposits. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. Margins of some organs have minimal postfixation dehydration (e.g., mesenteric fat in slide 1A).

Measures of physiologic condition
Hepatocellular glycogen, none (slides 1A, 2A, 3A, 4A, 5A, 6A)
Mesenteric adipose tissue depletion, none (slides 1A, 2A, 3A, 4A, 5A, 6A)
This pattern in the measures of physiologic condition is consistent with healthy growing fish (abundant mesenteric fat) that recently stopped feeding (no glycogen deposits).

Intestinal ceca (slide 1A) - The lumen of the intestinal ceca is filled and slightly distended by homogeneous deeply eosinophilic protein. Narrow tentacles of protein extend between villi to the crypts. Scattered fairly evenly through the luminal protein are small colonies of Gram-negative bacteria and macrophages distended by protein and occasional bacteria. Gram positive structures, each about 4 µm in diameter, are scattered primarily in the proteinaceous tentacles. About half of the polar capsule-like structures are within cells (no more than two per cell), whereas the rest of the polar capsule-like structures are free in the lumen. Some granules of eosinophilic granular cells are Gram positive. The intestinal epithelium is intact, columnar, and seems to be about normal. The proteinaceous material in the lumen (slide 1A) contains no GMS-positive material (i.e., no fungi). Intestinal ceca in slide 4A have abundant protein in the lumen and tentacles of protein between the villi, but no significant bacterial growth in the protein. Intestinal ceca in slide 3A have tentacles of protein between the villi, but the main lumen is not distended by protein.

Submission 2006-00595 **Case Report**

Virology

3 samples inoculated onto tissue culture - all negative.

* Results faxed on Mar 21/06.

Molecular Diagnostics/PCR

Samples A, B, C:

Infectious Hematopoietic Necrosis virus Negative by PCR.
Viral Hemorrhagic Septicemia virus Negative by PCR.

Diagnosis

1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 2A, 3A, 6A), moderate (slide 1A)
1b. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1A, 2A, 3A, 5B)
1c. Liver: hepatic necrosis, acute, multifocal, mild (slides 1A, 3A)
1d. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, with intracellular hemosiderin and lipofuscin, mild (slides 1A. 2A)
1e. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 6A)
2a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1A, 2B, 3A, 4B, 6A)
2b. Spleen: capillary fibrin, bifocal, subcapsular, acute, mild (slide 3A)
2c. Spleen: parenchymal golden pigment, disseminated, intracellular, mild (slide 6A), moderate (slide 5A)
3a. Intestinal ceca: luminal protein, diffuse, with scattered Gram-negative bacterial colonies and myxosporeans, mild (slides 3A, 4A), moderate (slide 1A)
3b. Intestinal ceca: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate (slide 6A)
4. Intestine: bacterial overgrowth, moderate (slide 3A)
5a. Heart: myocardial karyomegaly, multifocal, mild (slides 1A, 5A)
5b. Heart: epicarditis, multifocal, lymphoplasmacytic, mild (slide 3B)
5c. Heart: endothelial cell hypertrophy and hyperplasia, focal, mild (slide 6A)
6a. Swimbladder: serositis and vasculitis, fibrinous, with submucosal congestion and serosal edema, acute, multifocal, moderate (slide 4A)
6b. Swimbladder: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate (slide 5A)

Final Comments

These salmon have a number of lesions that are common in pen-reared Atlantic salmon in British Columbia. However, I have not previously seen the combination of intestinal ceca filled with protein, small colonies of Gram-negative bacteria, and myxosporeans (fish #6, slide 1A). It might be worth looking at these fish again, particularly if the sampling was related to increased mortality. The lesion is significant and probably contributed significantly to morbidity. Our Auditing and Surveillance Program does not routinely sample intestinal ceca, but ceca are commonly included with diagnostic cases. The protein probably is a result of leakage of blood plasma into the lumen. Lack of erythrocytes in the lumen is evidence that the lesion is not a result of hemorrhage. Possible causes include a bacterial infection, or exposure to endothelial toxins (endogenous or exogenous). The myxosporeans might have come from the feed.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins) or come from outside the fish (e.g., from the water or the feed). Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, Piscirickettsia salmonis). This case has no obvious organisms, and PCR results rule out VHSV. Proliferative lesions in the biliary system provide evidence for a toxic cause for the hepatic necrosis.

Pigment in the liver could be lipofuscin, hemosiderin, or both. Pigment in the spleen is probably just lipofuscin. Accumulation of lipofuscin in the liver and spleen is a non-specific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. Conditions that lead to moderate to abundant lipofuscin accumulation have been associated with decreased growth and survival in several studies. Hemosiderin accumulation in the liver is evidence of increased turnover of red blood cells.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other salmon it might be related to increased protein needed as part of an inflammatory response.

Peritonitis (spleen, swimbladder, and intestinal ceca) is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Multifocal fibrin deposits in the spleen (slide 3A) are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin (e.g., an early vaccine reaction). I have also seen this response in salmon that are PCR positive for VHSV. Lack of remodelling of the fibrin is consistent with these deposits forming just before death.

Swimbladder hemorrhage is an uncommon manifestation of viral and bacterial infections. It has been described as part of "Hemorrhagic smolt syndrome (HSS)" associated with a new alphavirus in Norwegian Atlantic salmon (Nylund et al. 2003) and in largemouth bass infected with largemouth bass virus (Hanson et al. 2001). As far as I know, alphaviruses have not been reported from cultured salmon in British Columbia. Petechiae are more commonly reported than frank hemorrhage. Ferguson (“Systemic Pathology of Fish,” 1989) reports that petechiae and ecchymoses are "particularly obvious with the endotheliotropic rhabdoviruses."

Bacterial overgrowth in the intestine is evidence of decreased intestinal motility. Bacteria are normally not seen on routine preparations of intestine from salmon. Decreased intestinal motility is also consistent with poor digestion.

Mild myocardial karyomegaly is fairly common in cultured salmonids, but the cause and significance is unknown. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease). Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Hypertrophy and

hyperplasia of endothelial cells in the heart is consistent with a systemic immune stimulation, probably a bacterial infection.

Fibrinous serositis and vasculitis in the outer wall of the swimbladder is evidence of systemic toxins; likely differentials include a bacterial infection and acute vaccine reactions. Congestion of the swimbladder propria-submucosa might be secondary to vasculitis.

Literature Cited:

Hanson, LA; Petrie-Hanson, L; Meals, KO; Chinchar, VG; Rudis, M 2001. Persistence of largemouth bass virus infection in a northern Mississippi Reservoir after a die-off. Journal of Aquatic Animal Health. 13(1):27-34.

Nylund, A; Plarre, H; Hodneland, K; Devold, M; Aspehaug, V; Aarseth, M; Koren, C; Watanabe, K 2003. Haemorrhagic smolt syndrome (HSS) in Norway: Pathology and associated virus-like particles. Diseases of Aquatic Organisms 54(1):15-27.

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00064 **Date** 06-Jan-2006 **Report** 16-Jan-2006

Report Copies

Submitter: 11036 Mainstream Canada (M)
Owner 15630 Mainstream 05-07 Cliff Bay
Farm:
Vet Clinic:
Attending

Specimen: Other **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted one agar plate for culture and sensitivity.

Internal hemorrhage on pylorus, swim bladder, and flesh. Kidney slightly swollen

Report to Jeanine Sumner. Submission form requests to see attached PO#, however, there is no PO# attached.

Bacteriology

See attached sheet for results.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00689 **Date** 23-Feb-2006 **Report** 03-Mar-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 15913 #5595, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue-Fresh f Forma **Count** 3 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-00689

Case Report

History/Symptoms

Submitted 2 histo and 3 viral samples. Virology (2 fish pools: 6 fish in total). Two histo for follow up to case #5584. Three samples fro virology and PCR for IHN and VHS. Tissues for virology have not been frozen.

Mortality has increased, many fish with mouth rot and septicemia. Samples sent in to rule out any viral agents.

PO# CL5403.

Histopathology

Two cassettes of formalin-fixed tissues were submitted for histopathology. Sides were labelled as follows:

Slide 1 - liver, heart, head kidney, spleen, intestinal ceca, and mesenteric fat
Slide 2 - liver, heart, head kidney, spleen, stomach, intestinal ceca, and mesenteric fat
All major organs on each slide were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is excellent for all organs. Margins of some organs have minimal postfixation dehydration (e.g., liver in slide 1). Sections have no acid-hematin deposits.

Measures of physiologic condition
Hepatocellular glycogen, none (slides 1, 2)
Mesenteric adipose tissue depletion, none (slides 1, 2)
This pattern in the measures of physiologic condition is consistent with healthy growing fish (abundant mesenteric fat) that recently stopped feeding (no glycogen deposits).

Virology

3 samples inoculated onto tissue culture - all negative.

* Results faxed on Mar 21/06.

Molecular Diagnostics/PCR

Samples 1-2, 3-4 and 5-6: IHN and VHS Virus negative by PCR.

Submission 2006-00689 **Case Report**

Diagnosis

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 1)
1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 1, 2)
1c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1, 2)
2a. Spleen: ellipsoid capillary hypertrophy, diffuse, mild (slide 2)
2b. Spleen and mesenteric fat: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, mild (slides 1, 2)
3. Heart: endothelial cell hypertrophy and hyperplasia, multifocal, mild (slides 1, 2)

Final Comments

Changes in these fish are consistent with immune stimulation. Various changes are most consistent with vaccinated fish that have a systemic bacterial infection.

Biliary preductular cell hyperplasia in the liver is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins) or come from outside the fish (e.g., from the water or the feed). Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other salmon it might be related to increased protein needed as part of an inflammatory response.

Hypertrophy and hyperplasia of endothelial cells in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Hypertrophy of the ellipsoid capillaries in the spleen is also consistent with systemic immune stimulation

Granulomatous peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

/bb/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00770 **Date** 02-Mar-2006 **Report** 13-Mar-2006

Report Copies

Submitter: 11036 Mainstream Canada (M)
Owner 15944 Mainstream 06-06 PO7510888
Farm:
Vet Clinic:
Attending Dr. Peter McKenzie

Specimen: Other **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-00770

Case Report

History/Symptoms

Submitted one agar plate for culture and sensitivity.

Hatcher has history of BKD. Fish are about 200-300g.

K,L,S slightly swollen. Popeye.

PO# 7510888

Submitted by Jeanine Sumner.

Bacteriology

Bact. plate 4-2: Vibrio logei.
Bact. plate 2-3: Yersinia ruckeri.

Yersinia sensitive to: Florfenicol, Romet 30, Tri-sulfas, Sulfa-methox-trimeth. and Oxytetracycline.

* Results faxed Mar. 13/06.

/bb

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00837 **Date** 06-Mar-2006 **Report** 09-Mar-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 15981 #5579, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Other **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-00837

Case Report

History/Symptoms

Submitted one bacteriology sample taken from muscle lesion. Please ID.

PO# CL9202.

Atlantic, regular, 2004, saltwater.

Bacteriology

See attached sheet for culture and sensitivity results.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00838 **Date** 06-Mar-2006 **Report** 13-Mar-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 15982 #5601/5608, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 4 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

History/Symptoms

Submitted four pooled samples for virology - PCR for IHN and VHS. Samples are from the same site. Samples 5601 (1 and 2) and 5601 (3 and 4) have been previously frozen. Samples 5608 (pen 6 and 10) and 5608 (pen 1 and 4) are fresh, not frozen.

Please quote PO # CL9201 on the invoice.

Atlantic, regular, 2006, saltwater.

Virology

4 samples inoculated onto tissue culture - all negative.

* Results faxed on Mar 29/06.

Molecular Diagnostics/PCR

Infectious Hematopoietic Necrosis Virus negative by PCR.
Viral Hemorrhagic Septicemia Virus negative by PCR.

* Results faxed Mar. 13/06.

/bb/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00868 **Date** 07-Mar-2006 **Report** 09-Mar-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16003 #5610, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Formalized **Count** 4 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-00868

Case Report

History/Symptoms

Submitted 4 tissue samples for histology.

Please send copy of report to Dr. Diane Morrison, Cilka LaTrace, Brad Boyce, and Meghan Penney. PO# MP6116.

Additional history received by e-mail from Dr. Morrison: bacteriology yielded no growth.

Histopathology

Four full cassettes of formalin-fixed tissues were submitted for histopathology. Cassettes were processed into paraffin; after processing, organs were arbitrarily separated and embedded into 2 blocks (resulting in slide A and slide B). Sides were labelled as follows:
Slide 1A (5610-) - liver, gill, heart
Slide 1B - trunk kidney, head kidney, spleen, swimbladder (?), intestinal ceca, and mesenteric fat
Slide 2A (5610-2) - liver, spleen, gill
Slide 2B - trunk kidney, head kidney, heart, intestinal ceca, and mesenteric fat
Slide 3A (5610-3) - liver, gill
Slide 3B - heart, trunk kidney, head kidney, spleen, intestinal ceca, and mesenteric fat
Slide 4A (5610-) - trunk kidney, gill, heart,
Slide 4B - liver, spleen, head kidney, intestinal ceca, and mesenteric fat
All major organs on each slide were examined. Organs not listed below have no significant lesions.

Quality control: Tissue preservation is good to excellent for most organs; the tips of intestinal ceca sometimes have mild autolysis (this is common). Liver autolysis is none for all fish (slides 1A, 2A, 3A, 4B). Some large foci of erythrocytes (e.g., spleen in slide 1B) have precipitates of acid hematin; however, most sections have none or minimal acid hematin deposits. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. Tissues have no postfixation dehydration.

Measures of physiologic condition
Hepatocellular glycogen, none (slides 1A, 2A, 3A, 4B)
Mesenteric adipose tissue depletion, none (slides 1B, 2B, 3B, 4B)
This pattern in the measures of physiologic condition is consistent with healthy growing fish (abundant mesenteric fat) that recently stopped feeding (no glycogen deposits).

Submission 2006-00868 **Case Report**

Diagnosis

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 1A, 2A, 3A)
1b. Liver: hepatocellular eosinophilic cytoplasmic inclusions, multifocal, mild (slides 1A, 2A, 3A)
1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 2A, 3A)
1d. Liver: vasculitis, lymphohistiocytic, with fibrin, focal, moderate (slide 3A)
1e. Liver: hepatic necrosis, acute, multifocal, mild (slide 3A)
1f. Liver: hepatic necrosis, peracute, zonal, moderate (slide 4B)
1g. Liver: yellow-brown sinusoidal macrophages, disseminated, mild (slide 4B)
2a. Trunk kidney: intracytoplasmic eosinophilic granules in endothelial cells, diffuse, mild (slide 1B)
2b. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, mild (slide 2B)
3a. Head kidney: intracytoplasmic eosinophilic granules in endothelial cells, diffuse, moderate (slide 1B)
3b. Head kidney: nephritis, interstitial, granulomatous, multifocal, mild (slides 1B, 2B, 3B)
4a. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, mild (slide 3B), moderate (slide 2A)
4b. Spleen: protein (fibrin?), diffuse, mild (slide 4B)
5. Intestinal ceca and mesenteric fat: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, mild (slide 3B)
6. Heart: Kudoa thyrsites pseudocyst, focal, 70 x 40 µm, mild (slide 4A)
7. Intestinal ceca: crypt epithelial cell hyperplasia and karyorrhexis, diffuse, moderate (slide 4B)

Submission 2006-00868 **Case Report**

Final Comments

These fish have several nonspecific changes consistent with a bacterial or viral infection; consider toxin exposure as a less likely differential.

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins) or come from outside the fish (e.g., from the water or the feed). Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

I occasionally see homogeneous cytoplasmic inclusions in hepatocytes of fish that have been exposed to toxins, although this change is not described in common fish pathology books. Hepatocellular inclusions might be phagocytosed cellular debris or plasma protein, or accumulation of protein synthesized by hepatocytes; viral inclusions are unlikely. Transmission electron microscopy might help determine the nature of this change, but TEM is not available at the Animal Health Centre. The deposits might be related to inflammation; e.g., lymphohistiocytic vasculitis in the liver in slide 3A. Consider an underlying bacterial cause like Yersinia ruckeri.

Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., VHSV). The pattern in slide 3A is fairly common, with discrete foci that have hepatocellular pyknosis and hypereosinophilic cytoplasm. By comparison, the pattern in slide 4B is not common. In 4B, hepatocytes in early stages of necrosis form a reticular pattern composed of bands of degenerating hepatocytes; each band is about 60 µm wide. Fish livers lack the distinct lobular pattern of mammals, but the pattern in slide 4B is almost certainly related to the vascular supply (afferent and efferent vessels cannot be differentiated unless the vessels are injected with dye).

Pigment in the liver (slide 4B) could be lipofuscin, hemosiderin, or both. Accumulation of lipofuscin in the liver is a non-specific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. Lipofuscin in slide 4B is evidence that this fish had suffered previous bouts of hepatic necrosis. Hemosiderin accumulation in the liver is evidence of increased turnover of red blood cells.

In the kidney, endothelial cell cytoplasm distended by eosinophilic granules is a distinctive finding in Atlantic salmon. As a differential, these granules might be part of eosinophilic granular cells; however, granule size seems too large and variable for eosinophilic granular cells. I have seen these granules associated with a case of Piscirickettsia salmonis, but other cases have no known cause.

Granulomatous inflammation in the head kidney and trunk kidney is poorly defined and is more likely a result of a chronic infection with Yersinia ruckeri or other bacteria than with Renibacterium salmoninarum, the cause of bacterial kidney disease. Bacteriology or PCR is needed for a more definitive diagnosis.

Peritonitis (spleen, intestinal ceca, and mesenteric fat) is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. In slide 4B, the red pulp is expanded my poorly defined bands of protein that might be fibrin. Fibrin deposits are consistent with inflammation elsewhere in the fish (e.g., the liver and kidney).

Kudoa thyrsites is a myxosporean that is most common in skeletal muscle, but sometimes occurs in heart muscle. This example has characteristic stellate spores with four unequal polar capsules converging on one end. This case has no associated inflammation.

Hyperplasia and karyorrhexis of crypt epithelial cells of the intestinal ceca is a distinctive finding. Hyperplasia occurs as a result of increased demand for epithelial cells, and karyorrhexis occurs during normal turnover (apoptosis) or pathologic conditions; the specific cause in fish 4 in unknown.

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2006-00914 **Date** 10-Mar-2006 **Report** 27-Mar-2006

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 16021 #5610, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 3 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2006-00914

Case Report

History/Symptoms

Submitted 3 (pooled) samples for virology and PCR for IHN and VHS.
Samples have been previously frozen.

Species - Atlantic. Sex - Regular. Saltwater.

Please quote PO #CL9205 for accounting purposes.

Submitted by Cilka LaTrace.

Virology

3 pools inoculated onto tissue culture - all negative.

* Results faxed on Apr 5/06.

Molecular Diagnostics/PCR

Samples Pen 7: 1-2, 3-4, 5-6 - IHNV and VHSV Negative by PCR.

* Results faxed on Mar 27/06.

/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2007-01013 **Date** 13-Mar-2007 **Report** 19-Mar-2007

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 18703 Log #6086, PO# CL1352
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 6 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2007-01013

Case Report

History/Symptoms

Submitted fresh salmon tissue for PCR IHN and VHS testing.

6 virology samples collected on Mar 8/07 and frozen.

Sex: regular
Saltwater
Tank ID: pens 1-6
Log no.: 6086
PO#: CL 1352

Virology

6 samples inoculated onto tissue culture - all negative.

* Results faxed on Apr 11/07.

Molecular Diagnostics/PCR

Samples A (6086-1) and C (6086-3) are Viral Hemorrhagic Septicemia Virus positive by PCR.

Samples B (6086-2), D (6086-4), E (6086-5) and F (6086-6) are Viral Hemorrhagic Septicemia Virus negative by PCR.

Samples A (6086-1), B (6086-2), C (6086-3), D (6086-4), E (6086-5), and F (6086-6) are Infectious Hematopoetic Necrosis Virus negative by PCR.

/sr/mm

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2007-01043 **Date** 14-Mar-2007 **Report** 16-Mar-2007

Report Copies

Submitter: 11036 Mainstream Canada (M)
Owner 11036 Mainstream Canada (M)
Farm:
Vet Clinic: 9420 Sea to Sky Veterinary Service
Attending

Specimen: Tissue - Formalized **Count** 1 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2007-01043 **Case Report**

History/Symptoms

Submitted formalized salmon tissue for histology. Unusual swimming pattern. No obvious internal lesions, fairly skinny fish. Farm name: Bedwell.
PO# 6922
Submitted by: Sean Day

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology. All cassettes were labelled "Bedwell Mar 8/07". Tissues from each cassette were embedded in paraffin and the resultant block was sectioned 3 times: face and two deeper levels, each 250 µm deeper to the previous section (this was done in an attempt to include more of the brainstem in the sections examined)
Slide 1 - brain (3 pieces), spleen (2 pieces), head kidney, trunk kidney (3 pieces).
Slide 2 - brain (2 pieces), spleen (2 pieces), trunk kidney (4 pieces), and mesenteric fat (near 1 piece of spleen).
All organs were examined. Organs not listed below have no significant lesions.

Quality control: Trunk kidney autolysis: none (slides 1, 2). Organs have no postfixation dehydration and no acid hematin deposits.

Diagnosis

1. Trunk kidney: renal tubular epithelial necrosis with regeneration, multifocal, subacute, mild (slide 1, 1 of 3 pieces), moderate (slide 2, 1 of 4 pieces)
2. Brain (nuclei of the torus semicircularis, deep to the optic tectum): encephalitis, diffuse, lymphohistiocytic, with neuropil and intraneuronal microsporidia ("Microsporidium cerebralis"), mild (slide 2, deeper level 2), moderate (slide 1, deeper levels 1 and 2)
3a. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slide 1, both pieces; slide 2, 1 piece)
3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1, one piece)
4. Head kidney: moderate numbers of intracytoplasmic eosinophilic granules in endothelial cells, diffuse, moderate (slide 1)
5. Mesenteric fat: vascular congestion, diffuse, severe (slide 2)

Final Comments

These fish have several lesions consistent with septicemia: renal tubular necrosis, eosinophilic granules in the kidney, and vascular congestion of mesenteric fat. The primary differential this time of year is VHSV, but bacteria or other viruses cannot be ruled out without further diagnostic work.

Inflammation associated with microsporidians in the brains is consistent with the clinical signs of "unusual swimming pattern". Indeed, the first sections did not contain obvious organisms, and I ordered deeper sections only because the history was suggestive of Microsporidium cerebralis. In future cases, I think the best way to ensure that appropriate parts of the brain are included in the first (and usually only) section examined is to remove the brain in one piece and then cut the brain mid-sagittally before placing the cut surfaces face down on the bottom of the cassette (the face at the bottom of the cassette is the face that will be sectioned for the slide. Parasite spores often occur in clusters, but they do not seem to form distinct xenomas (as occur with another microsporidian parasite, Loma salmonae). The brain microsporidian species in these fish has not been officially described, but it was given the temporary name of Microsporidian cerebralis when described as part of a case report by Brocklebank et al. (1995).

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Renal tubular epithelial necrosis was fairly common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 7.5%; n = 495) and Pacific salmon (prevalence = 2.2%; n = 134); the cause was not determined in many cases. Differentials include viral hemorrhagic septicemia virus (VHSV) and exposure to toxins (e.g., bacterial toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin).

The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. Small amounts of splenic lipofuscin are consistent with the observations that these were "fairly skinny fish". Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Accumulation of eosinophilic granules in the cytoplasm of cells lining small vessels in the kidney is a distinctive finding in Atlantic salmon. These granules probably accumulate in response to systemic immune stimulation. The granules might be part of eosinophilic granular cells or endothelial cells; granule size seems too large and variable for eosinophilic granular cells. I have seen these granules associated with Piscirickettsia salmonis, but other cases (like this one) have no known cause. Eosinophilic granular cells have been associated with experimental infection of rainbow trout with Listonella anguillarum (Lamas et al. 1991), but I have not seen this pattern of inflammation described in Atlantic salmon exposed to Listonella anguillarum.

Distension of blood vessels in the fatty mesenteries of the abdominal cavity is a classic sign of toxins in the blood; common causes include bacterial or viral infections.

Literature Cited:

Brocklebank, J.R., Speare, D.J., and M.L. Kent. 1995. Microsporidian encephalitis of farmed Atlantic salmon (Salmo salar) in British-Columbia. Canadian Veterinary Journal 36 (10): 631-633.

Lamas, J., Bruno, D.W., Santos, Y., Anadon, R., and A.E. Ellis. 1991. Eosinophilic granular cell response to intraperitoneal injection with Vibrio anguillarum and its extracellular products in rainbow trout, Oncorhynchus mykiss. Fish Shellfish Immunol. 1(3):187-194.

/sr

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2007-01256 **Date** 28-Mar-2007 **Report** 30-Mar-2007

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 18828 Log 6102, PO# CL 1359, Marine Harvest
Farm:
Vet Clinic:
Attending

Specimen: Tissue-Fresh f Forma **Count** 5 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Submission 2007-01256

Case Report

History/Symptoms

Submitted one histo cassette and four virology samples.

Histo cassettes labelled 6102 - 1, sorry, no gross lesions noted. Four samples for PCR for IHNV and VHSV. A Salmonicida isolated from 1 of 12 bacteriology samples taken (44 fish sampled in total) on March 22, 2007.

Histopathology

Formalin-fixed tissues were submitted in a cassette for histopathology. The resultant slide contains a section of spleen, liver, heart, head kidney, trunk kidney, intestinal ceca and mesenteric fat; step sections were stained with H&E and Twort's Gram stain. All organs were examined. Organs not listed below have no significant lesions.

Quality control: Intestinal autolysis: moderate (this can be minimized by submitting pieces of ceca that are no more than 4 times longer than the diameter of the ceca; both ends of the submitted piece should be cut transversely to speed penetration of fixative. Large foci of erythrocytes (e.g., hepatic vessels in slide 1A) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and tissues become acidic during fixation. Organs have no postfixation dehydration.

Measure of physiologic condition
Hepatocellular glycogen: none
Mesenteric fat, moderate amounts
This pattern in the measure of physiologic condition is consistent with a fish that was not been eating normally for the past several days.

Virology

4 samples inoculated onto tissue culture - all negative.

* Results faxed on Apr 24/07.

Molecular Diagnostics/PCR

Samples A (6102-1), B (6102-2), C (6102-3) and D (6102-4) are Viral Hemorrhagic Septicemia Virus and Infectious Hematopoetic Necrosis Virus Negative by PCR.

Submission 2007-01256 **Case Report**

Diagnosis

1a. Liver: hepatitis, granulomatous, multifocal, moderate
1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
1c. Liver: hepatocellular fatty change (lipidosis), multifocal, mild
2. Heart, peripheral compact layer of ventricle: myocarditis, granulomatous, diffuse, severe
3. Intestinal ceca: enteritis and peritonitis, granulomatous, multifocal, moderate
4a. Spleen: parenchymal edema, diffuse, mild
4b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
5. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate

Final Comments

The most common organism associated with disseminated granulomas in salmon is Renibacterium salmoninarum, the cause of bacterial kidney disease. However, because the Gram stain contains no organisms (several fields were searched using the 100× oil immersion lens), other differentials should be considered: Yersinia ruckeri and a vaccine reaction. The section has no Aeromonas salmonicida.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response.

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Splenic parenchymal edema is a change I noticed recently in a case from the Provincial Fish Health Auditing Program from which Yersinia ruckeri was cultured. Both splenic parenchymal edema and the culture of Yersinia ruckeri are uncommon in our auditing program; I suspect that other bacteria can cause similar lesions. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease. Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 22%; n = 495) and Pacific salmon (prevalence = 38%; n = 134). Ferguson ("Systemic Pathology of Fish," 1989) reports an association of renal protein droplets and high ammonia levels in salmonids. Roberts ("Fish Pathology, Third Edition," 2001) describes the change, but offers no cause.

/sr

Ministry of
Agriculture, Food and Fisheries
1767 Angus Campbell Road

Abbotsford BC V3G 2M3 Telephone: (604) 556-

Facscimile: (604) 556-3010
Toll=Free: 1-800-661-9903

3003

ANIMAL HEALTH CENTRE

AAVLD - Accredited Laboratory

Case Report

Submission 2007-00182 **Date** 16-Jan-2007 **Report** 22-Jan-2007

Report Copies

Submitter: 9439 Marine Harvest Canada (M)
Owner 18208 PO#CL6666, Marine Harvest Canada
Farm:
Vet Clinic:
Attending

Specimen: Tissue - Fresh **Count** 2 **Flock Herd Size:**
Species: Atlantic Salmon **Age**
Breed: **Sex:**

Feed: **Feed**

Vaccination

Treatmen

Diagnosis

Gary D. Marty, D.V.M., Ph.D.;
Diplomate, A.C.V.P
Fish Pathologist

Case Report

History/Symptoms

Submitted fresh salmon tissue for PCR IHNV and VHSV testing.

ID: 6014 #1 and 6014#2

Gross lesions suspect of VHS

Gross Pathology

N/A

Virology

Two samples inoculated onto tissue culture - both negative.

* Results faxed on Feb 15/07.

Molecular Diagnostics/PCR

Samples A (6014 #1) and B (6014 #2) are VHSV Positive by PCR.
Samples A (6014 #1) and B (6014 #2) are IHNV Negative by PCR.

/mm