

**ANIMAL HEALTH CENTRE**

AAVLD - Accredited Laboratory

Ministry of  
Agriculture, Food and Fisheries  
1767 Angus Campbell Road

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

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

**Case Report**

**Submission** 2005-01065      **Date** 31-Mar-2005      **Report** 05-Apr-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 13865 #5252, 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

### **History/Symptoms**

Submitted 2 cassettes for histology. Log #5252.

Species - Atl., Regular, Saltwater entry - 2005. Netpen 2U.  
Mortality low but increased in silvers.

No signs of infectious disease but there is an increase in mortality and morbidity of silvers from one pen. No significant growth on bacteriology. Tissue imprints reveal no apparent findings.

Two histo. cassettes labelled CUR 1 and CUR 2. CUR 1 is from a moribund silver with cataracts. CUR 2 is from a fresh dead silver.

Submitted by Cilka LaTrace/ Diane Morrison.

### **Histopathology**

2 cassettes of tissues were submitted for histopathology

Slide 1 (CUR13 3/24/5): gill, brain, heart, skeletal muscle, head kidney, trunk kidney, liver, intestinal ceca, mesenteric fat, and spleen.

Slide 2 (CUR1 3/24/5): gill, heart, skeletal muscle (3 pieces), head kidney, trunk kidney, liver, intestine, intestinal ceca, mesenteric fat, and spleen.

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

Quality control: In slide 1, tissue preservation for most organs is good (liver and intestine have mild autolysis). In slide 2, tissue preservation is excellent for all organs. Tissues have no evidence of postfixation dehydration.

Measure of physiologic condition:

Hepatocellular glycogen depletion, severe (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 (severe glycogen depletion).

### **Diagnosis**

1. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate (slide 1).
2. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 1, 2).
3. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 2).



**Final Comments**

All 3 abnormalities are fairly common in cultured Atlantic salmon. 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 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. 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).

/sr



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## Case Report

**Submission** 2005-01073      **Date** 01-Apr-2005      **Report** 07-Apr-2005

**Report**      **Copies**

**Submitter:** 8447      Stolt Sea Farm Inc.  
**Owner** 12340      Koskimo  
**Farm:**  
**Vet Clinic:** 9348      Canadian Animal Hth Mgmt Serv.  
**Attending**      Dr. B. Cox

**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

### **History/Symptoms**

Submitted 2 sets of tissue for histology (05-35).

Both fish had gross lesions in the liver.

Submitted by Brad Boyce.

Bill to PO # 56921.

LAB NOTE:

Cassettes labelled as 04-35, not 05-35.

### **Histopathology**

Histopathology: 2 sets of preserved tissues were submitted for histopathology

Slide 1-1: gill

Slide 1-2: liver (2 pieces), intestine, skin/ skeletal muscle

Slide 1-3: heart, trunk kidney (2 pieces), intestinal cecum, mesenteric fat, and spleen

Slide 1-4: brain

Slide 2-1: heart, trunk kidney, head kidney, spleen, and liver

Slide 2-2: skin/ skeletal muscle, intestine, and mesenteric fat

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

Quality control: In set 1, tissue preservation for most organs is fair; the gill and one piece of liver have severe autolysis. In set 2, tissue preservation for most organs is good, but the intestine has moderate autolysis.

Measures of physiologic condition:

Hepatocellular glycogen depletion, severe (slides 1-2, 2-1).

Mesenteric adipose tissue depletion, none (slides 1-3, 2-2).

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

### **Diagnosis**

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 1-2), severe (slide 2-1).
2. Skeletal muscle: myonecrosis, white fibers, peracute, multifocal, mild (slide 1-2).
3. Heart: endocarditis, focal, lymphoplasmacytic, mild (slide 2-1).
4. Head kidney: nephritis, interstitial, granulomatous, multifocal, mild (slide 2-1).



**Final Comments**

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Myonecrosis in the fish in slide 1-2 is limited to scattered individual fibers with flocculent cytoplasm and loss of cross striations. This pattern is consistent with agonal contractions.

The epidermis is missing from part of the surface of skin in slides 1-2 and 2-2; lack of filamentous bacteria supports the hypothesis that this loss is an artifact rather than an ulcer. Single large vacuoles in neuron cell bodies in the brain (slide 1-4) are probably normal.

Lymphoplasmacytic inflammation in the heart is evidence of chronic immune stimulation (e.g., low grade bacterial infection), but the cause is otherwise unknown.

The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.

/sr



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**Case Report**

**Submission** 2005-01121      **Date** 05-Apr-2005      **Report** 07-Apr-2005

**Report**      **Copies**

**Submitter:** 11899 Grieg Seafoods B.C. Ltd.

**Owner** 11899 Grieg Seafoods B.C. Ltd.

**Farm:**

**Vet Clinic:**

**Attending** Dr. Barry Milligan

**Specimen:** Tissue-Fresh f Forma      **Count**      **Flock Herd Size:** 0

**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**

Farm name: Muehal at South. Age: 2004.

Recently submitted samples from Campbell River without the AHC submission form contained within. I would like to have virology performed on the fresh tissues and IHN PCR if possible. I would like histology on the tissue within the two histosettes labelled MS Apr. 4. I am faxing the form as well as this letter (2 pages total). In addition to the samples noted in the body of the form, I also included 3 additional histosettes.

1) AP Feb 28 (2003G Atlantic salmon skin samples). At harvest there has intermittently been a superficial rash. Upon inspection, I believe it to be superficial hemorrhaging as a result of inappropriate handling. If possible, I would like confirmation.

2) Unlabelled histosette (2004G Atlantic salmon samples). Includes tissues from 3 moribund non-performers with signs of cataracts. Incidence of cataracts appears highly correlated with poor performing stock.

3) WP Mar 13 (2003G Atlantic salmon samples from one harvest fish). Samples include generalized 2 to 4mm tubercles seen throughout visceral organs and peritoneum. The stock had a history of BKD.

Submission form history:

Inappetant 5-6 days - largely due to presence of sea lions (50+) hunting slowly at SW face and gaping (moribund fish sampled). Note similar signs seen in last few months with large volumes of freshwater from Gold River.

PM lesions: pale gills/livers on several fish (moribund). Sampled - some part of pale liver likely due to inappetance. No signs of external or internal hemorrhaging.

3 X multiorgan tissue samples and 2 histo cassettes labelled.

- 1) virology on tissue samples Apr. 04.
- 2) and IHN PCR if available.
- 3) histology (see attached letter).





**Histopathology**

5 cassettes of preserved tissues were submitted for histopathology. Three of the cassettes were too full; the tissues were split and processed onto two slides (A and B).

Slide 1A (MSAPR4): mesenteric fat; 2 pieces each of spleen, liver, and trunk kidney

Slide 1B (MSAPR4): gill, liver

Slide 2 (AF FE28): skin (4 pieces), skeletal muscle (included with 3 of the skin pieces)

Slide 3A (MS): gill, liver, head kidney, and trunk kidney

Slide 3B (MS): trunk kidney (3 pieces), Corpuscle of Stannius (1), and spleen (2 pieces)

Slide 4 (WP MAR 15): skeletal muscle (2 pieces), and bony skin

Slide 5A: section of 3 eyes (only 1 section includes the lens)

Slide 5B: 2 pieces each of liver and trunk kidney (with several Corpuscles of Stannius)

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

Quality control: Tissue preservation for most organs is fair to good.

Measures of physiologic condition:

Hepatocellular glycogen depletion, severe (slide 1A, 1B, 3A, 5B).

Mesenteric adipose tissue depletion, none (slides 1A).

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**

3 pooled samples inoculated onto tissue culture - all negative.

\* Results faxed Apr. 28/05.

**Molecular Diagnostics/PCR**

IHN Virus negative by PCR.

VHS Virus negative by PCR.

\* Results faxed Apr. 11/05.



**Diagnosis**

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 1A)
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 1A)
- 1c. Liver: hepatic necrosis, acute, multifocal, mild (slide 1B)
- 2a. Spleen and fatty mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1A)
- 2b. Spleen: parenchymal golden pigment, disseminated, intracellular, mild (slide 1A, 3B)
- 3a. Skin: superficial ulcer, diffuse, moderate (slide 2)
- 3b. Skin: acanthosis and dermal fibroplasia, diffuse moderate (slide 4)
4. Skeletal muscle: myositis, granulomatous, multifocal, coalescing, with central necrosis, consistent with Bacterial Kidney Disease, severe (slide 4)
5. Eye: phacolysis, choroidal hyperplasia, and abundant scleral eosinophilic granular cells, severe (slide 5A)

**Final Comments**

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). 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.

The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin 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 have been associated with decreased growth and survival in several studies.

The epidermis is missing from the entire surface of one of the skin biopsies in slide 2. The other pieces have only part of the surface of skin missing. None of the pieces have filamentous bacteria on the denuded surface; therefore, some of these changes might be artefacts rather than an ulcer. The changes are consistent with the clinical impression of acute ulcers. In slide 4, epidermal hyperplasia and dermal fibroplasia are evidence of chronic irritation; this section has no evidence of bacterial kidney disease.

Granulomatous inflammation in the skeletal muscle is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* is the most common organism associated with these lesions.

Lesions in the eye are consistent with the gross impression of cataracts. Cataracts in farmed Atlantic salmon are most commonly associated with nutritional causes.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated (Mutoloki et al. 2004).

S. Mutoloki, S. Alexandersen, and Ø. Evensen. 2004. Sequential study of antigen persistence and concomitant inflammatory reactions relative to side-effects and growth of Atlantic salmon (*Salmo salar* L.) following intraperitoneal injection with oil-adjuvanted vaccines. *Fish & Shellfish Immunology* 16(5):633-644.

/sr





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**Case Report**

**Submission** 2005-01187      **Date** 07-Apr-2005      **Report** 18-Apr-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13944      A.3.2-36

**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**

Provincial Surveillance Program. Specimens: subculture A.3.2-36(7). For bacteriology - identification of isolates and C & S.

**Bacteriology**

Bact. isolate - *Brochothrix thermosphacta* isolated.

Sensitive to: Florfenicol only.

\* Results faxed Apr. 18/05.

/bb

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**Case Report**

**Submission** 2005-01192      **Date** 07-Apr-2005      **Report** 18-Apr-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 13849 #5253, Marine Harvest  
**Farm:**  
**Vet Clinic:**  
**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



**History/Symptoms**

PO #508397

Atlantic salmon. Brookstock. Saltwater. Sample size: gills.

Continuation of case #5253, 2005-1032. Gills for histopathology. Continued mortality at sea site. Gill damage visible on dead fish.

**Histopathology**

Four gills (slides 1 - 4) submitted for histopathology. All gills on each slide were examined. Gills not listed below had no significant lesions.

Quality control: Tissue preservation for most gills is excellent. The margins of the gills in slides 3 and 4 have evidence of mild dehydration after fixation.

**Diagnosis**

1. Gill: lamellar fusion, multifocal, with intralesional protozoa (20-25 µm diameter) consistent with *Paramoeba* sp. (Amoebic gill disease), moderate (slide 4)
2. Gill: branchitis and filament bone thickening, focal, with abundant eosinophilic granular cells, small numbers of multinucleate giant cell macrophages, and lamellar epithelial hypertrophy, moderate (slide 1)
3. Gill: lamellar fusion, multifocal, mild (slide 1), moderate with capillary thrombosis (slide 2)
4. Gill: telangiectasis, focal, with thrombosis and recanalization, chronic, mild (slide 1), moderate (slide 2)

**Final Comments**

Amoebic gill disease is a serious, and often limiting condition, which is most commonly associated with prolonged periods of high water temperature [reference: Roberts "Fish Pathology, Third Edition," 2001; p. 71]. Paramoeba are best diagnosed on wet mount because they tend to fall off the gill during tissue processing; I recommend confirming my diagnosis by examination of wet mounts of affected gills. The few organisms in the affected section (slide 4) are mostly in small pockets created by fused lamella. Significant infections with Paramoeba are associated with multifocal pale areas on the gill; affected fish may gasp at the surface. Heavy infections are most common in fish that are already compromised by other diseases or smoltification problems. Lesions in the other gills might be a result of Paramoeba infection, but other sections lack clearly identifiable organisms.

Telangiectasis in the gill most commonly results from trauma (e.g., handling). Lamellar fusion results from disruption of the normal lamellar surface mucus film; it has been associated with fish breathing at the surface during periods of low oxygen and with exposure to heavy metals.

Inflammation in the gill (slide 1) is evidence of chronic immune stimulation. Inflammation in slide 1 is limited to a single piece of a filament, about 2 mm long. Changes are similar to the previous case (2005-01032), except that this case includes evidence of a healing microfracture in the associated filament (i.e., the focus of bone thickening in the filament).

/sr



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**Case Report**

**Submission** 2005-01199      **Date** 08-Apr-2005      **Report** 18-Apr-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 13949 #5258, 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

**History/Symptoms**

Log No. 5258  
Date: April 7/05  
Species: Atlantic  
Sex: regular  
Sample size: 5 samples for virology, 3 histo cassettes  
Saltwater entry: 2004  
Netpen/tank ID: all  
Saltwater  
Mortality: steady  
Samples for virology labeled: 1-3; 4-6; 7-9; 10-12; 13-15. Three histo cassettes from moribund fish with net rub, reddening of belly and around vent. No other visible lesions.

Contact: Cilka LaTrace or Dr. Diane Morrison  
Please quote PO #508400

**Histopathology**

Three cassettes of preserved tissues were submitted for histopathology.  
Slide 1 (GC-1 4/5/05): Trunk kidney, head kidney, heart, spleen, liver, intestine, intestinal ceca, mesenteric fat, exocrine pancreas, skeletal muscle, and gill.  
Slide 2 (GC-2 4/5/05): Trunk kidney, head kidney, heart, spleen, liver, intestine, intestinal ceca, mesenteric fat, exocrine pancreas, skeletal muscle, brain, and gill.  
Slide 3 (GC-3 4/5/05): Trunk kidney, head kidney, heart, liver, intestine, intestinal ceca, mesenteric fat, exocrine pancreas, skeletal muscle, brain, and gill.

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

Quality control: Tissue preservation for most organs is excellent. The margins of many organs have evidence of mild dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cells types stains poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Measures of physiologic condition:  
Hepatocellular glycogen depletion, severe (slides 1, 2, and 3)  
Mesenteric adipose tissue depletion, none (slides 1, 2, and 3)

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**

5 pooled samples inoculated onto tissue culture - all negative.

\* Results faxed May 4/05.



**Molecular Diagnostics/PCR**

Samples 1-3, 4-6, 7-9, 10-12, 13-15, VHS and IHN Virus Negative by PCR.

**Diagnosis**

1. Spleen and surrounding fatty mesenteries: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, mild (slides 1, 2, 3).
2. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1, 3).
3. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 2, 3), severe (slide 1).
4. Skeletal muscle: myonecrosis, peracute, multifocal, mild (slides 1, 3).
5. Heart: endocarditis, regionally diffuse, with a thin layer of macrophages and lymphocytes, moderate (slide 1).

**Final Comments**

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.

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

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.

The pattern of inflammation in the heart (slide 1) is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface throughout most of the atrium are rarely more than 2 cell layers thick.

PCR results faxed to submitter on April 15, 2005.

/sr





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**Case Report**

**Submission** 2005-01200      **Date** 08-Apr-2005      **Report** 15-Apr-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 13950 #5259, Marine Harvest (PO #508398)  
**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**

Log No. 5259. Date: April 7/05. Species: Atlantic. Sample size: 8 samples for virology  
Sex: regular. Saltwater entry: 2005. Netpen/tank ID: all. Mortality: increased this dive

Samples are labelled:

Virology 1-3; 4-6; 7-9; 10-12; 13-15; and W-A; W-B; W-C for tissue culture and PCR for IHN and PCR for VHSV.

Contact: Cilka LaTrace or Dr. Diane Morrison

**Virology**

8 pooled samples inoculated onto tissue culture - all negative.

\* Results faxed May 4/05.

**Molecular Diagnostics/PCR**

Samples 1-3, 4-6, 7-9, 10-12, 13-15, W-A, W-B, W-C: IHN Virus negative by PCR.

Samples 4-6, 7-9, W-C: VHS Virus positive by PCR.

Samples 1-3, 10-12, 13-15, W-A, W-B: VHS Virus negative by PCR.

\* Results faxed Apr. 15/05.

/bb



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**Case Report**

**Submission** 2005-01201      **Date** 08-Apr-2005      **Report** 19-Apr-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 13951 #5260, Marine Harvest (PO #508399)  
**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

**History/Symptoms**

Log No. 5260  
Date: April 7/05  
Species: Atlantic  
Sex: regular  
Saltwater entry: 2005  
Netpen/Tank ID: all  
Saltwater  
Mortality: Increased  
Sample size: 6 virology

Six pools virology for tissue culture and PCR for IHN and PCR for VHSV. 8 histo samples cassettes - 1 to 5 moribund - distended brain vessels; bact pending 16-18. Fresh dead with similar (more severe) congested vessels of the brain.

Contact: Cilka LaTrace or Dr. Diane Morrison.



### **Histopathology**

Eight cassettes of preserved tissues were submitted for histopathology.

Slide 1 (5260-1): Trunk kidney, head kidney, heart, spleen, liver, intestine, intestinal ceca, mesenteric fat, exocrine pancreas, skeletal muscle, brain, and gill.

Slide 2 (5260-2): Trunk kidney, head kidney, heart, spleen, liver, intestine, intestinal ceca, mesenteric fat, exocrine pancreas, skeletal muscle, brain, and gill

Slide 3 (5260-3): Trunk kidney, head kidney, heart, liver, intestine, intestinal ceca, mesenteric fat, exocrine pancreas, skeletal muscle, brain, and gill

Slide 4 (5260-4): Trunk kidney, head kidney, heart, spleen, liver, intestine, intestinal ceca, mesenteric fat, exocrine pancreas, skeletal muscle, brain, and gill.

Slide 5 (5260-5): Trunk kidney, head kidney, heart, spleen, liver, intestine, intestinal ceca, mesenteric fat, exocrine pancreas, skeletal muscle, brain, and gill.

Slide 16 (5260-16): Trunk kidney, head kidney, heart, spleen, liver, intestine, intestinal ceca, mesenteric fat, exocrine pancreas, skeletal muscle, brain, and gill.

Slide 17 (5260-17): Trunk kidney, head kidney, heart, liver, intestine, intestinal ceca, mesenteric fat, exocrine pancreas, skeletal muscle, brain, and gill.

Slide 18 (5260-18): Trunk kidney, head kidney, heart, spleen, liver, intestine, intestinal ceca, mesenteric fat, exocrine pancreas, skeletal muscle, brain, and gill.

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

Quality control: Tissue preservation for most organs is excellent (the tips of villi are autolyzed in some intestine sections, particularly in slides 16-18). The margins of many organs have evidence of mild dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cells types stains poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Measures of physiologic condition:

Hepatocellular glycogen depletion, severe (slides 1, 2, 3, 4, 5, 16, 17, and 18)

Mesenteric adipose tissue depletion, none (slides 1, 2, 3, 4, 5, 17, and 18), mild (slide 16)

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**

6 pooled samples inoculated onto tissue culture - all negative.

\* Results faxed May 4/05.





**Molecular Diagnostics/PCR**

Samples 7-9, 13-15, 16-18 VHS Virus Positive by PCR

Samples 1-3, 4-6, 10-12 VHS Virus Negative by PCR

Samples 1-3, 4-6, 7-9, 10-12, 13-15, 16-18 IHN Virus Negative by PCR

\*PCR results faxed to submitter on April 15, 2005.

**Diagnosis**

- 1a. Trunk kidney: necrotic epithelial cells in renal tubules, multifocal, acute, mild (slides 1, 2, 3, 4, 16, 17, and 18)
- 1b. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 3)
- 2a. Spleen and/or surrounding fatty mesenteries: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, mild (slides 1, 2, 4, and 5), moderate (slide 16)
- 2b. Spleen and surrounding fatty mesenteries: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, severe (slides 3, 17, and 18)
- 3a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1, 2, 3, 4, and 17)
- 3b. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 2, 4)
- 3c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 4, 5, 17, and 18), moderate, (slide 16), severe (slide 17)
- 3d. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 5)

**Final Comments**

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). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamycin).

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.

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). 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. 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response.

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

Gross differences in redness around the brain (moribund fish 1-5 vs. dead fish 16-18) might have been a result of vascular congestion (fish 18) or postmortem passive congestion (fish 16 and 17).

/sr



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

Facsimile: (604) 556-3010  
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**Case Report**

**Submission** 2005-01244      **Date** 12-Apr-2005      **Report** 28-Apr-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13966      A.3.2-36 (1-7)

**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**

Sample ID: A.3.2-36 (1-7)

Provincial Surveillance Program samples. Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia samples. Culture any PCR positive samples.

ADDENDUM: April 19, 2005

Received samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the spleen sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of the organs have minimal evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cells types stains poorly or not at all). Postfixation dehydration most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, none (all organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, minimal
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: 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. The cause and significance of myocardial karyomegaly 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). Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, none (all organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: epicarditis, regionally diffuse, lymphocytic, mild
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, minimal
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1c. Liver: hepatitis, histiocytic, focal (80 µm in diameter), mild
- 2. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: nephritis, interstitial, histiocytic, multifocal (each about 80 µm in diameter), mild

Comment: The focus of macrophages in the liver (histiocytic hepatitis) might be a response to localized bacterial infection or dead cells. The foci of macrophages in the head kidney are probably in response to a persistent antigen (e.g., early reaction or *Renibacterium salmoninarum* infection), but the inciting cause is unknown.

Slide 4: autolysis, mild (other organs) to moderate (liver)

- 1. Liver: pericholangitis, lymphocytic, multifocal, mild
- 2a. Heart: endocarditis, multifocal, with endothelial cell hypertrophy and small foci of lymphocytes, moderate
- 2b. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: splenitis, focal, granulomatous, mild



4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: 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. The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection (*Renibacterium salmoninarum*). Inflammatory cells lining the endocardial surface are rarely more than 2 cell layers thick. Granulomatous inflammation in the spleen is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* is the most common organism associated with these lesions.

Slide 5: autolysis, none (other organs) to mild (liver)

1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

1b. Liver: hepatitis, perivascular, lymphocytic, focal, mild

2. Heart: no significant lesions

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, minimal

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

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

Slide 6: autolysis, mild (other organs) to moderate (liver)

1. Liver: no significant lesions

2a. Heart: endocarditis, multifocal, with endothelial cell hypertrophy and small foci of lymphocytes and macrophages, moderate

2b. Heart: myocardial karyomegaly, multifocal, mild

2c. Heart: epicarditis, regionally diffuse, lymphocytic, mild

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: see comments on previous slides.

Slide 7: autolysis, mild (other organs) to moderate (trunk kidney) and severe (liver)

1. Liver: no significant lesions

2a. Heart: mural thrombosis, focal, mild

2b. Heart: endocarditis, focal, histiocytic, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, minimal

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

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

### **Molecular Diagnostics/PCR**

Samples 1-4, 5-7: VHS, IHN, ISA, IPN, *Piscirickettsia salmonis* negative by PCR.

\* Results faxed Apr. 28/05.

/bb





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1767 Angus Campbell Road

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

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

**Case Report**

**Submission** 2005-01245      **Date** 12-Apr-2005      **Report** 28-Apr-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13967      A.3.2-31 (1-4)

**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**

Sample ID: A.3.2-31 (1-4)  
Date: April 11/05

Provincial Surveillance Program samples. Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia samples. Culture any PCR positive samples.

ADDENDUM: April 19, 2005

Received samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: some of the spleen sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have minimal evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cells types stains poorly or not at all). Postfixation dehydration most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, none (all organs)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen and mesenteric fat: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. The cause and significance of myocardial karyomegaly 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). Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, none (all organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1c. Liver: parenchymal golden pigment, disseminated, intracellular, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen and mesenteric fat: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, moderate
4. Trunk kidney: moderate numbers of eosinophilic granular cells in interstitial tissue, diffuse
5. Head kidney: moderate numbers of eosinophilic granular cells in interstitial tissue, diffuse

Comment: 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). The golden pigment in the liver is most likely lipofuscin. Accumulation of lipofuscin 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 have been associated with decreased growth and survival in several studies.

Increased numbers of eosinophilic granular cells in the kidney is a distinctive finding in Atlantic salmon. 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*. Increased numbers of eosinophilic granular cells are sometimes associated with chronic parasitic infections, but again, the inciting cause was not included in the sections examined.

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.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, mild
2. Heart: myocardial karyomegaly, multifocal, mild



- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

Comment: Hepatic peritonitis is consistent with a reaction to foreign material; peritonitis is common in fish that have been vaccinated.

Slide 4: autolysis, mild (other organs) to moderate (liver)

- 1. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2a. Heart: myocardial karyomegaly, multifocal, moderate
- 2b. Heart: epicarditis, regionally diffuse, lymphocytic, mild
- 3. Spleen: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

Comment: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

#### **Molecular Diagnostics/PCR**

Samples 1-2, 3-4: VHS, IHN, ISA, IPN, *Piscirickettsia salmonis*: negative by PCR.

\* Results faxed Apr. 29/05.

/bb



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1767 Angus Campbell Road

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

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**Case Report**

**Submission** 2005-01247      **Date** 12-Apr-2005      **Report** 29-Apr-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13969      A.3.2-34 (1-7)

**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**

Sample ID: A.3.2-34 (1-7)  
Date: April 11/05

Provincial Surveillance Program samples. Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia samples. Culture any PCR positive samples.

ADDENDUM: April 19, 2005

Received samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the spleen sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have minimal evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cells types stains poorly or not at all). Postfixation dehydration most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, none (all organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2a. Heart: mural thrombosis, multifocal, mild
- 2b. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: 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. Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection. The cause and significance of myocardial karyomegaly 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). Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, none (all organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: mural thrombosis, multifocal, mild
- 3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin 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 have been associated with decreased growth and survival in several studies.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 2a. Heart: mural thrombosis, focal (200 x 150 µm), mild
- 2b. Heart: myocardial karyomegaly, multifocal, mild
- 2c. Heart: endocarditis, multifocal, lymphoplasmacytic, mild
- 3. Spleen: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, moderate
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

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

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multiple small foci in an area 5 mm in diameter, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild



2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicaemia virus, *Piscirickettsia salmonis*). Proliferative lesions in the biliary system are evidence for a toxic cause for the hepatic necrosis. 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).

Slide 5: autolysis, none (other organs) to mild (liver)

- 1a. Liver: pericholangitis, lymphocytic, multifocal, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart: endocarditis, focal, with endothelial cell hypertrophy and a thin layer of macrophages, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: nephritis, granulomatous, focal (a single multinucleate giant cell), mild
5. Head kidney: no significant lesions

Comment: 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. The single multinucleate giant cell in the kidney is evidence of a persistent antigen (e.g., bacterial cell wall). The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface in the affected focus are less than 2 cell layers thick.

Slide 6: autolysis, none (other organs) to mild (liver)

- 1a. Liver: pericholangitis, lymphocytic, multifocal, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart: endocarditis, multifocal, with endothelial cell hypertrophy and a thin layer of macrophages, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: see comments on previous slides.

Slide 7: autolysis, none (heart) to mild (other organs)

- 1a. Liver: pericholangitis, lymphocytic, multifocal, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart: epicarditis, regionally diffuse, lymphocytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.



**Molecular Diagnostics/PCR**

Samples 1-4, 5-7: VHS, IHN, ISA, IPN, Piscirickettsia salmonis: negative by PCR.

\* Results faxed Apr. 29/05.

/bb

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1767 Angus Campbell Road

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

Facsimile: (604) 556-3010  
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**Case Report**

**Submission** 2005-01248      **Date** 12-Apr-2005      **Report** 29-Apr-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13970      A.3.2-33 (1-3)

**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**

Sample ID:A.3.2-33 (1-3)  
Date: April 11/05

Provincial Surveillance Program samples. Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia samples. Culture any PCR positive samples.

ADDENDUM: April 19, 2005

Received samples for routine histology processing and analysis.



**Histopathology**

Quality control/quality assurance: the spleen sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have minimal evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cells types stains poorly or not at all). Postfixation dehydration most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, none (all organs)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: hepatitis, perivascular, lymphocytic, focal, mild
- 2. Heart: endocarditis, multifocal, with endothelial cell hypertrophy and a thin layer of macrophages, mild
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Lymphocytic inflammation around a vessel in the liver is evidence of chronic immune stimulation, e.g., from a bacterial infection. The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface in affected foci are rarely more than 2 cell layers thick.

Slide 2: autolysis, none (all organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1c. Liver: pericholangitis, lymphocytic, multifocal, mild
- 2. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

Comment: 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). 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. The cause and significance of myocardial karyomegaly 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).

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, granulomatous, multifocal, moderate
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1c. Liver: pericholangitis, lymphocytic, multifocal, mild
- 1d. Liver: hepatic necrosis, acute, focal, mild
- 2a. Heart: epicarditis, regionally diffuse, lymphocytic, mild
- 2b. Heart: endocarditis, focal, with endothelial cell hypertrophy and a thin layer of macrophages, mild
- 3. Spleen: no significant lesions
- 4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, moderate
- 5. Head kidney: no significant lesions

Comment: The most common organism associated with granulomatous nephritis and hepatitis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation. Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., *Renibacterium salmoninarum*). Lack of proliferative lesions in the biliary system is evidence against a chronic



toxic cause for the hepatic necrosis. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

**Molecular Diagnostics/PCR**

Samples 1-3: VHS, IHN, ISA, IPN, *Piscirickettsia salmonis* negative by PCR.

\* Results faxed May 2/05.

/bb

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Ministry of  
Agriculture, Food and Fisheries  
1767 Angus Campbell Road

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

Facsimile: (604) 556-3010  
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**Case Report**

**Submission** 2005-01332      **Date** 19-Apr-2005      **Report** 24-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14010      A.2.4-27 (1-12)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**Count** 12

**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**

Sample ID: A.2.4-27 (1-12)  
Date: April 18/05

Provincial Surveillance Program samples. Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis samples. Culture any PCR positive samples.

**Histopathology**

See case report 2005-1533 for histopathology results.

**Molecular Diagnostics/PCR**

Samples 1-4, 5-8, 9-12 IHN, VHS, ISA, IPN, Piscirickettsia salmonis Negative by PCR.

/sr

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**Case Report**

**Submission** 2005-01333      **Date** 19-Apr-2005      **Report** 18-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14011      A.2.4-28 (1-4)

**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**

Sample ID: A.2.4-28 (1-4)  
Date: April 18/05

Provincial Surveillance Program samples. Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis samples. Culture any PCR positive samples.

Samples also received for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the spleen sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have evidence of dehydration after fixation (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.

Slide 1: autolysis, moderate (other organs) to severe (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: interstitial cell hyperplasia, diffuse, mild
5. Head kidney: not included on the slide

COMMENT: 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).

Slide 2: autolysis, mild (other organs) to moderate (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: leukocytic karyorrhexis, multifocal, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

COMMENT: 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. The cause and significance of myocardial karyomegaly 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). The presence of degenerating nuclei (karyorrhexis) in the spleen is evidence of increased cell turnover, possibly as part of an active inflammatory response.

Slide 3: autolysis, mild (other organs) to moderate (liver)

- 1a. Liver: hepatitis, granulomatous, moderate
- 1b. Liver: hepatocellular single cell necrosis (apoptosis), disseminated, acute, moderate
- 1c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 2a. Heart: multifocal, granulomatous, mild
- 2b. Heart: myocardial karyomegaly, multifocal, mild
- 2c. Heart: mural thrombosis, multifocal, mild
3. Spleen: splenitis, multifocal, coalescing, granulomatous, severe
4. Trunk kidney: nephritis, interstitial, multifocal, coalescing, granulomatous, severe
5. Head kidney: not included on the slide

COMMENT: Granulomatous inflammation in all organs is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* is the most common organism associated with these lesions. Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection. In this case, it might be related to infection with *Renibacterium salmoninarum*.

Hepatocellular single cell necrosis (apoptosis) can occur 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). Exposure to toxins (endogenous or exogenous) might also increase the rate of apoptosis. 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response.

Slide 4: autolysis, mild (other organs) to moderate (liver)

1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

1b. Liver: hepatocellular single cell necrosis (apoptosis), disseminated, acute, moderate

2. Heart: no significant lesions

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

COMMENT: see comments on other fish above.

### **Molecular Diagnostics/PCR**

Samples 1-2, 3-4 IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* Negative by PCR.

/sr

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**Case Report**

**Submission** 2005-01334      **Date** 19-Apr-2005      **Report** 20-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14012      A.3.3-38 (1-6)

**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

**History/Symptoms**

Sample ID: A3.3-38 (1-6)  
Date: April 18/05

Provincial Surveillance Program samples. Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis samples. Culture any PCR positive samples.

**Histopathology**

As per case 2005-1530.

**Molecular Diagnostics/PCR**

Samples 1-3, 4-6 IHN, VHS, ISA, IPN, Piscirickettsia salmonis Negative by PCR.

**Final Comments**

See case report 2005-1530 for histopathology comments.

/sr

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**Case Report**

**Submission** 2005-01335      **Date** 19-Apr-2005      **Report** 01-Jun-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14013      A.3.3-39 (1-11)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**Count** 11

**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**

Sample ID: A.3.3-39 (1-11)  
Date: April 18/05

Provincial Surveillance Program samples. Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis samples. Culture any PCR positive samples.

**Histopathology**

Refer to case 2005-1534 for histopathology findings.

**Molecular Diagnostics/PCR**

Samples 1-3, 5-8, 9-11 IHN, VHS, ISA, IPN, Piscirickettsia salmonis Negative by PCR.

/sr

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**Case Report**

**Submission** 2005-01336      **Date** 19-Apr-2005      **Report** 02-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14014      A.3.3-40 (1-5)

**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

**History/Symptoms**

Sample ID: A.3.3-40 (1-5)

Date: April 18/05

Provincial Surveillance Program samples. Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis samples. Culture any PCR positive samples.

Also received samples for routine histology processing and analysis.



**Histopathology**

Quality control/quality assurance: the spleen sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have evidence of dehydration after fixation (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.

Slide 1: autolysis, none (other organs) to mild (liver)

1a. Liver: pericholangitis, lymphocytic, multifocal, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2. Heart: no significant lesions

3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

4a. Trunk kidney: renal tubular dilation, with intratubular necrotic epithelial cells, focal, moderate

4b. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: no significant lesions

COMMENT: 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. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. 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. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies. Dilation of renal components is evidence of impaired renal function, but the cause is unknown. Necrotic tubular epithelial cells in the dilated tubules are evidence that the dilation is causing tubular necrosis, but necrotic tubules are not included in the slide. 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.

Slide 2: autolysis, none (other organs) to mild (trunk kidney and liver)

1. Liver: no significant lesions

2. Heart: no significant lesions

3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

COMMENT: see slide #1.

Slide 3: autolysis, none (other organs) to mild (liver)

1. Liver: no significant lesions

2. Heart: no significant lesions

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

COMMENT: These organs had no lesions to explain the death of this fish.

Slide 4: autolysis, none (other organs) to mild (liver)

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2. Heart: no significant lesions

3. Spleen: no significant lesions

4. Trunk kidney: nephritis, interstitial, granulomatous, focal, moderate



5. Head kidney: not included on the slide

COMMENT: 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). The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.

Slide 5: autolysis, mild (other organs) to moderate (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: interstitial cell hyperplasia, diffuse, moderate
5. Head kidney: not included on the slide

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

#### **Molecular Diagnostics/PCR**

Samples: 1-3, 4-5 IHN, VHS, IPN, ISA, *Piscirickettsia salmonis* Negative by PCR.

\*PCR results faxed on May 2, 2005.

/sr



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**Case Report**

**Submission** 2005-01337      **Date** 19-Apr-2005      **Report** 02-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14015      A.3.3-41 (1-9)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**Count** 9

**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**

Sample ID: A.3.3-41 (1-9)

Date: April 18/05

Provincial Surveillance Program samples. Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis samples. Culture any PCR positive samples.

**Histopathology**

See case report 2005-1531 for histopathology comments.

**Molecular Diagnostics/PCR**

Samples: 1-5, 6-9 IHN, VHS, IPN, ISA, Piscirickettsia salmonis Negative by PCR.

\*PCR results faxed on May 2, 2005.

/sr

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**Case Report**

**Submission** 2005-01338      **Date** 19-Apr-2005      **Report** 02-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14016      A.3.3-43 (1-8)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**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

**History/Symptoms**

Sample ID: A.3.3-43 (1-8)

Date: April 18/05

Provincial Surveillance Program samples. Request Virology - PCR for IHN, ISA, IPN, VHS and *Piscirickettsia salmonis* samples. Culture any PCR positive samples.

**Histopathology**

See case report 2005-1528 for histopathology comments.

**Molecular Diagnostics/PCR**

Samples 1-4, 5-8 IHN, VHS, IPN, ISA, *Piscirickettsia salmonis* Negative by PCR.

\*PCR results faxed on May 2, 2005.

/sr



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**Case Report**

**Submission** 2005-01339      **Date** 19-Apr-2005      **Report** 02-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14017      A.3.3-46 (1-4)

**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**

Sample ID: A.3.3-46 (1-4)

Date: April 18/05

Provincial Surveillance Program samples. Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis samples. Culture any PCR positive samples.

**Histopathology**

See case report 2005-1532 for histopathology results.

**Molecular Diagnostics/PCR**

Samples: 1-2, 3-4 IHN, VHS, IPN, ISA, Piscirickettsia salmonis Negative by PCR.

\*PCR results faxed on May 2, 2005.

/sr

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**Case Report**

**Submission** 2005-00134      **Date** 17-Jan-2005      **Report** 19-Jan-2005

**Report**      **Copies**

**Submitter:** 11036 Mainstream Canada (M)

**Owner** 11036 Mainstream Canada (M)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Other

**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 cassettes for histology: BA 114, BA 106, BA 105-1, and BA 105-2.

This site has Atlantic smolts of 170 grams that entered the sea in the end of November. The water temperature is 8.2 C. These fish show internally severe petechial hemorrhage on pyloric cecae, a few of these fish also have some hemorrhage on body wall.

If you have any questions, please contact the following: sorka.cerna@mainstreamcanada.com  
francisco.miranda.morales@mainstreamcanada.com

### **Histopathology**

BA105-1 (Slide 1): gill, intestinal ceca, exocrine pancreas and fatty mesenteries, spleen, head kidney, trunk kidney, liver

BA105-2 (Slide 2): gill, intestinal ceca, exocrine pancreas and fatty mesenteries, spleen, trunk kidney, liver

BA106-1 (Slide 3): gill, stomach, intestinal ceca, exocrine pancreas and fatty mesenteries, spleen, trunk kidney, liver

BA114 (Slide 4): gill, stomach, intestine, intestinal ceca, exocrine pancreas and fatty mesenteries, spleen, trunk kidney, liver

All organs were examined on each slide. Organs without findings listed below had no significant lesions.

Quality control/quality assurance: Tissue preservation is good for all organs in slide 1, but fair to poor for most organs in other slides (i.e., mild autolysis in most organs, moderate to severe autolysis of intestinal villi and gill). The margins of the kidney (slides 1, 2, 3), liver (slides 1, 2, 4), spleen (slide 4), and gill (slides 1, 4) have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cell types stains poorly or not at all). Most commonly this occurs when tissues are removed from liquid for more than a few minutes (e.g., during shipment); other causes include fixation in formalin that is too concentrated (e.g., 100% formalin instead of 10% formalin), or transfer to ethanol that is too concentrated (e.g., >70% ethanol) before processing to paraffin.

Measures of physiologic condition

Hepatocellular glycogen depletion, severe (slides 1, 2, 3, 4)

Mesenteric adipose tissue depletion, none (slides 1, 2, 3, 4)

The pattern in the measures of physiologic condition is consistent with fish that were healthy and growing (abundant mesenteric fat), but stopped eating normally within the past few days (severe glycogen depletion).



**Diagnosis**

1. Spleen, liver, intestinal ceca, and surrounding mesenteries (stomach in slide 3): peritonitis, lymphohistiocytic, multifocal, with abundant fine fibrocellular fronds, moderate (slide 1, 4), severe (slide 2, 3)
2. Trunk Kidney: renal tubular epithelial necrosis, multifocal, acute, moderate (slide 1)
3. Trunk Kidney - renal interstitial congestion and hemorrhage, diffuse, severe (slide 1)
4. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 4), moderate (slide 2)
5. Liver: pericholangitis, lymphocytic, multifocal, moderate (slide 1)
6. Liver: hepatitis, granulomatous, multifocal, mild (slide 1)
7. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1, 4), moderate (slide 3)
8. Liver: vasculitis and perivasculitis, lymphocytic, focal, mild (slide 2, 3)
9. Mesenteric fat: congestion and hemorrhage, multifocal, mild (slide 3), moderate (slides 2, 4)
10. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 4)

**Final Comments**

These fish have evidence of chronic immune stimulation (e.g. from a bacterial or viral infection, and/or a vaccine reaction). Consider further workup to include virology (IHN, VHS, and ISA PCR) and bacteriology (culture and PCR for *Renibacterium salmoninarum*). I recommend ISA because hemorrhagic kidney (e.g., slide 1) is one of the classic signs associated with infection with Infectious Salmon Anemia.

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). Causes in fish include exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as Gentamicin). In this case, necrosis might be related to interstitial congestion and hemorrhage (lack of perfusion of certain tubules, with the possibility of reperfusion damage). Hemorrhage in the renal interstitium is indirect evidence of damage to vascular walls; however, the nature of the damage is not evident in the vessels included in the section.

Splenic, hepatic, and mesenteric peritonitis is consistent with a foreign body reaction, probably in response to vaccine adjuvant, but possibly secondary to a bacterial infection. The inflammation in slide 2 surrounds spaces that range from 60  $\mu$ m to 1 mm in diameter); whatever was in the spaces was lost during processing of the tissue to a slide.

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 juvenile salmon it might be related to increased protein needed as part an inflammatory response.

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. Lymphocytic inflammation in and around blood vessels is also evidence of chronic immune stimulation, probably of vascular and bacterial origin.

Granulomatous inflammation in the liver is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* is the most common organism associated with these lesions; these lesions have also been associated with chronic *Yersinia ruckeri* infection.

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).

Congestion and hemorrhage of mesenteric fat would appear grossly as petechiae, and petechiae are consistent with a bacterial or viral infection.

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.

/bb





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**Case Report**

**Submission** 2005-01342      **Date** 19-Apr-2005      **Report** 02-May-2005

**Report**      **Copies**

**Submitter:** 12847      Heritage Salmon

**Owner** 12847      Heritage Salmon

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Other

**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

**History/Symptoms**

PO #16365.

Five slant cultures labelled: 05-14-2, 05-15-3, 05-15-4, 05-16-1, 05-17. Please use case numbers (corresponding) on report. Please report to Dr. Peter McKenzie.

Please ID bacterial cultures and reference/sequence.

**Bacteriology**

Plate 05-16-1 - *Vibrio* sp.

Plate 05-17 - *Photobacterium* sp.

Plate 05-15-3 - *Vibrio tubiashii*.

Plate 05-15-4 - *Vibrio splendidus*.

Plate 05-14-2 - *Vibrio logei*.

Isolates were all negative for *Vibrio ordali*, *Listonella anguillarum* type 1 and type 2 serology.

\* Results faxed May 2/05.

/bb



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**Case Report**

**Submission** 2005-01416      **Date** 26-Apr-2005      **Report** 05-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14062      A.3.3-38

**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**

Provincial Surveillance Program. Submitted one culture plate for Bacteriology - identification of isolates and C & S.

ID - Subculture A.3.3-38 (3).

**Bacteriology**

Culture plate - Pseudoalteromonas sp.

Due to poor growth of this isolate, antibiotic sensitivities were unable to be performed.

\* Results faxed May 5/05.

/bb

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**Case Report**

**Submission** 2005-01417      **Date** 26-Apr-2005      **Report** 05-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14063      A.3.3-40

**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**

Provincial Surveillance Program. Submitted one culture plate for Bacteriology - identification of isolates and C & S.

ID - Subculture A.3.3-40 (3).

**Bacteriology**

Culture plate - Photobacterium phosphoreum.

Sensitive to: Erythromycin, Florfenicol, Romet 30, Sulfa-methox-trimeth. and Tetracycline.

\* Results faxed May 5/05.

/bb

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**Case Report**

**Submission** 2005-01418      **Date** 26-Apr-2005      **Report** 05-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14064      A.3.3-39

**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**

Provincial Surveillance Program. Submitted one culture plate for Bacteriology - identification of isolates and C & S.

ID - Subculture A.3.3-39 (4).

**Bacteriology**

Culture plate - *Yersinia ruckeri* identified as *Yersinia ruckeri* serotype II.

Sensitive to: Florfenicol, Romet 30, Sulfa-methox-trimeth. and Tetracycline.

\* Results faxed May 5/05.

/bb

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**Case Report**

**Submission** 2005-01419      **Date** 26-Apr-2005      **Report** 05-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14065      A.2.4-28

**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**

Provincial Surveillance Program. Submitted one culture plate for Bacteriology - identification of isolates and C & S.

ID - Subculture A.2.4-28 (1).

**Bacteriology**

Culture plate - Photobacterium phosphoreum isolated.

Sensitive to: Florfenicol, Romet 30, Sulfa-methox-trimeth. and Tetracycline.

\* Results faxed May 5/05.

/bb

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**Case Report**

**Submission** 2005-01443      **Date** 28-Apr-2005      **Report** 29-Apr-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 14080 #5262, Marine Harvest

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Other

**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 histo. cassettes.

Species - ALT. Broodstock. Saltwater entry - 2002. Netpen/tank ID - all.

Grossly white gills with excess mucus, some necrotic areas, some pale raised (inflamed) areas. No other visible lesions. Tentative confirmation of amoeba on gill wet mounts.

Marine Harvest Log 5262.

Submitted by Cilka La/Trace or Dr. Diane Morrison.

Additional history: Diane Morrison reported that this is a continuation of cases 2005-01192 (suspect amoebic gill disease) and 2005-01032. She included two photographs of the gills of affected fish; the tips of several filaments were white and thickened, about 1 mm thicker than normal. The site was treated for sea lice about one month ago, but numbers of sea lice currently are low.

### **Histopathology**

Cassettes of gill (slides 1-4) were submitted wrapped in tissue paper.

Slide 1 (4/26/05 SS1-1)

Slide 2 (4/26/05 ss1-1A)

Slide 3 (SS1 #3)

Slide 4 (SS1 - #3)

All pieces of gill on each slide were examined. Slides not listed below have no significant lesions.

Quality control: Tissue preservation for most gills is excellent. The margins of many organs have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cells types stains poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes. This problem can be avoided by keeping tissues immersed in fluid at all times. Once tissues have fixed in 10% neutral buffered formalin for 24 hours, they can be safely transferred to physiologic saline or tap water for shipment. Options for shipping include leak-proof plastic jars or whirlpak bags. If whirlpak bags are used, I suggest putting the tissues/cassettes in a small whirlpak bag with just enough saline or water to keep the tissues immersed; then put the small whirlpak bag in a larger whirlpak bag along with a paper towel (in case the smaller bag leaks).

### **Diagnosis**

1. Gill: eosinophilic granular cells in the loose connective tissue of filaments, moderate numbers (slides 1 and 3), abundant (slides 2 and 4)
2. Gill: lamellar hyperplasia and fusion, focal, mild (slide 2), moderate (slide 3)



**Final Comments**

Increased numbers of eosinophilic granular cells in the gill have been associated with chronic disease. Increased numbers of eosinophilic granular cells was a feature of the previous submission (2005-01192) that had suspect *Paramoeba* species, and this lesion has also been associated with parasitic copepods. Gill lamellar fusion with lamellar hypertrophy may be a result of local exposure to a parasite. Unfortunately, the inciting cause was not included in the sections examined. *Paramoeba* are best diagnosed on wet mount because they tend to fall off the gill during tissue processing.

If similar lesions are submitted for histopathology in the future, please submit the entire gill arch from an affected fish using the shipping recommendations listed in the "quality control" section above. Please e-mail me a photo of the appearance of the fresh gill, and note with the history that the new case is linked to this case. Also, please include a request to have me examine the tissue before it is processed (normally, my technician simply puts the Animal Health Centre case # on the cassette and sends it for processing). I will trim and decalcify the tissue so that I am sure to get a section of what is on the photo (it might require examining several sections cut at different levels through the paraffin block).

/bb





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**Case Report**

**Submission** 2005-01498      **Date** 03-May-2005      **Report** 13-May-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14111 #5270, Marine Harvest (PO #636414)  
**Farm:**  
**Vet Clinic:**  
**Attending**

**Specimen:** Other      **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

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**Case Report**

**Submission** 2005-01501      **Date** 03-May-2005      **Report** 10-Jun-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14113 #5263, Marine Harvest (PO #636412)  
**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

**History/Symptoms**

Log No. 5263  
Species: Atlantic  
Sex: Regular  
Sample size: 5 samples  
Saltwater entry: 2005  
Netpen/tank ID: All  
Saltwater

5 samples for virology and PCR for IHNV. Samples are labelled as 1-3; 4-6; 7-9; 10-12; 13-15.

Contact: Cilka LaTrace.

**Virology**

No virus isolated.

\*Virology results faxed to submitter on June 10, 2005.

**Molecular Diagnostics/PCR**

Samples 1-3, 4-6, 7-9, 10-12, 13-15 IHN Virus Negative by PCR.

\*Results faxed to submitter on June 10, 2005.

/sr



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## Case Report

**Submission** 2005-01502      **Date** 03-May-2005      **Report** 10-Jun-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14114 #5265, Marine Harvest (PO #636412)  
**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

**History/Symptoms**

Log No. 5265  
Species: Atlantic  
Sex: Regular  
Sample size: 5 virology  
Netpen/tank ID: All  
Saltwater

5 samples for virology (tissue culture) and PCR for IHN and PCR for VHSV. Samples are labelled as: 1-3; 4-6; 7-9; 10-12; 13-15.

Contact: Cilka La Trace or Dr. Diane Morrison.

**Virology**

No virus isolated.

\*Virology results faxed on June 10, 2005.

**Molecular Diagnostics/PCR**

1-3, 4-6, 7-9, 10-12, 13-15 IHN Virus Negative by PCR.  
1-3, 4-6, 7-9, 10-12, 13-15 VHS Virus Negative by PCR.

\*PCR results faxed on June 10, 2005.

/sr



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**Case Report**

**Submission** 2005-01514      **Date** 04-May-2005      **Report** 18-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14121      A3.1-30 (1-2)

**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



### **History/Symptoms**

Sample ID: A3.1-30 (1-2)

Provincial Surveillance Program samples.

Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis. Culture any PCR positive samples.

Samples for routine histology and processing and analysis

### **Histopathology**

Quality control/quality assurance: the sections have no foci of acid hematin. The margins of some organs have evidence of dehydration after fixation (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.

Slide 1: autolysis, mild (other organs) to moderate (trunk kidney and liver)

1. Liver: sinusoidal fibrin, multifocal, acute, mild
2. Heart: epicarditis, regionally diffuse, lymphocytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

COMMENT: 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. Lack of remodeling of the fibrin is consistent with these deposits forming just before death. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: parenchymal golden pigment, disseminated, intracellular, mild
- 1c. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

COMMENT: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. The golden pigment in the liver 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. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies. 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).



**Molecular Diagnostics/PCR**

Samples 1-2: IHN, VHS, IPN, ISA, *Piscirickettsia salmonis* Negative by PCR.

/sr

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**Case Report**

**Submission** 2005-01515      **Date** 04-May-2005      **Report** 18-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14122      A3.2-32 (1-5)

**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

**History/Symptoms**

Sample ID: A3.2-32 (1-5)

Provincial Surveillance Program samples.

Request Virology - PCR for IHN, ISA, IPN, VHS and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the sections have no foci of acid hematin. The margins of some organs have evidence of dehydration after fixation (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.

Slide 1: autolysis, none (all organs)

1. Liver: pericholangitis, lymphocytic, multifocal, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: not included on the slide

COMMENT: 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. 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.

Slide 2: autolysis, none (all organs)

1. Liver: no significant lesions
2. Heart: epicarditis, regionally diffuse, lymphocytic, moderate
3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

COMMENT: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 3: autolysis, none (all organs)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

COMMENT: 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response.

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, subacute, multifocal, moderate, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*)
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1c. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: epicarditis, regionally diffuse, lymphocytic, moderate
3. Spleen: not included on the slide
- 4a. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate
- 4a. Trunk kidney: interstitial eosinophilia (protein leakage from vessels), diffuse, moderate, with 1- $\mu$ m-diameter bacteria in macrophages consistent with *Piscirickettsia salmonis*
5. Head kidney: interstitial eosinophilia (protein leakage from vessels), diffuse, moderate, with 1- $\mu$ m-diameter bacteria in macrophages consistent with *Piscirickettsia salmonis*

COMMENT: Changes in the liver, head kidney, and trunk kidney are consistent with the PCR diagnosis of



*Piscirickettsia salmonis*. 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). In this case, hyperplasia and basophilic staining probably are related to *Piscirickettsia salmonis* infection.

Slide 5: autolysis, none (all organs)

1. Liver: no significant lesions

2. Heart: no significant lesions

3. Spleen: no significant lesions

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: no significant lesions

COMMENT: these organs have no lesions to explain the death of this fish.

### **Molecular Diagnostics/PCR**

Samples 1-3, 4-5: IHN, VHS, IPN, ISA Negative by PCR

Sample 4-5 *Piscirickettsia salmonis* Positive by PCR

Sample 1-3 *Piscirickettsia salmonis* Negative by PCR

/sr



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**Case Report**

**Submission** 2005-01517      **Date** 04-May-2005      **Report** 20-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14124      A2.3-23 (1-5)

**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

**History/Symptoms**

Sample ID: A2.3-23 (1-5)

Provincial Surveillance Program samples.

Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis. Culture any PCR positive samples.

**Histopathology**

See case report 2005-1526 for histopathology comments.

**Molecular Diagnostics/PCR**

Samples 1-3, 4-5 IHN, VHS, IPN, ISA, Piscirickettsia salmonis Negative by PCR.

/sr

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**Case Report**

**Submission** 2005-01518      **Date** 04-May-2005      **Report** 18-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14125      A2.3-26 (1-4)

**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

**History/Symptoms**

Sample ID: A2.3-26 (1-4).

Provincial Surveillance Program samples.

Request Virology - PCR for IHN, ISA, IPN, VHS and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the spleen sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have evidence of dehydration after fixation (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.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2a. Heart: endocarditis, multifocal, with endothelial cell hypertrophy and a thin layer of macrophages and lymphocytes, moderate
- 2b. Heart: epicarditis, regionally diffuse, lymphocytic, mild
3. Spleen: no significant lesions
4. Trunk kidney: interstitial cell hyperplasia, diffuse, mild
5. Head kidney: no significant lesions

COMMENT: 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). The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface throughout most of the ventricle are rarely more than 2 cell layers thick. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. 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).

Slide 2: autolysis, none (other organs) to mild (liver)

- 1a. Liver: sinusoidal fibrin, multifocal, acute, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: epicarditis and endocarditis, multifocal, lymphocytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

COMMENT: 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. Lack of remodeling of the fibrin is consistent with these deposits forming just before death. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 3: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

COMMENT: The cause and significance of myocardial karyomegaly 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).

Slide 4: autolysis, none (other organs) to mild (liver)

1. Liver: pericholangitis, lymphocytic, multifocal, mild
2. Heart: epicarditis, regionally diffuse, granulomatous, lymphocytic, moderate



3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate
4. Trunk kidney: nephritis, interstitial, granulomatous, neutrophilic, multifocal, severe
5. Head kidney: nephritis, interstitial, granulomatous, neutrophilic, multifocal, moderate

COMMENT: The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation. Large numbers of neutrophils in these lesions provides evidence that the lesions might have expanded substantially in the day before this fish died. Epicarditis is evidence of chronic immune stimulation; differentials include a bacterial infection (e.g., *Renibacterium salmoninarum*) and a reaction to a vaccine. A 50- $\mu$ m-diameter focus of pale crystalline-like material in the heart is also consistent with vaccine material. 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.

### **Molecular Diagnostics/PCR**

Samples 1-2, 3-4 IHN, VHS, IPN, ISA, *Piscirickettsia salmonis* Negative by PCR.

/sr

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**Case Report**

**Submission** 2005-01519      **Date** 04-May-2005      **Report** 19-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14126      A2.3-25 (1-5)

**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



**History/Symptoms**

Sample ID: A2.3-25 (1-5)

Provincial Surveillance Program samples.

Request Virology - PCR for IHN, ISA, IPN, VHS and *Piscirickettsia salmonis*. Culture any PCR positive samples.

**Histopathology**

Quality control/quality assurance: the spleen and liver #5 sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cells types stains poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, mild (other organs) to moderate (trunk kidney and liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: pericholangitis, lymphocytic, multifocal, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: no significant lesions
4. Trunk kidney: interstitial hemorrhage, diffuse, with hematopoietic cell atrophy, moderate (consistent with VHSV)
5. Head kidney: not included on the slide

COMMENT: Renal interstitial hemorrhage is an uncommon manifestation of infection with viral hemorrhagic septicemia virus (VHSV). The finding is more commonly associated with infectious salmon anemia virus (ISAV), but the positive PCR for VHSV and negative PCR for ISAV rule out ISAV as the cause. 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). 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. The cause and significance of myocardial karyomegaly 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).

Slide 2: autolysis, none (other organs) to mild (trunk kidney and liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, regionally diffuse, with fibrocellular fronds, mild
4. Trunk kidney (2 pieces): no significant lesions
5. Head kidney: not included on the slide

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

Slide 3: autolysis, none (heart) to mild (other organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: epicarditis, focal, lymphocytic, mild
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

COMMENT: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. 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. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Slide 4: autolysis, none (all organs)

1. Liver: no significant lesions



2. Heart: mural thrombosis, focal, mild
3. Spleen: peritonitis, chronic, regionally diffuse, with fibrocellular fronds and fibrin, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

COMMENT: Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus (e.g., VHSV), bacterial, or parasitic infection. The addition of fibrin to the splenic peritonitis is consistent with VHSV infection.

Slide 5: autolysis, none (all organs)

- 1a. Liver: vasculitis, fibrinous, multifocal, with sinusoidal congestion, acute, moderate
- 1b. Liver: hepatocellular fatty change (lipidosis), multifocal, moderate
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, regionally diffuse, with fibrocellular fronds and fibrin, moderate
- 4a. Trunk kidney: renal tubular epithelial necrosis, focal, subacute, with regeneration, mild
- 4b. Trunk kidney: glomerulonephritis, fibrinous, focal, acute, mild
5. Head kidney: not included on the slide

COMMENT: Sinusoidal congestion (peliosis) is an uncommon manifestation of infection with viral hemorrhagic septicemia virus (VHSV). The finding is more commonly associated with infectious salmon anemia virus (ISAV), but the positive PCR for VHSV and negative PCR for ISAV rule out ISAV as the 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). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamycin). Fibrinous glomerulonephritis in this fish also is consistent with VHSV infection.

### **Virology**

Viral Hemorrhagic Septicemia virus Positive  
Tissue culture positive for VHS (confirmed by PCR).

\* Results faxed on Jun 3/05.

### **Molecular Diagnostics/PCR**

Samples 1-3, 4-5 ISA, IPN, IHN, *Piscirickettsia salmonis* Negative by PCR.  
Samples 1-3, 4-5 VHS Virus Positive by PCR.

/sr/mm



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**Case Report**

**Submission** 2005-01520      **Date** 04-May-2005      **Report** 18-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14127      A2.3-24 (#1-6)

**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

**History/Symptoms**

Sample ID: A2.3-24 (#1-6)

Provincial Surveillance Program samples.

Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis. Culture any PCR positive samples.

**Histopathology**

See case 2005-1522 for Histopathology results.

**Virology**

Two samples inoculated onto tissue culture - both negative.

**Molecular Diagnostics/PCR**

Samples 1-3, 4-6 ISA, IPN, IHN, Piscirickettsia salmonis Negative by PCR.

Samples 1-3, 4-6 VHS Virus Positive by PCR

/sr/mm

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**Case Report**

**Submission** 2005-01521      **Date** 04-May-2005      **Report** 09-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14128      A2.3-23 #4

**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**

Samples ID: A2.3-23 #4

Provincial Surveillance Program. For bacteriology - identification of isolates and C & S.

\*Bacteriology results faxed on May 9, 2005.

**Bacteriology**

Bact. plate - *Aeromonas salmonicida* ss *salmonicida*.

Sensitive to: Erythromycin, Florfenicol, Romet 30, Tri-sulfas and Sulfa-methox-trimeth.

\* Results faxed May 9/05.

/bb

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**Case Report**

**Submission** 2005-01522      **Date** 04-May-2005      **Report** 18-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14129      A2.3-24 (#1-6)

**Farm:**

**Vet Clinic:**

**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

**History/Symptoms**

Sample ID: A2.3-24 (#1-6)

Provincial Surveillance Program. Samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the spleen and liver sections contain foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have evidence of dehydration after fixation (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.

Slide 1: autolysis, none (other organs) to mild (liver, intestinal ceca)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: no significant lesions
3. Spleen and intestinal ceca: peritonitis, chronic, multifocal, with fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions
6. Intestinal ceca: no significant lesions

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

Slide 2: autolysis, none (other organs) to mild (liver)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: epicarditis, granulomatous, focal (200 x 250 µm), mild
3. Spleen and intestinal ceca: peritonitis, granulomatous, multifocal, with intralesional vacuoles (up to 200 µm in diameter) and fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: not included in the section

COMMENT: 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response. Epicarditis is consistent with a reaction to foreign material like vaccine material.

Slide 3: autolysis, mild (other organs) to moderate (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: peritonitis, granulomatous, multifocal, with fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

COMMENT: see comments on previous fish. None of the first three fish had lesions consistent with the PCR positive VHSV result.

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

COMMENT: 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response.



Slide 5: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: endocarditis, focal, lymphohistiocytic, mild
3. Spleen: no significant lesions
4. Trunk kidney: peritonitis, granulomatous, focal (200 x 150 µm), mild
5. Head kidney: no significant lesions

COMMENT: Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Granulomatous peritonitis on the margin of the trunk kidney might be a vaccine reaction.

Slide 6: autolysis, mild (other organs) to moderate (liver and intestinal ceca)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
2. Heart: mural thrombosis, focal, mild
3. Spleen: peritonitis, granulomatous, regionally diffuse, with fibrocellular fronds, severe
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: not included on the slide
6. Intestinal ceca: no significant lesions

COMMENT: Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus (e.g., VHSV), bacterial, or parasitic infection. 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.

### **Molecular Diagnostics/PCR**

See case 2005-1520 for PCR results.

/sr



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**Case Report**

**Submission** 2005-01523      **Date** 04-May-2005      **Report** 20-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14130      A3.1-29 (1-5)

**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



**History/Symptoms**

Sample ID: A3.1-29 (1-5)  
Provincial Surveillance Program.

Virology - PCR for IHN, ISA, IPN, VHS and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: some spleen sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cell types stain poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, granulomatous, multifocal, severe
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: epicarditis and endocarditis granulomatous, multifocal, moderate
3. Spleen: splenitis, granulomatous, multifocal, mild
4. Trunk kidney: not included on the slide

5. Head kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, severe

COMMENT: The most common organism associated with disseminated granulomas in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response.

Slide 2: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, granulomatous, multifocal, severe
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
2. Heart: epicarditis and endocarditis granulomatous, multifocal, moderate
3. Spleen: splenitis, granulomatous, multifocal, moderate
4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, severe
5. Head kidney: not included on the slide

COMMENT: The most common organism associated with disseminated granulomas in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease.

Slide 3: autolysis, mild (other organs) to moderate (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: epicarditis and endocarditis granulomatous, multifocal, mild
- 3a. Spleen: splenitis, granulomatous, multifocal, mild
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, severe
5. Head kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, severe

COMMENT: Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections. Lack of any granulomatous inflammation in the liver makes *Renibacterium salmoninarum* a less likely differential for the liver lesions. The most common organism associated with disseminated granulomas in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 4: autolysis, none (other organs) to mild (liver)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
2. Heart: no significant lesions
3. Spleen: not included on the slide
4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, with fibrosis and neutrophils, severe
5. Head kidney: capsulitis, lymphohistiocytic, diffuse, moderate



COMMENT: The kidney lesions probably are a result of infection with *Renibacterium salmoninarum*, the cause of bacterial kidney disease.

Slide 5: autolysis, mild (other organs) to moderate (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: no significant lesions

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

COMMENT: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in

### **Molecular Diagnostics/PCR**

Samples 1-3, 4-5 ISA, IPN, IHN, VHS, *Piscirickettsia salmonis* Negative by PCR.

/sr

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**Case Report**

**Submission** 2005-01526      **Date** 04-May-2005      **Report** 20-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14133      A2.3-23 (#1-5)

**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

**History/Symptoms**

Provincial Surveillance Program. Samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: erythrocytes in most of the sections have diffuse thin deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cell types stain poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, none (all organs)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

COMMENT: 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, none (all organs)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: not included on the slide
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

COMMENT: 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).

Slide 3: autolysis, none (heart) to mild (other organs)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

COMMENT: see comments on other fish.

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: endocarditis (bulbus arteriosus), diffuse, with a thin layer of eosinophilic granular cells, mild
3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

COMMENT: The pattern of granulomatous inflammation in the heart sometimes occurs in fish with a bacterial infection. Eosinophilic granular cells line the endocardial surface throughout part of the bulbus arteriosus, but they are never more than 2 cell layers thick.

Slide 5: autolysis, none (other organs) to mild (liver)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild





- 2. Heart: no significant lesions
  - 3. Spleen: not included on the slide
  - 4. Trunk kidney: no significant lesions
  - 5. Head kidney: no significant lesions
- COMMENT: see comments on other fish.

**Molecular Diagnostics/PCR**

See case report 2005-01517 for PCR results.

/sr

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**Case Report**

**Submission** 2005-01528      **Date** 04-May-2005      **Report** 20-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14135      A3.3-43(1-8)

**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

**History/Symptoms**

Sample ID: A3.3-43 (1-8)

Provincial Surveillance Program. Samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: erythrocytes in most of the sections have diffuse thin deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cell types stain poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, mild
- 1b. Liver: hepatitis, granulomatous, multifocal, moderate
- 1c. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1d. Liver: pericholangitis, lymphocytic, multifocal, mild
- 2. Heart: epicarditis and endocarditis granulomatous, diffuse, moderate
- 3. Spleen: splenitis, granulomatous, multifocal, mild
- 4a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, severe
- 4b. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, mild
- 5. Head kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, severe

COMMENT: The most common organism associated with disseminated granulomas in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. Large numbers of small Gram positive rods in all organs (Twort's stain) is also consistent with *Renibacterium salmoninarum*. Hepatic and renal tubular necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections. In this case, necrosis is probably related to *Renibacterium salmoninarum* 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response. 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.

Slide 2: autolysis, none (all organs)

- 1a. Liver: pericholangitis, granulomatous, focal, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1c. Liver: hepatic necrosis, acute, multifocal, mild
- 2. Heart: epicarditis and endocarditis granulomatous, diffuse, mild to moderate
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5a. Head kidney: nephritis, interstitial, granulomatous, focal, mild
- 5b. Head kidney: perivascular neutrophils, multifocal, mild

Comment: Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicaemia virus, *Piscirickettsia salmonis*). In combination with granulomatous inflammation in other areas, this fish might be infected with *Renibacterium salmoninarum*, but other bacteria cannot be ruled out (e.g., *Yersinia ruckeri*). Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Perivascular neutrophils in the head kidney are evidence of an acute inflammatory response, probably in response to a bacterial infection.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, granulomatous, multifocal, moderate
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: not included on the slide



- 4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, moderate
- 5. Head kidney: no significant lesions
- Comment: see comments on other fish.

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

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

Slide 5: autolysis, none (other organs) to mild (liver)

- 1. Liver: no significant lesions
- 2. Heart: no significant lesions
- 3. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: see comments on other fish.

Slide 6: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1c. Liver: pericholangitis, lymphocytic, multifocal, mild
- 2a. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild
- 2b. Heart: endocarditis, diffuse, with focal thrombosis and multifocal endothelial cell hypertrophy and a thin layer of macrophages, mild
- 3. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface throughout most of the ventricle are rarely more than 2 cell layers thick.

Slide 7: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, moderate
- 1b. Liver: hepatitis, granulomatous, multifocal, moderate
- 1c. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1d. Liver: pericholangitis, lymphocytic, multifocal, mild
- 2. Heart: endocarditis granulomatous, diffuse, moderate
- 3a. Spleen: splenitis, granulomatous, multifocal, mild
- 3b. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, mild
- 4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, severe
- 5. Head kidney: nephritis, interstitial, granulomatous, multifocal, moderate

Comment: The most common organism associated with disseminated granulomas in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease.

Slide 8: autolysis, none (other organs) to mild (liver)



1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
  2. Heart: epicarditis, regionally diffuse, lymphohistiocytic, moderate
  3. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, moderate
  4. Trunk kidney: no significant lesions
  5. Head kidney: no significant lesions
- Comment: see comments on other fish.

**Molecular Diagnostics/PCR**

See case report 2005-1338 for PCR results.

/sr



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

Facsimile: (604) 556-3010  
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**Case Report**

**Submission** 2005-01530      **Date** 04-May-2005      **Report** 20-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14136      A3.3-38(1-6)

**Farm:**

**Vet Clinic:**

**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

**History/Symptoms**

Sample ID: A3.3-38 (1-6)

Provincial Surveillance Program. Samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: erythrocytes in most of the sections have diffuse thin deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cell types stain poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, granulomatous, multifocal, severe
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
2. Heart: epicarditis and endocarditis granulomatous, diffuse, mild
3. Spleen: splenitis, granulomatous, multifocal, coalescing, severe
- 4a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, severe
- 4b. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, mild
5. Head kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, severe

COMMENT: The most common organism associated with disseminated granulomas in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. Renal tubular necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections. In this case, necrosis is probably related to *Renibacterium salmoninarum* 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response.

Slide 2: autolysis, mild (other organs) to severe (liver)

1. Liver: pericholangitis and perivascularitis, lymphocytic, multifocal, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

COMMENT: Lymphocytic inflammation around bile ductules and blood vessels in the 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 or the vasculature.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, granulomatous, multifocal, severe
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
2. Heart: epicarditis and endocarditis granulomatous, diffuse, mild
3. Spleen: splenitis, granulomatous, multifocal, moderate
- 4a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, with mural thrombosis; severe
- 4b. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, mild
5. Head kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, severe

COMMENT: see comments on other fish.

Slide 4: autolysis, none (other organs) to mild (trunk kidney and liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions



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

Slide 5: autolysis, none (other organs) to mild (liver)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

COMMENT: Hepatocyte cytoplasm has an unusual morphology. The fine apical vacuoles resemble the morphology of exocrine pancreas with zymogen granule depletion. This might be within the range of normal for Atlantic salmon liver.

Slide 6: autolysis, none (other organs) to mild (liver)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild
- 3a. Spleen: splenitis, granulomatous, focal, moderate
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: nephritis, interstitial, granulomatous, bifocal, mild

COMMENT: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Granulomatous splenitis and nephritis is probably a result of infection with *Renibacterium salmoninarum*, but other bacteria (e.g., *Yersinia ruckeri*) cannot be ruled out. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

### **Molecular Diagnostics/PCR**

See case report 2005-1334 for PCR results.

/sr



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1767 Angus Campbell Road

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

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**Case Report**

**Submission** 2005-01531      **Date** 04-May-2005      **Report** 24-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14137      A3.3-41(1-9)

**Farm:**

**Vet Clinic:**

**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

**History/Symptoms**

Sample ID: A3.3 - 41 (1-9)

Provincial Surveillance Program. Samples for routine histology processing and analysis.



**Histopathology**

Quality control/quality assurance: some spleen sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have evidence of dehydration after fixation (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.

Slide 1: autolysis, none (heart) to mild (other organs)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

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

Slide 2: autolysis, none (all organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

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

Slide 3: autolysis, none (all organs)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

COMMENT: see comments on other fish.

Slide 4: autolysis, none (all organs)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: epicarditis, multifocal, lymphohistiocytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

COMMENT: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 5: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: pericholangitis, lymphocytic, multifocal, moderate
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild



4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

COMMENT: 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.

Slide 6: autolysis, none (other organs) to mild (liver)

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: epicarditis, focal, lymphohistiocytic, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

COMMENT: see comments on other fish.

Slide 7: autolysis, none (other organs) to mild (liver)

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2a. Heart: endocarditis, bifocal, lymphohistiocytic, mild

2b. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild

2c. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

COMMENT: Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. The cause and significance of myocardial karyomegaly 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).

Slide 8: autolysis, none (other organs) to mild (liver)

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: endocarditis, focal, lymphohistiocytic, mild

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

COMMENT: see comments on other fish.

Slide 9: autolysis, none (heart) to mild (other organs)

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

6a. Mesenteric Fat: peritonitis, chronic, multifocal, with fibrocellular fronds, mild

6b. Mesenteric Fat: small vessel congestion, diffuse, moderate

COMMENT: 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. In pen-reared salmon, hepatic



lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several

**Molecular Diagnostics/PCR**

See case report 2005-1337 for PCR results.

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

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**Case Report**

**Submission** 2005-01532      **Date** 04-May-2005      **Report** 24-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14138      A3.3-42(1-4)

**Farm:**

**Vet Clinic:**

**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

**History/Symptoms**

Sample ID: A3.3-42 (1-4)

Provincial Surveillance Program. Samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the spleen sections contain no foci of acid hematin, and tissues have no evidence of significant dehydration after fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: 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. 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. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). 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.

Slide 2: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, severe
- 2a. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild
- 2b. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: peritonitis, granulomatous, regionally diffuse, with fibrin deposition and occasional fine fibrocellular fronds, moderate
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

Comment: The cause and significance of myocardial karyomegaly 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). Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Fibrin deposition is evidence of an active component to the inflammation that might have significantly contributed to the death of this fish. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 3: autolysis, mild (other organs) to moderate (liver)

- 1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: no significant lesions
- 4. Trunk kidney: small numbers of eosinophilic granular cells in interstitial tissue
- 5. Head kidney: moderate numbers of eosinophilic granular cells in interstitial tissue

Comment: Increased numbers of eosinophilic granular cells in the kidney is a distinctive occasional finding in Atlantic salmon. 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*. Increased numbers of eosinophilic granular cells are sometimes associated with chronic parasitic infections, but again, the inciting cause was not included in the sections examined.





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.

Slide 4: autolysis, none (other organs) to mild (liver)

1a. Liver: hepatitis and peritonitis, granulomatous, multifocal, with mural thrombosis, severe

1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild

2. Heart: epicarditis and endocarditis, granulomatous, diffuse, with mural thrombosis, severe

3. Spleen: splenitis, granulomatous, multifocal, with fibrinous exudates, severe

4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, severe

5. Head kidney: not included on the slide

Comment: The most common organism associated with disseminated granulomas in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. The liver is covered by granulomatous inflammation and a band of fibrin that is up to 400  $\mu\text{m}$  thick. 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response.

/sr



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**Case Report**

**Submission** 2005-01533      **Date** 04-May-2005      **Report** 24-May-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14139      A2.4-27 (1-12)

**Farm:**

**Vet Clinic:**

**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

**History/Symptoms**

Sample ID: A2.4-27 (1-12)

Provincial Surveillance Program. Samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: clusters of erythrocytes contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have evidence of dehydration after fixation (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. Postfixation dehydration is severe in the spleen and liver in slide 10.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

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

Slide 2: autolysis, mild (other organs) to moderate (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, moderate
- 2a. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages, moderate

2b. Heart: epicarditis, regionally diffuse, lymphoplasmacytic, moderate

2c. Heart: mural thrombosis, multifocal, mild

3. Spleen, intestinal cecum, and surrounded fatty mesenteries: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, severe

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

COMMENT: 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). Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. The pattern of endocarditis in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection. Inflammatory cells lining the endocardial surface throughout most of the ventricle and atrium are rarely more than 2 cell layers thick. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: peritonitis, chronic, focal, with fibrocellular fronds, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

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

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, with hepatocellular cytoplasmic eosinophilic bodies, moderate.
- 1b. Liver: biliary preductular cell hyperplasia, multifocal, mild



2. Heart: epicarditis, focal, lymphocytic, neutrophilic, mild
3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

COMMENT: Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections. These types of lesions are associated with infection with viral hemorrhagic septicaemia virus and *Piscirickettsia salmonis*, but the negative PCR results make these organisms unlikely. A Twort's Gram stain revealed no bacteria associated with hepatic necrosis. Preductular cell hyperplasia provides evidence for a toxic cause for the hepatic necrosis. 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). Epicarditis is evidence of subacute immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 5: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: pericholangitis, lymphocytic, focal, mild
2. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild
3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: not included on the slide

COMMENT: 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. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. 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.

Slide 6: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, multifocal, mild
- 1b. Liver: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 2a. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages, mild
- 2b. Heart: epicarditis, regionally diffuse, lymphohistiocytic, moderate
3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

COMMENT: see comments on other fish.

Slide 7: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: not included on the slide
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions
6. Gill: no significant lesions
7. Intestine and surrounding fatty mesenteries: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate

COMMENT: see comments on other fish.

Slide 8: autolysis, mild (other organs) to moderate (gill) and severe (liver)

- 1a. Liver: biliary preductular cell hyperplasia, multifocal, mild





- 1b. Liver: pericholangitis, lymphocytic, focal, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, severe
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions
6. Gill: no significant lesions

COMMENT: The cause and significance of myocardial karyomegaly 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).

Slide 9: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, multifocal, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1c. Liver: hepatic necrosis, acute, multifocal, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

COMMENT: see comments on other fish.

Slide 10: autolysis, none (other organs) to mild (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: not included in the slide
5. Head kidney: not included in the slide

COMMENT: these organs had no lesions to explain the death of this fish.

Slide 11: autolysis, none (other organs) to mild (liver and gill)

- 1a. Liver: biliary preductular cell hyperplasia, multifocal, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: epicarditis, focal, lymphocytic, mild
3. Spleen and intestine: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, severe
4. Trunk kidney: not included on the slide
5. Head kidney: no significant lesions
6. Gill: no significant lesions

COMMENT: see comments on other fish.

Slide 12: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, moderate
4. Trunk kidney: not included in the slide
5. Head kidney: not included in the slide

COMMENT: see comments on other fish.



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Facsimile: (604) 556-3010  
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**Case Report**

**Submission** 2005-01534      **Date** 04-May-2005      **Report** 01-Jun-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14140      A3.3-39(1-11)

**Farm:**

**Vet Clinic:**

**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

**History/Symptoms**

Sample ID: A3.3-39 (1-11)

Provincial Surveillance Program. Samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: sections have no foci of acid hematin. The margins of some organs have minimal evidence of dehydration after fixation (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.

Slide 1: autolysis, moderate (other organs) to severe (liver)

1. Liver: no significant lesions
  2. Heart: no significant lesions
  3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
  4. Trunk kidney: no significant lesions
  5. Head kidney: not included on the slide
  6. Teeth, bone, and cartilage: 2 x 1 mm focus of mixed bacteria (probably postmortem growth)
- COMMENT: Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, moderate (other organs) to severe (liver)

1. Liver: no significant lesions
  2. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages and eosinophilic granular cells, moderate
  3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
  4. Trunk kidney: no significant lesions
  5. Head kidney: not included on the slide
- COMMENT: The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface throughout most of the bulbus arteriosus are rarely more than 2 cell layers thick.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
  - 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
  - 1c. Liver: hepatitis, perivascular, lymphocytic, multifocal, mild
  - 2a. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages and eosinophilic granular cells, moderate
  - 2b. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild
  3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate
  4. Trunk kidney: no significant lesions
  5. Head kidney: not included on the slide
- COMMENT: 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response. 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). Lymphocytic inflammation around vessels in the liver is evidence of chronic immune stimulation, e.g., from a bacterial infection. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 4: autolysis, mild (other organs) to moderate (liver)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages and eosinophilic granular cells, mild
3. Spleen: peritonitis, granulomatous, regionally diffuse, moderate



- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions
- COMMENT: see comments on other fish.

Slide 5: autolysis, moderate (other organs) to severe (liver)

- 1. Liver: no significant lesions
- 2. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages and eosinophilic granular cells, mild
- 3. Spleen: peritonitis, granulomatous, regionally diffuse, severe
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide
- COMMENT: see comments on other fish.

Slide 6: autolysis, mild (other organs) to moderate (liver)

- 1. Liver: peritonitis, granulomatous, focal, mild
- 2. Heart: no significant lesions
- 3. Spleen: not included on the slide
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide
- COMMENT: These organs have no lesions to help explain the death of this fish.

Slide 7: autolysis, mild (other organs) to moderate (liver)

- 1. Liver: no significant lesions
- 2. Heart (bulbus arteriosus): endocarditis, diffuse, with a thin layer of eosinophilic granular cells, mild
- 3. Spleen: peritonitis, granulomatous, diffuse, severe
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions
- COMMENT: see comments on other fish.

Slide 8: autolysis, none (all organs)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions
- COMMENT: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Slide 9: autolysis, none (all organs)

- 1. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: splenitis, granulomatous, focal, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions
- COMMENT: The focus of granulomatous splenitis contains a single multinucleate giant cell and a 40-µm-diameter; a vaccine reaction is the most likely differential.

Slide 10: autolysis, moderate (other organs) to severe (liver)

- 1. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2. Heart: no significant lesions





- 3. Spleen: peritonitis, granulomatous, multifocal, moderate
  - 4. Trunk kidney: no significant lesions
  - 5. Head kidney: not included on the slide
- COMMENT: see comments on other fish.

Slide 11: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 2. Heart: bulbus arteriosus endocardial eosinophilia, multifocal, mild
- 3. Spleen: peritonitis, granulomatous, diffuse, moderate
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

COMMENT: Endocardial foci of eosinophilia in the bulbus arteriosus might represent fibrin deposition, or they might be within the range of normal.

### **Molecular Diagnostics/PCR**

Refer to case 2005-1335 for PCR results.

/sr

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1767 Angus Campbell Road

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

Facsimile: (604) 556-3010  
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## Case Report

**Submission** 2005-01567      **Date** 06-May-2005      **Report** 13-May-2005

**Report**      **Copies**

**Submitter:** 9283      Pan Fish Canada (Omega Salmon  
Group Ltd.)

**Owner** 9283      Pan Fish Canada (Omega Salmon  
Group Ltd.)

**Farm:**  
**Vet Clinic:**  
**Attending**

**Specimen:** Tissue - Formalized

**Species:** Atlantic Salmon

**Breed:**

**Count** 1

**Flock Herd Size:**

**Age**

**Sex:**

**Feed:**

**Feed**

**Vaccination**

**Treatmen**

**Diagnosis**

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

**History/Symptoms**

I just wanted to let you know that I shipped off some histo samples today. There is gill, pyloric ceca, kidney, liver and spleen from 5 fish. These are Atlantic salmon smolts that went to sea about a month ago. We have BKD causing some mortality at the hatchery and things have gotten much worse since they went to sea. All the morts clearly have BKD, but I just wanted to make sure that there wasn't anything else going on.

**Histopathology**

Preserved tissues were submitted for histopathology in five cassettes.

Slide 1 (AP1): gill, heart, spleen, liver, trunk kidney, intestine and surrounding mesenteric fat

Slide 2 (AP2): gill, heart, spleen (2 pieces), liver, trunk kidney, intestine and surrounding mesenteric fat

Slide 3 (AP3): gill, heart, spleen, liver, trunk kidney, stomach, intestinal cecum and surrounding mesenteric fat

Slide 4 (AP4): gill, heart, spleen, liver, trunk kidney, intestine and surrounding mesenteric fat

Slide 5 (AP5): gill, heart, spleen, liver, trunk kidney, stomach, intestinal cecum and surrounding mesenteric fat

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

Quality control: Tissue preservation for most organs is good. Tips of intestinal villi sometimes have moderate to severe autolysis. Sections have no evidence of dehydration after fixation.

Measures of physiologic condition:

Hepatocellular glycogen depletion, severe (slides 1, 2, 3, 4, 5)

Mesenteric adipose tissue depletion, none (slides 2, 3), mild (slide 1, 5)

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

**Diagnosis**

- 1a. Liver: hepatitis, granulomatous, multifocal, with necrosis, mild (slide 3), moderate (slides 1, 2), severe (slide 4, 5)
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 2, 3, 4, 5), moderate (slide 1)
- 1c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 2, 4, 5), moderate (slide 1, 3)
- 1d. Liver: hepatitis, perivascular, pericholangial, lymphocytic, multifocal, with karyorrhexis, mild (slide 3, 4), moderate (slide 2, 5)
- 2a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, with necrosis, severe (slide 1, 2, 3, 4, 5)
- 3a. Exocrine pancreas: pancreatic necrosis and granulomatous inflammation, multifocal, mild (slide 2), moderate (slide 1, 3, 5), severe (slide 4)
- 4b. Heart: epicarditis and endocarditis, multifocal, granulomatous, mild (slide 3), moderate (slide 2, 5); with thrombosis, moderate (slide 1, 4)
- 5a. Gill arch: branchitis, granulomatous, focal, mild (slide 2, 3, 4); multifocal, mild (slide 1), moderate (in filaments, slide 5)
- 5b. Gill: superficial branchial filamentous bacteria and algae, multifocal, mild (slide 2)
- 5c. Gill: lamellar subepithelial edema, multifocal, mild (slide 4)
- 6. Stomach: gastritis, submucosal, granulomatous, multifocal, coalescing, mild (slide 3)
- 7a. Spleen: splenitis, granulomatous, multifocal, coalescing, with necrosis, moderate (slide 5), severe (slide 1, 2, 3, 4)
- 7b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 3)



**Final Comments**

All five fish had overwhelming granulomatous inflammation in multiple organs, consistent with the clinical impression of infection with *Renibacterium salmoninarum*, the cause of bacterial kidney disease. Necrosis of exocrine pancreatic cells is probably also associated with Bacterial kidney disease. Other changes are relatively minor, but might provide clues for other causes of morbidity.

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). 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response. Lymphocytic inflammation around bile ductules and vessels in the 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. All three of these lesions can occur independently of *Renibacterium salmoninarum* infection.

Features of the foci of filamentous bacteria and algae between the gill filaments (slide 2 only) are most consistent with organisms that grew somewhere else and then got lodged in the gills. Once wedged in the gills, they would tend to increase morbidity. Consider water conditions that might have led to exposure to these organisms. Separation of gill lamellar epithelium from underlying pillar cells can be a result of edema and it is a common postmortem artifact. 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.

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

/sr





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**Case Report**

**Submission** 2005-00158      **Date** 19-Jan-2005      **Report** 01-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13312      A. 2.4 - 5 (1-8)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**Count** 8

**Flock Herd Size:**

**Species:** Atlantic Salmon

**Age**

**Breed:** Atlantic Salmon

**Sex:**

**Feed:**

**Feed**

**Vaccination**

**Treatmen**

**Diagnosis**

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

**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 2.4 - 5 (1-8).

Addendum (Jan 20/05): samples submitted for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the sections contain moderate precipitates of acid hematin; this might be a result of transferring tissues to ethanol followed by return to formalin as part of processing the tissue into paraffin. Alternatively, tissues might not have been fixed in neutral buffered formalin.

Slide 1: autolysis, mild (other organs) to moderate (liver)

1. Liver: pericholangitis, lymphocytic, multifocal, mild
2. Heart: no significant lesions
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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. The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin 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. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Slide 2: autolysis, mild (other organs) to moderate (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: epicarditis, regionally diffuse, lymphocytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: 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). Epicarditis is evidence of chronic immune stimulation, but the cause is otherwise unknown. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2a. Heart: epicarditis, regionally diffuse, lymphocytic, mild
- 2b. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. The cause and significance of myocardial karyomegaly 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).

Slide 4: autolysis, mild (other organs) to severe (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: pericholangitis, lymphocytic, multifocal, mild
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2a. Heart: epicarditis, regionally diffuse, lymphocytic, mild
- 2b. Heart: endocarditis, multifocal, lymphoplasmacytic, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions



5. Head kidney: no significant lesions

Comment: Lymphoplasmacytic inflammation in the heart is evidence of chronic immune stimulation (e.g., low grade bacterial infection), but the cause is otherwise unknown.

Slide 5: autolysis, none (all organs)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: no significant lesions

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: moderate numbers of eosinophilic granular cells in interstitial tissue

5. Head kidney: moderate numbers of eosinophilic granular cells in interstitial tissue

Comment: Increased numbers of eosinophilic granular cells in the kidney is a fairly common finding in Atlantic salmon. 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*. Increased numbers of eosinophilic granular cells are sometimes associated with chronic parasitic infections, but again, the inciting cause was not included in the sections examined.

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.

Slide 6: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, severe

2. Heart (2 pieces): endocarditis, multifocal, lymphoplasmacytic, mild

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: none

Slide 7: autolysis, none (other organs) to mild (liver)

1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild

1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2. Heart: epicarditis, focal, lymphohistiocytic, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: not included on the slide

5. Head kidney: no significant lesions

Comment: 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 juvenile salmon it might be related to increased protein needed as part an inflammatory response. Epicarditis is evidence of chronic immune stimulation, but the cause is otherwise unknown.

Slide 8: autolysis, none (all organs)

1. Liver: peritonitis, chronic, focal, with fibrocellular fronds, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, severe

2a. Heart: myocardial karyomegaly, multifocal, mild

2b. Heart: increased endocardial cellularity, multifocal, moderate

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: not included in the section

Comment: Hepatic peritonitis is probably part of a vaccine reaction (same as splenic peritonitis). Increased



cellularity of the endothelium of the heart is a result of small, deeply basophilic nuclei lining the vascular spaces; the cells most likely are thrombocyte. Their significance is unknown, but they may reflect a state of abnormal coagulation. The heart contains no obvious thrombi.

**Molecular Diagnostics/PCR**

Samples 1-4, 5-8: ISA, IPN, IHN, VHS Virus, *Piscirickettsia salmonis* negative by PCR.

\* Results faxed Feb. 16/05.

/bb

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1767 Angus Campbell Road

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

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**Case Report**

**Submission** 2005-00160      **Date** 19-Jan-2005      **Report** 01-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13326      A. 2.4 - 7 (1-2)

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 2.4 - 7 (1-2).

Addendum: Jan 21/05 - samples submitted for routine histology.

### **Histopathology**

Quality control/quality assurance: the sections contain moderate precipitates of acid hematin; this might be a result of transferring tissues to ethanol followed by return to formalin as part of processing the tissue into paraffin. Alternatively, tissues might not have been fixed in neutral buffered formalin.

Slide 1: autolysis, mild (other organs) to severe (liver)

1. Liver: no significant lesions
- 2a. Heart: epicarditis, regionally diffuse, lymphocytic, neutrophilic, mild
- 2b. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: Lymphocytic epicarditis is evidence of chronic immune stimulation, and the addition of neutrophils is evidence of a more acute component (e.g., active bacterial infection). The cause and significance of myocardial karyomegaly 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). The liver has an unusual pattern of autolysis in which most of the hepatocyte nuclei are swollen and pale; the normal pattern is for autolytic nuclei to contract and become hyperbasophilic.

Slide 2: autolysis, mild (other organs) to moderate (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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.



**Molecular Diagnostics/PCR**

Samples 1-2: ISA, IPN, VHS Virus, Piscirickettsia salmonis negative by PCR.

\* Results faxed Feb. 16/05.

/bb

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Ministry of  
Agriculture, Food and Fisheries  
1767 Angus Campbell Road

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

Facsimile: (604) 556-3010  
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**Case Report**

**Submission** 2005-00161      **Date** 19-Jan-2005      **Report** 01-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13315      A. 2.4 - 8 (1-4)

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 2.4 - 8 (1-4).

Addendum: Jan 21/05 - samples submitted for routine histology.

**Histopathology**

Quality control/quality assurance: the sections contain moderate precipitates of acid hematin; this might be a result of transferring tissues to ethanol followed by return to formalin as part of processing the tissue into paraffin. Alternatively, tissues might not have been fixed in neutral buffered formalin.

Slide 1: autolysis, mild (other organs) to moderate (liver)

1. Liver: peritonitis, chronic, focal, with fibrocellular fronds, moderate
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, regionally diffuse, with fibrocellular fronds, severe
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Hepatic peritonitis is probably part of a vaccine reaction (same as splenic peritonitis).

Slide 2: autolysis, none (other organs) to mild (liver)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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 juvenile salmon it might be related to increased protein needed as part an inflammatory response. 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).

Slide 3: autolysis, mild (other organs) to moderate (trunk kidney, liver)

- 1a. Liver: parenchymal golden pigment, disseminated, intracellular, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild
3. Spleen: peritonitis, chronic, regionally diffuse, with fibrocellular fronds, moderate
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: The golden pigment in the liver most likely is lipofuscin. Accumulation of lipofuscin 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 have been associated with decreased growth and survival in several studies. Epicarditis is evidence of chronic immune stimulation, but the cause is otherwise unknown. 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.

Slide 4: autolysis, none (other organs) to mild (liver)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, regionally diffuse, with fibrocellular fronds, mild
4. Trunk kidney: renal tubular epithelial necrosis, regionally diffuse, acute, with regeneration, moderate
5. Head kidney: no significant lesions

Comment: 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). Causes in fish include exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as Gentamicin).



**Molecular Diagnostics/PCR**

Samples 1-2, 3-4: ISA, IPN, IHN, VHS Virus, Piscirickettsia salmonis negative by PCR.

\* Results faxed Feb. 16/05.

/bb



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

**Case Report**

**Submission** 2005-00162      **Date** 19-Jan-2005      **Report** 25-Jan-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13316      A. 2.4 -5 (2)

**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

**History/Symptoms**

Submitted two subcultures for bacteriology - identification of isolates and culture and sensitivity.

Provincial Surveillance Program Samples.

A. 2.4 - 5 (2).

**Bacteriology**

Bact plate A2.4-5 (2): *Vibrio* sp. negative for *Vibrio ordalli*, *Vibrio anguillarum* type 1 and 2 serology.

*Vibrio* sensitive to: Erythromycin, Florfenicol, Romet 30, Tri-sulfas, Sulfa-methox-trimeth and Tetracycline.

\* Results faxed Jan. 25/05.

/bb

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**Case Report**

**Submission** 2005-01740      **Date** 17-May-2005      **Report** 14-Jun-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 14269 #5275, 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 for tissue culture and IHNV - PCR, and VHSV - PCR.

Please quote PO# 636419 for accounting purposes.

**Virology**

Three samples inoculated onto tissue culture - all negative.

**Molecular Diagnostics/PCR**

IHN Virus Negative by PCR

VHS Virus Negative by PCR

Recheck of samples of IHN, VHS and Piscirickettsia salmonis (virologist request) - Negative by PCR

/sr

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**Case Report**

**Submission** 2005-01763      **Date** 18-May-2005      **Report** 20-May-2005

**Report**      **Copies**

**Submitter:** 12847      Heritage Salmon  
**Owner** 14282      Heritage Salmon #05-23  
**Farm:**  
**Vet Clinic:** 12847      Heritage Salmon  
**Attending**      Dr. Peter McKenzie

**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

### **History/Symptoms**

Submitted 2 histology cassettes labeled 2-1, 2-2 in Ethanol 35% for histology.

Increasing mortality from population. High mortality in crowding situations. Internal hemorrhage.

\*\* Please use our internal reference #05-23 on report. PO# H16388.

\*\* Please report to Dr. Peter McKenzie

### **Histopathology**

two cassettes of preserved tissues were submitted immersed in 35% ethanol for histopathology.

Slide 1 (2-1): gill, spleen, liver, trunk kidney, and mesenteric fat

Slide 2 (2-2): gill, spleen, liver, trunk kidney, stomach, and mesenteric fat

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

Quality control: Tissue preservation for most organs is fair to poor (i.e., mild to severe autolysis). Some tissues have evidence of dehydration after fixation. This most commonly results when preserved tissues are removed from liquid for more than a few minutes. Other potential causes include fixation in formalin that is too concentrated (e.g., 100% formalin instead of 10% formalin), or transfer to ethanol that is too concentrated (e.g., >70% ethanol) before processing to paraffin. Sections have no acid hematin.

Measures of physiologic condition

Hepatocellular glycogen depletion, severe (slides 1 and 2)

Mesenteric adipose tissue depletion, none (slides 1 and 2)

This pattern in the measures of physiologic condition is consistent with a previously healthy fish (mesenteric fat) that recently stopped feeding normally (severe glycogen depletion).

### **Diagnosis**

1a Liver: hepatic necrosis, acute, focal, mild (slide 2)

1b Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 1), moderate (slide 2)

1c. Liver: hepatitis, perivascular, lymphocytic, neutrophilic, mild (slide 2)

### **Final Comments**

Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus). Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Consider bacteriology and virology, if not already done. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Perivascular hepatitis (slide 2) is consistent with a systemic bacterial infection.

/sr



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**Case Report**

**Submission** 2005-01767      **Date** 18-May-2005      **Report** 27-May-2005

**Report**      **Copies**

**Submitter:** 12847      Heritage Salmon  
**Owner** 14284      Heritage Salmon #05-22 (Cypress  
Harbour)

**Farm:**  
**Vet Clinic:** 12847      Heritage Salmon  
**Attending**      Dr. Peter McKenzie

**Specimen:** Other      **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 agar plates (subcultures) labelled 05-22 - 115A, 115B, 115C, and 115D for culture and sensitivity.

Internal Hemorrhage on swim bladder, flesh, and adipose tissue.

Internal Reference #05-22. PO# H16387.

**Bacteriology**

Due to poor growth, antibiotic sensitivities were unable to be performed on sample 1115A. *Vibrio* sp. were negative for *Vibrio anguillarum* type I and II and *Vibrio ordalii* serology.

See attached sheets for sensitivities.

\* Results faxed May 27/05.

/bb

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**Case Report**

**Submission** 2005-01779      **Date** 19-May-2005      **Report** 24-May-2005

**Report**      **Copies**

**Submitter:** 9420      Sea to Sky Veterinary Service

**Owner** 14290      SS-120

**Farm:**

**Vet Clinic:** 9420      Sea to Sky Veterinary Service

**Attending**      Dr. S. Saksida

**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

### **History/Symptoms**

Submitted formalized fish tissue for histology.

Grossly no abnormal findings, feed in gut. Possibly anoxia (low Do levels) Kun 17; kun 19.

### **Histopathology**

Two cassettes of preserved tissues wrapped in moist paper towels were submitted for histopathology.

Slide 1 (O-1, 17 KUN): gill, spleen, liver, heart, trunk kidney, intestinal ceca and mesenteric fat

Slide 2 (O-2, KUN 19): gill, liver, heart, brain (includes 5-mm-diameter pituitary gland), trunk kidney, intestinal ceca and mesenteric fat

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

Quality control: Tissue preservation for most organs is good (i.e., autolysis none to mild). The gills have mild to moderate autolysis. Tissues have no significant dehydration after fixation or acid hematin deposits.

Measures of physiologic condition

Hepatocellular glycogen depletion, severe (slides 1 and 2)

Mesenteric adipose tissue depletion, none (slides 1 and 2)

This pattern in the measures of physiologic condition is consistent with a previously healthy fish (mesenteric fat) that recently stopped feeding normally (severe glycogen depletion).

### **Diagnosis**

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 1)

1a. Liver: sinusoidal fibrin, multifocal, acute, mild (slide 2)

2. Spleen: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, moderate (slide 1)

**Final Comments**

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).

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. Consider bacteriology and virology, if not already done. A fish with VHSV would be more susceptible to low dissolved oxygen levels (low DO as a cause of death rarely results in microscopic lesions).

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. (Mutoloki et al. 2004)

Literature Cited:

S. Mutoloki, S. Alexandersen, and Ø. Evensen. 2004. Sequential study of antigen persistence and concomitant inflammatory reactions relative to side-effects and growth of Atlantic salmon (*Salmo salar* L.) following intraperitoneal injection with oil-adjuvanted vaccines. *Fish & Shellfish Immunology* 16(5):633-644.

/sr



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**Case Report**

**Submission** 2005-01781      **Date** 19-May-2005      **Report** 08-Jul-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14292      A 3.2 - 35 (1-8)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**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

**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 3.2 - 35 (1-8).

ADDENDUM: June 30, 2005

Samples submitted for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of some organs have evidence of dehydration after fixation (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.

Slide 1: autolysis, none (all organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: peritonitis, granulomatous, focal, with occasional fine fibrocellular fronds, mild
2. Heart: endocarditis, multifocal, with endothelial cell hypertrophy and a thin layer of macrophages, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

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

The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface throughout most of the ventricle are rarely more than 2 cell layers thick.

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.

Slide 2: autolysis, none (all organs)

- 1a. Liver: macrophage aggregates, multifocal, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds and intralesional vacuoles up to 100 µm in diameter (vaccine material?), mild
- 4a. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
- 4b. Trunk kidney: renal tubular dilation, focal (area about 300 µm in diameter), mild
5. Head kidney: no significant lesions

Comment: Multiple foci of macrophage aggregates in the liver are an uncommon change.

The change is different from granulomatous inflammation; it might be related to a chronic low-grade bacterial infection. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Dilation of renal components is evidence of focally impaired renal function, but the cause is unknown.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: pericholangitis, lymphocytic, multifocal, mild
2. Heart:
3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds and intralesional vacuoles up to 200 µm in diameter (vaccine material?), severe
- 4a. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
- 4b. Trunk kidney: renal tubular dilation, focal (area about 300 µm in diameter), mild





5. Head kidney: no significant lesions

Comment: 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.

Slide 4: autolysis, none (other organs) to mild (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: mural thrombosis, bifocal, mild

3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus (e.g., VHSV), bacterial, or parasitic infection. The PCR results rule out VHSV as a cause.

Slide 5: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: no significant lesions

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (vaccine reaction)

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: no significant lesions

Comment: see comments on other fish (above).

Slide 6: autolysis, none (other organs) to mild (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: endocarditis, multifocal, with a thin layer of lymphocytes, mild

3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds and intralesional vacuoles up to 600 µm in diameter (vaccine material?), moderate

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: Lymphocytic inflammation in the heart is evidence of chronic immune stimulation (e.g., low grade bacterial infection), but the cause is otherwise unknown. Small droplets (5 - 20 µm in diameter) of intracellular homogeneous eosinophilic material are included with the granulomatous peritonitis; this is an unusual feature of vaccine-associated peritonitis. Some cells have a single larger droplet, whereas other cells contain multiple smaller droplets. This material might be part of the vaccine, or it might be a result of overproduction of protein by plasma cells.

Slide 7: autolysis, none (all organs)

1a. Liver: macrophage aggregates, multifocal, mild

1b. Liver: peritonitis, granulomatous, focal, with occasional fine fibrocellular fronds, mild (probable vaccine reaction)

2. Heart: no significant lesions

3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds and intralesional vacuoles up to 200 µm in diameter (vaccine material?), moderate

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: nephritis, interstitial, granulomatous, focal (~200 µm in diameter), mild

Comment: Foreign refractive material in the hepatic macrophage aggregates might be of vaccine origin. Small droplets (5 - 20 µm in diameter) of intracellular homogeneous eosinophilic material are included with the granulomatous peritonitis; features are similar to slide 6. The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous



inflammation.

Slide 8: autolysis, none (other organs) to mild (liver)

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild

1b. Liver: hepatic necrosis, acute, multifocal, mild

2a. Heart: endocarditis, granulomatous, focal, mild

2b. Heart: endocarditis, multifocal, with endothelial cell hypertrophy and a thin layer of macrophages, mild

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicaemia virus and *Piscirickettsia salmonis*, but these are ruled out by the negative PCR results). Proliferative lesions in the biliary system provide evidence for a chronic toxic cause for the hepatic necrosis. The most common organism associated with granulomas in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. Because these sections included only a solitary granulomatous lesion (in the heart), other bacterial causes should also be considered (e.g., *Yersinia ruckeri*). 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).

#### **Molecular Diagnostics/PCR**

Samples 1-4, 5-8: IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* negative by PCR.

/bb



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

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**Case Report**

**Submission** 2005-01782      **Date** 19-May-2005      **Report** 13-Jul-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14293      A 3.4 - 44 (1-3)

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 3.4 - 44 (1-3).

ADDENDUM: June 30, 2005

Submitted samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the spleen in slide 1 sections contains minimal deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The margins of all organs have no evidence of dehydration after fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: pericholangitis, lymphocytic, multifocal, mild
2. Heart: no significant lesions
- 3a. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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. 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. 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.

Slide 2: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 2a. Heart: epicarditis and endocarditis, multifocal, lymphoplasmacytic, mild
- 2b. Heart: multifocal iron pigments, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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. Epicarditis and endocarditis are evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. The peripheral compact layer of the heart has several small foci of basophilic precipitates that tend to line myocardial fibres. These precipitates stain for positive for iron but negative for mineral (Von Kossa stain). The precipitates are not the same as yellow-brown staining of hemosiderin; instead, they some a type of iron salt that resembles siderotic plaques in the spleens of old dogs. I have not previously seen these deposits in Atlantic salmon, but they probably are of little significance.

Slide 3: autolysis, none (all organs)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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).





**Molecular Diagnostics/PCR**

Samples 1-3, IHN, VHS, ISA, IPN *Piscirickettsia salmonis* Negative by PCR.

/sr

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**Case Report**

**Submission** 2005-01783      **Date** 19-May-2005      **Report** 11-Jul-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14294      A 3.4 - 45 (1-2)

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.4 - 45 (1-2).

ADDENDUM: (June 30, 2005)

Submitted samples for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the spleen section in slide 2 contains foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The organs have no significant dehydration after fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, severe
2. Heart: mural thrombosis, focal, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection.

Slide 2: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin 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 have been associated with decreased growth and survival in several studies.

**Molecular Diagnostics/PCR**

Samples 1-2: IHN, VHS, ISA, IPN, Piscirickettsia salmonis negative by PCR.

/bb



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**Case Report**

**Submission** 2005-01784      **Date** 19-May-2005      **Report** 11-Jul-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14295      A 3.4 - 46 (1-4)

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.4 - 46 (1-4).

ADDENDUM: (June 30, 2005)

Samples submitted for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the sections have no significant acid hematin or dehydration after fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, severe
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart: endocarditis, multifocal, lymphoplasmacytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. 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). Lymphoplasmacytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, none (other organs) to mild (liver and trunk kidney)

1. Liver: no significant lesions
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart: endocarditis, multifocal, lymphoplasmacytic, mild
- 2c. Heart: epicarditis, regionally diffuse, lymphoplasmacytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, minimal
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 3: autolysis, mild (other organs) to moderate (liver)

1. Liver: peritonitis, chronic, focal, with fibrocellular fronds, moderate
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: renal tubular dilation and dilation of the urinary space, regionally diffuse, mild
5. Head kidney: no significant lesions

Comment: Hepatic peritonitis is consistent with a reaction to foreign material; peritonitis is common in fish that have been vaccinated. Dilation of renal components is evidence of impaired renal function, possibly due to a functional blockage, but the cause is unknown. The liver has an unusual pattern of multifocal, coalescing autolysis; cytoplasm of autolyzed hepatocytes is paler than the surrounding parenchyma. This pattern might be a result of bile leakage, or it might be a result of necrosis. Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections; the PCR results rule out common causes of hepatic necrosis: viral hemorrhagic septicemia virus and *Piscirickettsia salmonis*.

Slide 4: autolysis, mild (other organs) to moderate (liver)

- 1a. Liver: hepatitis, granulomatous, multifocal, with central foci of neutrophils and karyorrhexis, severe
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: epicarditis and endocarditis, granulomatous, diffuse, moderate
- 3a. Spleen: splenitis, granulomatous, multifocal, coalescing, with occasional foci of neutrophils in the centre of granulomatous inflammation, severe
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, severe





5. Head kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, severe

Comment: The most common organism associated with disseminated granulomas in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. Granulomatous foci in the kidney contain foci of radiating material (foci about 80 µm in diameter) consistent with vaccine material.

**Molecular Diagnostics/PCR**

Samples 1-2, 3-4: IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* negative by PCR.

/bb

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**Case Report**

**Submission** 2005-01851      **Date** 26-May-2005      **Report** 01-Jun-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 14326 #5276, Marine Harvest

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**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

**History/Symptoms**

Submitted 8 samples for tissue culture, PCR for IHN, and PCR for VHSV.

Samples labelled 1-3, 4-5, 7-9, 10-12, 13-15, 16-17, 18-20.

Please quote PO#636423.

**Virology**

8 samples inoculated onto tissue culture - all negative.

\* Results faxed June 22/05.

**Molecular Diagnostics/PCR**

Samples 1-3, 4-5, 7-9, 10-12, 13-15, 16-17, 18-20, 21-22:

IHN Virus negative by PCR.

VHS Virus negative by PCR.

\* Results faxed June 1/05.

/bb

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**Case Report**

**Submission** 2005-01854      **Date** 26-May-2005      **Report** 02-Jun-2005

**Report**      **Copies**

**Submitter:** 12847      Heritage Salmon  
**Owner** 14328      Heritage Salmon 05-25  
**Farm:**  
**Vet Clinic:** 12847      Heritage Salmon  
**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

### **History/Symptoms**

Submitted formalized fish tissue for histology.

Fish have been in sea water for - 3 months. No internal signs of disease. High number of fish exhausting "pop eye". Increasing mortality.

Please include internal reference number 05-25.

PO# 16392.

### **Histopathology**

5 cassettes of preserved tissues immersed in 35% ethanol were submitted for histopathology.

Slide 1 (May 10/pen 3, fish 1): gill, skin/skeletal muscle, trunk kidney, liver, heart, spleen, stomach, intestinal ceca and surrounding mesenteric fat

Slide 2 (May 10/pen 3, fish 2): gill, skeletal muscle with bone (rib?), trunk kidney, liver, heart, spleen, stomach, and surrounding mesenteric fat

Slide 3 (3113, 05/21): trunk kidney, liver, spleen

Slide 4 (3103, 05/21): trunk kidney, liver, spleen

Slide 5 (3103, 05/21): trunk kidney, liver, spleen

All organs on each slide were examined; organs not listed below had no significant lesions.

Quality control: Tissue preservation for most organs is good (i.e., autolysis none to mild). The intestinal ceca have mild to moderate autolysis. The margins of some organs have evidence of dehydration after fixation (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. Half of the liver in slide 3 has postfixation dehydration. Except for one small focus in the liver in slide 1, tissues have no significant deposits of acid hematin.

Measures of physiologic condition

Hepatocellular glycogen depletion, severe (slides 1, 2, 3, 4, and 5)

Mesenteric adipose tissue depletion, none (slides 1, 2, 3), not present for analysis (slides 4 and 5)

This pattern in the measures of physiologic condition is consistent with healthy fish (mesenteric fat) that stopped eating normally in the past few days (glycogen depletion).

### **Diagnosis**

1. Skeletal muscle: myonecrosis, peracute, multifocal, mild (slide 1)
- 2a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1, 4, 5), moderate (slide 2)
- 2b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 3)
- 2c. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 3)
- 2d. Liver: hepatitis, granulomatous, focal (200 µm in diameter), mild (slide 5)
- 3a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 2, 3, 5)
- 3b. Spleen: splenitis, granulomatous, focal (150 µm in diameter), mild (slide 4)
4. Trunk Kidney: nephritis, interstitial, granulomatous, multifocal, mild (slide 4)



**Final Comments**

These fish have a few lesions that would have contributed to morbidity. None of the lesions specifically relate to the high prevalence of exophthalmia (noted clinically). To get a better impression of the nature and cause of exophthalmia, consider submitting the eyes and surrounding skull for histopathology.

Myonecrosis in slide 1 is limited to scattered individual fibers with flocculent cytoplasm and loss of cross striations. This pattern is consistent with capture myopathy or agonal contractions.

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.

The most common organism associated with granulomatous nephritis, hepatitis, and splenitis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation. Consider PCR or bacterial culture for a definitive diagnosis.

/sr





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**Case Report**

**Submission** 2005-00189      **Date** 21-Jan-2005      **Report** 01-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13327      A 2.4 - 64 (1-2)

**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

### **History/Symptoms**

Submitted Provincial Surveillance Program samples for routine histology.

Please refer to case 2004-3858 for PCR results.

Sample ID: A 2.4 - 64 (2).

### **Histopathology**

Quality control/quality assurance: the sections contain occasional precipitates of acid hematin; this might be a result of transferring tissues to ethanol followed by return to formalin as part of processing the tissue into paraffin. Alternatively, tissues might not have been fixed in neutral buffered formalin.

Slide 1: autolysis, mild (other organs) to moderate (liver)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: epicarditis, regionally diffuse, lymphoplasmacytic, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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 juvenile salmon it might be related to increased protein needed as part an inflammatory response. Epicarditis is evidence of chronic immune stimulation, but the cause is otherwise unknown. Lack of associated inflammation is evidence that multiple foci of bacteria, up to 1 mm in diameter, are post mortem growth. Tissue preservation is remarkably good considering the size of the intracardiac bacterial colonies.

Slide 2: autolysis, none (other organs) to mild (liver)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. The trunk kidney interstitium contains abundant melanomacrophage centres; the significance of this difference from other Atlantic salmon is unknown, but it is probably minimal.

/bb



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**Case Report**

**Submission** 2005-01939      **Date** 02-Jun-2005      **Report** 09-Jun-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14389 #5273, Marine Harvest  
**Farm:**  
**Vet Clinic:**  
**Attending** Cilka LaTrace/ Dr. Diane Morrison

**Specimen:** Tissue - Fresh      **Count** 15      **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 15 samples for tissue culture IHNV - PCR and VHSV - PCR.

Samples are labelled as follows: 1-3, 4-6, 7-9, 10-12, 13-15, 16-18, 19-21, 21-24, 25-27, 28-30, 31-33, 34-36, 37-39, 40-42, 43-45.

No previous history of isolation of IHN or VHS from these fish.

**Virology**

Fish viruses negative.

Tissue culture - negative.

\* Results faxed June 29/05.

**Molecular Diagnostics/PCR**

Samples 1-3, 4-6, 7-9, 10-12, 13-15, 16-18, 19-21, 22-24, 25-27, 28-30, 31-33, 34-36, 37-39, 40-42, 43-45:  
IHN Virus and VHS Virus negative by PCR.

/bb

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## Case Report

**Submission** 2005-01940      **Date** 02-Jun-2005      **Report** 09-Jun-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14390 #5281, Marine Harvest  
**Farm:**  
**Vet Clinic:**  
**Attending** Cilka LaTrace/Dr. Diane Morrison

**Specimen:** Tissue - Fresh      **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

**History/Symptoms**

Submitted 8 samples for virology - tissue culture IHNV - PCR and VHSV - PCR.

Bags are labelled A-G, the eighth bag is brain tissue. VHS has been isolated at this site previously. Affected fish are only showing signs of congested vessels in the brain. Please check for isolation of virus from brain tissue.

Please quote PO# 636426.

**Virology**

Fish viruses negative.  
Tissue culture negative.

\* Results faxed June 29/05.

**Molecular Diagnostics/PCR**

Samples A, B, C, D, E, F, G, H: IHN Virus negative by PCR; VHS Virus negative by PCR.

/bb



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**Case Report**

**Submission** 2005-01982      **Date** 06-Jun-2005      **Report** 10-Jun-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 14411 #5285, 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 cassette.

Kidney from one fish with swollen kidney, deposit in ureters nephrocalcinosis? BKD?

\* For billing purposes, please quote PO# 636430.

**Histopathology**

One cassette of tissues preserved in 10% neutral buffered formalin was submitted wrapped in tissue for histopathology. The single slide contains a section of head kidney and 3 pieces of trunk kidney. Tissue preservation is good to excellent. Tissues have no evidence of postfixation dehydration or acid hematin accumulation.

**Diagnosis**

1. Trunk kidney: nephritis, interstitial, granulomatous, diffuse, with intralesional amorphous to globular pale gray-brown foreign material and rare small Gram positive bacilli consistent with *Renibacterium salmoninarum*, severe
2. Trunk kidney: dilated tubules, with intratubular globular pale gray-brown foreign material, Gram negative bacteria, histiocytes, neutrophils, and thrombi, severe
3. Trunk kidney: tubular epithelial necrosis, multifocal, acute, mild
4. Trunk kidney: renal tubular mineralization, focal, with dilated tubules and tubular epithelial hyperplasia, moderate
5. Trunk kidney: enlarged glomeruli (up to 300  $\mu$ m in diameter), multifocal, moderate
6. Head kidney: dilated pronephric duct, focal (3 mm in diameter), moderate
7. Head kidney: multiple glomeruli, with redundant vasculature, multifocal

**Final Comments**

The main changes in this kidney seem to relate more to foreign material (e.g., vaccine adjuvant) than to mineral (present in just one tubule) or *Renibacterium salmoninarum* (few observed with Gram stain).

Granulomatous inflammation in the trunk kidney seems to be primarily in response to the globular pale gray-brown foreign material (the material is weakly PAS positive). The most likely source of this material is a vaccine. A Twort's Gram stain highlights small numbers of Gram negative bacilli within some foci foreign material, consistent with bacteria in the vaccine; I cannot determine if the bacteria were viable at the time the kidney was sampled. Gram negative bacilli are also in the center of dilated and inflamed tubules. Three tubules contain small foci of short bacterial rods consistent with *Renibacterium salmoninarum*, but the lesions associated with the bacteria are not classic for bacterial kidney disease; it may be that the *Renibacterium salmoninarum* are an incidental finding. Bacterial culture is recommended, if not already done. Material in the tubules probably led to the tubular lesions, including scattered foci of bacteria. Necrosis of tubular epithelial cells is probably secondary to other renal lesions. A GMS stain of the tissues rules out the presence of fungus in the lesions.

Only one tubule has significant deposits of mineral (confirmed with Von Kossa stain); this tubule is nearly 1 mm in diameter. Renal mineralization is common in cultured fish species; when severe, the condition is termed nephrocalcinosis. The lesion is not considered fatal, although feed conversion may be adversely affected. The cause is unknown, 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.

The large glomeruli in the trunk kidney might be a proliferative response to chronic inflammation. Alternatively, they might be a result of a developmental abnormality

The large dilated tubule in the head kidney is probably a result of the inflammation "downstream" in the trunk kidney. The head kidney normally has only 1 pronephric glomerulus on each side, but tubules are relatively common. By comparison, this section contains 11 sections of glomeruli and no sections of tubules. Head kidney glomeruli are relatively large (up to 200 µm in diameter), as expected for pronephric glomeruli, but the vessels approaching/surrounding the glomeruli are redundant (several cross sections of the vessels surround a single glomeruli). These findings are unusual and might be a result of a developmental abnormality.

/sr



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**Case Report**

**Submission** 2005-02023      **Date** 08-Jun-2005      **Report** 14-Jun-2005

**Report**      **Copies**

**Submitter:** 12847      Heritage Salmon  
**Owner** 14435      05-26, Heritage Salmon  
**Farm:**  
**Vet Clinic:** 12847      Heritage Salmon  
**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

**History/Symptoms**

Submitted one culture plate labeled 05-26-1 for culture and sensitivity.

History of BKD. ERM typed in November 2004. Conducting OTC treatment.

Please use our internal reference 05-26 on the report. PO #H16400.

Please send results to Dr. Peter McKenzie.

**Bacteriology**

Isolate: *Pseudomonas fluorescens*. See attached report for sensitivity pattern.

/sr

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## Case Report

**Submission** 2005-02064      **Date** 10-Jun-2005      **Report** 17-Jun-2005

**Report**      **Copies**

**Submitter:** 12847      Heritage Salmon  
**Owner** 12847      Heritage Salmon  
**Farm:**  
**Vet Clinic:** 12847      Heritage Salmon  
**Attending**      Dr. Peter McKenzie

**Specimen:** Whole Animal      **Count** 60      **Flock Herd Size:**  
**Species:** Atlantic Salmon      **Age**  
**Breed:** Mowi      **Sex:**

**Feed:**      **Feed**

**Vaccination**

**Treatmen**

**Diagnosis**

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

**History/Symptoms**

Submitted 60 fish from 5M23, 3 pools for PCR testing BKD.

Fish in fresh water 12.4 degrees C. Three bags of 20 fish/bag - live sample.

**Gross Pathology**

Three groups of salmon (20 fish each) were received alive in plastic bags. Fish were deeply anesthetized in tricaine methane sulfonate in the water in which they were submitted. Total wet weight of the 20 fish in each group was 40.4 g, 44.2 g, and 42.3 g. Fish were analyzed for *Renibacterium salmoninarum* (by PCR) in three 20-fish pools based on the bag in which they were submitted.

**Molecular Diagnostics/PCR**

20 fish Pool #1 - *Renibacterium salmoninarum* Negative by PCR

20 fish Pool #2 - *Renibacterium salmoninarum* Negative by PCR

20 fish Pool #3 - *Renibacterium salmoninarum* Negative by PCR

Note - normal pooling of samples for PCR should be to a limit of 5 fish per pool.

\*PCR results faxed to submitter on June 17, 2005.

/sr



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

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**Case Report**

**Submission** 2005-00209      **Date** 24-Jan-2005      **Report** 31-Jan-2005

**Report**      **Copies**

**Submitter:** 11899      Grieg Seafoods B.C. Ltd.

**Owner** 13337      Williamson Passage

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Formalized

**Count** 1

**Flock Herd Size:** 250

**Species:** Atlantic Salmon

**Age**

**Breed:** Mowi X

**Sex:**

**Feed:**

**Feed**

**Vaccination**

**Treatmen**

**Diagnosis**

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

### **History/Symptoms**

Submitted formalized liver, spleen, pyloric cecae, kidney, and gill tissue for histology. Short lists - environmental stress \*, toxin, disease (virology, bacteriology submitted).

Population stressed two days previously. Mortality increased over background and same age ( 1 day previously for majority).

Submitted by Dr. Barry Milligan.

### **Histopathology**

Three slides were prepared from 3 cassettes that each contained multiple tissues. Slide numbers 1 - 3 were arbitrarily assigned to the cassettes.

Slide 1: spleen, head kidney, trunk kidney, liver, gill, and 5x3 mm section of a Corpuscle of Stannius

Slide 2: spleen, trunk kidney, liver, intestine, and gill

Slide 3: spleen, trunk kidney, liver, intestine, and gill

All organs were examined on each slide. Organs without findings listed below had no significant lesions.

Quality control/quality assurance: Tissue preservation is fair to good for all organs in all slides (i.e., mild to moderate autolysis).

Measures of physiologic condition

Hepatocellular glycogen depletion, severe (all slides)

Mesenteric adipose tissue depletion, none (slides 2, 3)

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

### **Diagnosis**

1. Trunk Kidney: nephritis, interstitial, granulomatous, multifocal, moderate (slide 3)
2. Spleen: parenchymal golden pigment, disseminated, intracellular (primarily expanding melanomacrophages), minimal (slide 3)
3. Liver: hepatocellular cytoplasmic protein droplets (cytosegresomes?), disseminated, mild (slide 2)
4. Liver: parenchymal golden pigment, intracellular (primarily expanding macrophages), peribiliary, disseminated, mild (slides 1, 2, 3)
5. Liver: hepatocellular single cell necrosis (apoptosis), disseminated, acute, mild (slide 3), moderate (slide 1)
6. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1, 3)
7. Liver: hepatocellular fatty change (lipidosis), regionally diffuse, mild (slide 2), moderate (slides 1, 3)
8. Liver: hepatitis, perivascular, lymphoplasmacytic, neutrophilic, multifocal, mild (slide 3)
9. Gill: branchitis, lymphoplasmacytic, with disseminated apoptosis of leukocytes, mild (slide 1)



**Final Comments**

These organs have several inflammatory and degenerative lesions. Granulomatous inflammation in the kidney is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* is the most common organism associated with these lesions. Cytosegresomes and lipofuscin are consistent with exposure to toxins.

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).

Cytosegresomes occur in liver cells that have been sublethally injured by a variety of insults, ranging from hypoxia through a variety of intoxications to malnutrition, specific deficiencies, and some viral infections. They may be formed when masses of cytoplasmic organelles are gathered and condensed, and are sequestered from remaining cytoplasm by membranes that fuse with lysosomes (autolysosomes). Intracellular digestion of cytosegresomes leaves remnants of undigested material, known as lipofuscin.

The golden pigment in the spleen and liver 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. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR); however, these fish lack the key feature of netpen liver disease--enlarged hepatocytes. Conditions that lead to moderate and abundant lipofuscin have been associated with decreased growth and survival in several studies.

Single cell hepatocellular necrosis can be a result of exposure to toxins. In the affected liver (slide 1), affected hepatocytes have brightly eosinophilic cytoplasmic droplets.

The source of the toxins could be endogenous (i.e., bacterial toxins) or exogenous (something in the water or feed). Hepatocellular single cell necrosis (apoptosis) can also occur 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).

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

Lymphoplasmacytic branchitis with apoptosis in the non-lamellar stratified squamous epithelium of the filament is evidence of chronic immune stimulation, but the change is otherwise nonspecific. A similar mechanism could have caused perivascular hepatitis. Exposure to bacteria is the most likely cause of the change.

/bb



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**Case Report**

**Submission** 2005-00210      **Date** 24-Jan-2005      **Report** 28-Jan-2005

**Report**      **Copies**

**Submitter:** 11899      Grieg Seafoods B.C. Ltd.

**Owner** 13338      Barnes Bay

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Formalized

**Count** 1

**Flock Herd Size:**

**Species:** Atlantic Salmon

**Age**

**Breed:** Mowi

**Sex:**

**Feed:**

**Feed**

**Vaccination**

**Treatmen**

**Diagnosis**

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

**History/Symptoms**

Submitted formalized liver, kidney, spleen (6 histo cassettes). Would like to determine if Rickettsial agents are present in addition to BKD.

Previous diagnosis of BKD. Two weeks Oxytetracycline treatment 3 weeks previously. Multi focal yellow white nodules throughout liver, kidney, in a small percent of fish (< 5% fresh mortalities).

Submitted by Dr. Barry Milligan.

**Histopathology**

Six slides were prepared from 6 cassettes that each contained multiple tissues and each was labelled "BB 01/18." Slide numbers 1 - 6 were arbitrarily assigned to the cassettes.

Slide 1: spleen, head kidney, trunk kidney, liver

Slide 2: spleen, head kidney, trunk kidney, liver

Slide 3: spleen, trunk kidney, liver

Slide 4: spleen, trunk kidney, liver

Slide 5: spleen, head kidney, trunk kidney, liver

Slide 6: spleen, head kidney, trunk kidney, liver

All organs were examined on each slide. Organs without findings listed below had no significant lesions.

Quality control/quality assurance: Tissue preservation is excellent for all organs in slides 1, 2, 4, 5, and 6 (i.e., no significant autolysis in any organ) and fair for all organs in slide 3 (i.e., mild to moderate autolysis).

Measure of physiologic condition

Hepatocellular glycogen depletion, severe (all slides)

Severe glycogen depletion is consistent with fish that were not eating normally within at least the past few days.





**Diagnosis**

1. Trunk Kidney: nephritis, interstitial, granulomatous, multifocal, mild (slide 5), severe (slide 4)
2. Trunk Kidney: interstitial golden pigment, disseminated, intracellular (primarily expanding melanomacrophages), mild (slide 3)
3. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 1, 2, 5), moderate (slide 3, 4, 6)
4. Spleen: splenitis, granulomatous, multifocal, coalescing, moderate (slide 3)
5. Spleen: parenchymal golden pigment, disseminated, intracellular (primarily expanding melanomacrophages), minimal (slides 2, 3, 4, 5), mild (slides 1, 6)
6. Spleen: granulomas, multifocal, 300 µm in diameter, with intralesional foreign material, mild (slides 2 and 5)
  
7. Spleen and surrounding fatty mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, moderate (slide 2)
8. Liver: hepatitis, granulomatous, multifocal, mild (slides 3, 5), severe (slide 4)
9. Liver: parenchymal golden pigment, peribiliary, intracellular (primarily expanding macrophages), mild (slides 1, 2, 4, 5, 6), moderate (slide 3)
10. Liver: hepatic necrosis, multifocal, acute, mild (slide 6), moderate (slide 4)
11. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1, 6), moderate (slide 2)
12. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 2, 5), moderate (slides 3, 4)
13. Liver: hepatocellular fatty change (lipidosis), regionally diffuse, mild (slide 5), moderate (slide 6)
14. Head kidney: granulomas, multifocal (100-400 µm in diameter), mild (slides 2, 5)
15. Head Kidney: interstitial cell hyperplasia, diffuse, mild (slide 2)
16. Trunk Kidney: interstitial cell hyperplasia, diffuse, mild (slide 5)



**Final Comments**

These organs have several inflammatory and degenerative lesions. 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 (consistent with clinical diagnosis). Coagulative hepatic necrosis in the liver (slide 4) might be secondary to widespread granulomatous inflammation. The liver in slide 6 has multifocal hepatic necrosis, but no evidence of *Renibacterium salmoninarum* infection; therefore, necrosis in this fish might be a result of infection with a virus or another type of bacteria.

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). 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 juvenile salmon it might be related to increased protein needed as part an inflammatory response.

The golden pigment in the spleen, liver, and kidney 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. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR); however, these fish lack the key feature of netpen liver disease--enlarged hepatocytes. Conditions that lead to moderate and abundant lipofuscin have been associated with decreased growth and survival in several studies.

The renal and splenic granulomas (slide 2) are consistent with reaction to injected foreign material (e.g., vaccine adjuvant). Splenic peritonitis is also consistent with a foreign body reaction, probably in response to vaccine adjuvant.

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).

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.

The slides have no evidence of rickettsia. However, small numbers of *Piscirickettsia* are difficult to detect using histopathology. If clinical signs are consistent with *Piscirickettsia*, consider submitting fresh (chilled but not frozen) samples for *Piscirickettsia* PCR (multiple fish can be pooled and analyzed as one sample).

/bb



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**Case Report**

**Submission** 2005-02111      **Date** 15-Jun-2005      **Report** 23-Jun-2005

**Report**      **Copies**

**Submitter:** 11899      Grieg Seafoods B.C. Ltd.

**Owner** 11899      Grieg Seafoods B.C. Ltd.

**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

### **History/Symptoms**

Submitted formalized Atlantic salmon tissue for histology.

Higher than expected starve-out rate at first feeding. Some fish appear to have buoyancy problems.

Post Mortem: decreased body condition, inappetance, opportunistic bacterial infections (*Pseudomonas*).

### **Histopathology**

Fourteen small salmon (about 1 g each) were submitted in three cassettes in 10% formalin. Tissues caudal to the anus were removed; the remaining body and head were submersed whole in Protocol B for decalcification for 35 minutes (smallest fish) to 45 minutes (largest fish), and then transected midsagittally before processing routinely into paraffin.

Fish were trimmed in order from smallest (slide 14) to largest (slide 1). Fish on slides 1, 11, and 14 had curved spines resulting in upturned tails (moderate to severe kyphosis).

Measures of tissue/section quality, physiologic condition, and lesions were scored and recorded in spreadsheet format. Autolysis was scored on the most autolyzed tissue on the slide; in most cases, this was the intestine, which commonly develops severe autolysis because the tissues decompose before the fixative penetrates the body wall. Because of the large number of fish in this case, microscopic findings are presented in digital format (Excel file) maintained on the Animal Health Centre server <2005-02111.xls> independent of the Animal Health Centre's Vetlab system (the Vetlab system cannot handle Excel spreadsheets). The spreadsheet contains diagnoses for lesions that commonly appear in salmon fry, even if they did not appear in fish from this case.

### **Diagnosis**

1. Vertebral column: kyphosis (upturned tails), mild (slide 1), moderate (slide 14), severe (slide 11)
2. Head: fungal hyphae invasion of pseudobranch, gill, and connective tissue and skeletal muscle of the head, regionally diffuse, severe (slide 1)
3. Liver: bile duct fibrosis, focal, with dilation to 150 µm in diameter, moderate (slide 7)
4. Intestine: bacterial overgrowth, moderate (slides 10, 11)
5. Gastrointestinal tract: intraluminal metal, focal (about 1 mm in diameter; block 3)
6. Others: see the spreadsheet included with this report



**Final Comments**

Lesions that probably contributed to morbidity included fungal infections fish 1, and biliary preductular hyperplasia in most of the fish.

Fungal infections with *Saprolegnia* sp. and related organisms (e.g., *Achlya*) are common in fish. *Saprolegnia* and related fungi are most commonly secondary invaders, and disease usually develops after any type of trauma to the skin. Underlying causes on fungal infections include handling, overcrowding, suboptimal water quality, and bacterial or viral infections. Death is thought to be related to osmotic imbalance secondary to the ulcers. The fungi most commonly grow in dense mats on the surface of the skin, but hyphae can invade the underlying musculature and cause myonecrosis. [Source: Fish Pathology, 3rd Edition. 2001. R.J. Roberts.] In the affected fish, hyphae invaded the structures of the head, but not the brain.

Most of the fish had biliary preductular cell hyperplasia: 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). Only the smallest fish had no clear evidence of biliary preductular cell hyperplasia; livers of these fish were not well preserved, making analysis for lesions difficult. Also, the smallest fish might have eaten less feed (i.e., circumstantial evidence of feed as the source of a significant toxin).

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in these fish). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, something in the water or feed, or aminoglycoside antibiotics such as gentamicin). In these fish, autolysis of the kidney decreased my confidence in my diagnosis of renal tubular epithelial necrosis (i.e., the alternate diagnosis is renal tubular epithelial autolysis).

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 in fish that are not eating; muscle tissue is broken down to provide nutrients for critical organ survival.

Bacterial overgrowth in the intestine (slides 10 and 11) is evidence of decreased intestinal motility. Bacteria are normally not seen on routine preparations of intestine from small salmon. Decreased intestinal motility is also consistent with poor digestion.

The piece of metal in the gastrointestinal tract of fish #3 is an interesting finding. The piece of metal sectioned easily: evidence that it is softer than the steel blades used for sectioning tissues. The piece of metal was not retained in the stained slide. The piece of metal might be an unusual finding of little significance. However, if the feed contains several of these metal pieces, they probably should be identified; lead and zinc can be toxic to fish.

/sr





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**Case Report**

**Submission** 2005-02142      **Date** 16-Jun-2005      **Report** 23-Jun-2005

**Report**      **Copies**

**Submitter:** 12847      Heritage Salmon  
**Owner** 12847      Heritage Salmon  
**Farm:**  
**Vet Clinic:** 12847      Heritage Salmon  
**Attending**      Dr. P. McKenzie

**Specimen:** Other      **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

### **History/Symptoms**

Submitted 10 histology cassettes labeled 05-35-1 through 05-35-10 (gill, heart, ceca, liver, spleen, flesh, kidney, brain) for histology.

Fish have been in salt water for 3 months. Increased numbers of pinhead dropouts. Just finished SLICE treatment, number of mouth rot mortality increasing. High mortality.

\*\* Please use our case number 05-35. \*\*

\*\* PO #17204.

### **Histopathology**

10 cassettes of tissues preserved in 10% neutral buffered formalin were submitted immersed in ethanol for histopathology.

Slide 1 - skeletal muscle (and skin?), liver, intestinal cecum and mesenteric fat, trunk kidney, heart, gill, and spleen

Slide 2 - skeletal muscle and skin, liver, intestinal cecum and mesenteric fat, head kidney, trunk kidney, heart, and gill

Slide 3 - skeletal muscle and skin, liver, intestinal cecum and mesenteric fat, head kidney, trunk kidney, heart, brain, and gill

Slide 4 - skeletal muscle and skin, liver, intestinal cecum and mesenteric fat, trunk kidney, heart, spleen, brain, and gill

Slide 5 - skeletal muscle and skin, liver, intestinal cecum and mesenteric fat, trunk kidney, heart, spleen, brain, and gill

Slide 6 - skeletal muscle, liver, intestinal cecum and mesenteric fat, head kidney, heart, gill, and spleen

Slide 7 - skeletal muscle and skin, liver, intestinal cecum and mesenteric fat, head kidney, trunk kidney, heart, spleen, brain, and gill

Slide 8 - skeletal muscle and skin, liver, intestinal cecum and mesenteric fat, head kidney, trunk kidney, heart, spleen, brain, and gill

Slide 9 - skeletal muscle, liver, intestinal cecum and mesenteric fat, head kidney, heart, spleen, brain, and gill

Slide 10 - skeletal muscle, liver, intestinal cecum and mesenteric fat, trunk kidney, heart, spleen, brain, and gill

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

Quality Control: Tissue preservation is fair to poor for most organs. Autolysis is particularly severe in the gill and intestine. The margins of most organs have no evidence of dehydration after fixation; small foci in the few affected organs have nuclei that stain dull blue, erythrocyte cytoplasm stains yellow instead of red, and cytoplasm of other cell types stains poorly or not at all).

### **Diagnosis**

1. Liver: sinusoidal congestion, focal (600 µm in diameter), mild (slide 4)
2. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 8)
3. Spleen: splenitis, multifocal, granulomatous, mild (slide 8)



**Final Comments**

Moderate to severe autolysis in these tissues may have prevented the diagnosis of some lesions. Alternatively, the fish may be dying primarily from complications of mouth rot, described clinically.

The focal nature of sinusoidal congestion in the liver (slide 4) is consistent with local trauma. Sinusoidal congestion has also been associated with viral infections (ISA and VHSV), but I would expect changes from viral infection to be more widespread. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Granulomatous inflammation in the spleen (slide 8) is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* is the most common organism associated with these lesions. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.

/sr



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## Case Report

**Submission** 2005-02200      **Date** 21-Jun-2005      **Report** 24-Jun-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14527 #5296, Marine Harvest  
**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

### **History/Symptoms**

Two fresh mortalities. Cassettes #41, 42.

Species - Atlantic. Broodstock. Netpen #41, 42. Freshwater.

Internally hemorrhages on liver, pyloric ceca, peritoneal surface of musculature; occasionally with belly rash.

Bact. of kidney negative for growth.

Currently no increase in mortality.

Submitted by Cilka LaTrace.

### **Histopathology**

Two cassettes of preserved tissues were submitted for histopathology wrapped in moist towel.

Slide 1 (June8/05 5296-41) - head kidney, trunk kidney, spleen, heart, liver, gill, intestinal ceca and mesenteric fat.

Slide 2 (June8/05 5296-42) - head kidney, trunk kidney, spleen, heart, liver, skeletal muscle, intestinal ceca and mesenteric fat.

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

Quality Control: In the fish from netpen 41, tissue preservation is excellent for most organs, but the intestinal ceca and gill have mild autolysis. In the fish from netpen 42, tissue preservation is good for most organs, but the intestinal ceca have moderate to severe autolysis. The margins of most organs have evidence of dehydration after fixation; small foci in the affected organs have nuclei that stain dull blue, erythrocyte cytoplasm stains yellow instead of red, and cytoplasm of other cell types stains poorly or not at all). Tissues are free of acid hematin deposits.

Measures of physiologic condition:

Hepatocellular glycogen depletion, severe (slides 1, 2)

Mesenteric adipose tissue depletion, none (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).

### **Diagnosis**

1. Trunk kidney: glomerulonephritis, mesangioproliferative, multifocal, mild (slide 1)
2. Trunk kidney and head kidney: renal tubular mineralization, multifocal, moderate (slides 1, 2)
3. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, with fibrin and fibrosis, moderate (slide 2)
4. Head kidney: interstitial cell hyperplasia, diffuse, moderate (slide 1)
5. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1, 2)
6. Liver: hepatocellular fatty change (lipidosis), multifocal, moderate (slide 2)
7. Liver: sinusoidal congestion (peliosis), multifocal, mild (slide 2)
8. Heart: myocardial karyomegaly, multifocal, moderate (slide 2)





**Final Comments**

The fish from netpen 42 has some lesions consistent with a viral infection. VHSV is the most common virus affecting pen-raised salmon in British Columbia, but some lesions in the liver are also consistent with ISAV. I recommend submitting fresh tissues for virology and PCR to rule out VHSV, ISAV, and IHNV.

Mesangioproliferative 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. Similar types of glomerulonephritis have been associated with cardiomyopathy syndrome (in Atlantic salmon), nephrocalcinosis, and infections with a number of bacteria and parasitic species.

Renal mineralization is common in cultured fish species; when severe, the condition is termed nephrocalcinosis. Here it affects primarily large tubules, especially in the head kidney. The lesion is not considered fatal, although feed conversion may be adversely affected. The cause is unknown, 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.

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared. Early fibrosis in this case is evidence that the basement membrane was damaged. In slide 2, lesions are separate from renal mineralization. Causes of these lesions in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin).

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).

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.

Mild myocardial karyomegaly is fairly common in cultured salmonids, but the cause and significance of 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).

Hepatic peliosis is evidence of sinusoidal damage. In BC Atlantic salmon, peliosis is an uncommon feature of infection with viral hemorrhagic septicemia virus. Peliosis is also one of the classic lesions associated with ISAV infections. Consider virology and PCR for VHSV, IHNV, and ISAV (if not already done). Peliosis has also been described in wild fish (dab) surveyed in the north Pacific (source: <http://www.cefas.co.uk/publications/aquatic/aemr41.pdf>), but the cause was not determined. I have seen peliosis in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data).

/sr



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

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**Case Report**

**Submission** 2005-02227      **Date** 22-Jun-2005      **Report** 14-Jul-2005

**Report**      **Copies**

**Submitter:** 11899 Grieg Seafoods B.C. Ltd.

**Owner** 11899 Grieg Seafoods B.C. Ltd.

**Farm:**

**Vet Clinic:**

**Attending** Dr. Milligan

**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**

From typed history:

Submitted samples from Campbell River

- 1) 5 histosettes multiple tissues for histology.
- 2) 3 bags of fish for virology and bacteriology (pen 12, pen 14, unidentified bag). I would like virology on a pool of 5 from each bag if possible (3 virology pools total) and individual bacteriology on 5 fish from each of the 3 bags (15 bacteriology total).
- 3) 5 bags of kidney tissue for BKD PCR

History of population is poor appetite, mortality elevated above background and poor weight gain for 3 months. The problem appears restricted to a population of approx. 200,000 fish with a separate origin. Of the population in question, there appears to be about 10 to 15% of the population affected. Bacterial stomatitis is occurring concurrently (fish have been treated with tribissen in the last week and those fish eating have apparently responded appropriately). On post mortem, there is decreased body condition and lack of feed in the digestive tracts with some fish exhibiting pale friable livers. See previous histo report on the fish in question (2005-00209). Please note that previous virology/bacteriology has not identified significant pathogens.

From handwritten history:

- 1) 5 histocassettes - multiple tissues from 10 fish
- 2) 5 pooled kidney tissues
- 3) fish, 5 from pen 12, 5 from pen 14, 5 from control pens. 3 bags.

Request:

- 1) histology X 5 histocassettes
- 2) PCR for BKD X 5 pools
- 3) virology pool from each bag (5 fish/pool) and 5 individual bacteriology/bag (3 virology pools and 15 bacteriology total)

2 mo. History of poor appetite. Elevated chronic mortality. Increased number of starve-outs. Poor body condition in approx. 10 - 15% of 2 pens (200,000) rest of population apparently healthy.

TX: mouth rot, tribissen last week

PM lesions: decreased body condition

Absence of feed in majority of fish. Concurrent bacterial stomatitis. Note previous bacteriology/virology did not find anything significant.



**Gross Pathology**

Three groups of salmon were received dead in three plastic bags labeled Pen, Pen 14, and Pen 12. The gel-ice packs included with the shipment were warm when received. Because fish tissues had significant postmortem autolysis, they were not cultured for bacteria. From each bag, kidney was removed from 5 fish for virus isolation. Spleen was removed from the five fish from the "Pen" bag but not from the other two bags (fish in those bags were too autolyzed to separate spleen tissue from intestine). Summary of tissues for virus isolation:

1. "Pen" - kidney and spleen pooled from 5 fish (8 extra fish were discarded)
2. "Pen 14" - kidney pooled from 5 fish
3. "Pen 12" - kidney pooled from 5 fish

Also submitted were 2 lock-seal plastic bags of kidney and 3 Whirl-pak bags of kidney. The five bags of kidney were analyzed for BKD by PCR.

**Histopathology**

- Slide 1A: heart, trunk kidney, gill (2 pieces), liver
- Slide 1B: heart, trunk kidney (2 pieces), gill, and intestinal cecum
- Slide 1C: liver, gill, and intestinal cecum (also stained with Schmorl's lipofuscin stain and an iron stain)
- Slide 2A: gill (2 pieces), heart, liver (small pieces), and intestinal cecum
- Slide 2B: trunk kidney, liver, gill, and intestinal cecum
- Slide 2C: liver, gill, and intestinal cecum (also stained with Schmorl's lipofuscin stain and an iron stain)
- Slide 3A: liver, spleen, trunk kidney, and intestinal ceca (2 pieces)
- Slide 3B: liver, heart, trunk kidney, and intestinal ceca
- Slide 3C: spleen, gill, trunk kidney, and intestinal ceca
- Slide 4A: trunk kidney, and heart (4 pieces)
- Slide 4B: eye, stomach, spleen, liver, and intestinal ceca
- Slide 4C: gill (2 pieces), trunk kidney (4 pieces), spleen, liver, intestinal ceca
- Slide 4D: gill and intestinal ceca
- Slide 5A: stomach, trunk kidney, liver, and intestinal ceca
- Slide 5B: spleen, liver, trunk kidney (2 pieces), heart, stomach, and intestinal ceca
- Slide 5C: gill (3 pieces)

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

Quality Control: Tissue preservation is fair to poor for most organs. Bacterial colonies in the heart (slide 1A) associated with no inflammation are probably a result of postmortem growth. Fungi on the margin of one piece of gill on slide 5C are probably also postmortem growth. Autolysis is not consistent. The liver in slide 1A is well preserved. Tissues have no evidence of dehydration after fixation or of acid hematin deposits.

Measures of physiologic condition:

Hepatocellular glycogen depletion: severe (slide 1A, 1B)

Mesenteric fat depletion: most samples lack good sections of mesenteric fat (either the fat is completely depleted, or mesenteric fat was not included in the sections); mild (slide 5A)

This pattern in the measures of physiologic condition in most fish is consistent with fish that have not been eating for at least several days (depletion of both hepatocellular glycogen and mesenteric fat).





**Virology**

3 samples inoculated onto tissue culture - all negative.

\* Results faxed on July 21/05.

**Molecular Diagnostics/PCR**

Addendum: July 27/05

Samples #1,3,5 Renibacterium salmoninarum Positive by PCR

Samples #2, 4 Renibacterium salmoninarum Negative by PCR

**Diagnosis**

- 1a. Liver: hydropic degeneration, diffuse, severe (slide 1A)
- 1b. Liver: hepatocellular karyomegaly, diffuse, mild (slides 1C, 2B, 2C, 3B, 4B, 5A, 5B), moderate (slide 1A)
- 1c. Liver: hepatitis, perivascular, lymphoplasmacytic, multifocal, mild (slide 1A, 1C, 2C, 5B)
- 1d. Liver: hepatocellular single cell necrosis, diffuse, mild (slide 2B, 2C, 3B, 4B, 5A, 5B), moderate (slide 4C), severe (slides 1C, 2A)
- 1e. Liver: lipofuscin-pigmented sinusoidal macrophages, diffuse, mild (slide 1C), moderate (slides 2B, 3A, 4B, 4C), severe (slides 2C, 3B, 5A, 5B)
- 1f. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 3A)
- 1g. Liver: pericholangitis, lymphoplasmacytic, multifocal, mild (slide 4B), moderate (slide 3B, 4C)
- 2. Intestinal cecum: peritonitis, regionally diffuse, granulomatous, with fine fibrovascular fronds, mild (slides 2C), moderate (slides 1B, 3A, 4C, 5A)
- 3. Trunk kidney: tubular epithelial cytoplasmic eosinophilic droplets, multifocal, mild (slide 3A, 5A, 5B)
- 4a. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages, multifocal, mild (slide 3B)
- 4b. Heart: mural thrombi, multifocal, mild (slide 4A)
- 5. Stomach: peritonitis, regionally diffuse, granulomatous, with fine fibrovascular fronds, moderate (slide 4B, 5B)
- 6. Spleen: peritonitis, regionally diffuse, granulomatous, with intralesional foreign material and fine fibrovascular fronds, mild (slide 5B), moderate (slide 4B, 5A)



**Final Comments**

Many features of these slides are consistent with netpen liver disease, most commonly associated with the algal toxin microcystin-LR in the water. Other toxins in the water or feed (e.g., aflatoxins) are less likely differentials. The liver in slide 1A has the most severe case of hydropic degeneration that I have ever seen.

The liver in slide 1C has the most severe case of single cell necrosis that I have ever seen. All liver sections other than 1A have a fairly consistent pattern similar to slide 1C: a combination of single cell necrosis, hepatocellular karyomegaly, sinusoidal lipofuscin accumulation, and variable amounts of lymphoplasmacytic inflammation. Hepatic megalocytosis can result from exposure to several types of toxicants, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. Hydropic degeneration in slide 1A might be a precursor to the more chronic lesions in the other fish. 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.

Peritonitis on the margin of several organs 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 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 (slide 3B) is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface throughout most of the ventricle are rarely more than 2 cell layers thick.

/sr/mm/sr



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**Case Report**

**Submission** 2005-02274      **Date** 24-Jun-2005      **Report** 18-Jul-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14563      A.3.3-37 (#1-6)

**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

**History/Symptoms**

Sample ID: A.3.3-37 (#1-6)

Provincial Surveillance Program samples. Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis.  
Culture any PCR positive samples.

ADDENDUM: (June 30, 2005)

Samples submitted for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the sections contain few foci of acid hematin. The margins of very few organs have evidence of dehydration after fixation (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.

Slide 1: autolysis, none (all organs)

- 1a. Liver: peritonitis, granulomatous, focal, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: epicarditis (severe, diffuse) and endocarditis (mild, multifocal), granulomatous
- 3a. Spleen: splenitis, granulomatous, multifocal, moderate
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, severe
- 4b. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: not included on the slide

Comment: The most common organism associated with disseminated granulomas in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. 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. 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. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, mild (other organs) to severe (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
- 3a. Spleen: splenitis, granulomatous, multifocal, moderate
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, mild
5. Head kidney: nephritis, interstitial, granulomatous, multifocal, moderate

Comment: see comments on fish #1.

Slide 3: autolysis, none (other organs) to mild (trunk kidney and liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
  - 1b. Liver: peritonitis, chronic, focal, with fibrocellular fronds, mild
  2. Heart: no significant lesions
  - 3a. Spleen: splenitis, granulomatous, multifocal, mild
  - 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate
  - 4a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, moderate
  - 4b. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild
  5. Head kidney: nephritis, interstitial, granulomatous, multifocal, mild
- Comment: see comments on fish #1. Hepatic peritonitis is consistent with a vaccine reaction.

Slide 4: autolysis, mild (other organs) to moderate (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: not included on the slide
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild





5. Head kidney: no significant lesions  
Comment: see comments on other fish.

Slide 5: autolysis, none (other organs) to mild (liver)

1a. Liver: yellow-brown pigmented macrophage aggregates, multifocal, mild

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: no significant lesions

3. Spleen: not included on the slide

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: 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.

Slide 6: autolysis, none (heart) to mild (other organs)

1. Liver: no significant lesions

2. Heart: no significant lesions

3. Spleen: not included on the slide

4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, mild

5. Head kidney: nephritis, interstitial, granulomatous, multifocal, moderate

Comment: see comments on other fish.

#### **Molecular Diagnostics/PCR**

Samples 1-3, 4-6: ISA, IHN, IPN, VHS, Piscirickettsia salmonis negative by PCR.

\* Results faxed July 19/05; corrected version faxed Aug. 8/05.

/bb



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**Case Report**

**Submission** 2005-02321      **Date** 29-Jun-2005      **Report** 06-Jul-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14584 #5304, Marine Harvest  
**Farm:**  
**Vet Clinic:**  
**Attending** Cilka LaTrace or 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

**History/Symptoms**

Submitted 3 fish histo. cassettes.

Log No. 5304

Species: Atlantic

Sex: Regular

Saltwater entry: 2005

Netpen/Tank ID: All

Suspect Netpen Liver Disease. TDX: NPLDz

**Histopathology**

3 cassettes of preserved tissues were submitted for histopathology wrapped in moist towel.

Slide 1 (5-5304-1, 6/21/05) - head kidney, trunk kidney, spleen, heart, liver, gill, skin with skeletal muscle, stomach, intestinal ceca and mesenteric fat

Slide 2 (5-5304-2, 6/21/05) - head kidney, trunk kidney, heart, liver, gill, skin with skeletal muscle, intestinal ceca and mesenteric fat

Slide 3 (5-5304-3, 6/21/05) - head kidney, trunk kidney, heart, liver, gill, skeletal muscle with skin, intestinal ceca and mesenteric fat

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

Quality Control: Tissue preservation is good for most organs, but the intestinal ceca and gill have mild autolysis. Intestinal ceca in slide 3 surrounded by granulomatous inflammation have severe autolysis. The margins of most organs have evidence of dehydration after fixation; small foci in the affected organs have nuclei that stain dull blue, erythrocyte cytoplasm stains yellow instead of red, and cytoplasm of other cell types stains poorly or not at all). Tissues are free of acid hematin deposits.

Measures of physiologic condition

Hepatocellular glycogen depletion, severe (slides 1, 2, and 3)

Mesenteric adipose tissue depletion, moderate (slide 3), severe (slides 1, 2)

This pattern in the measures of physiologic condition is consistent with fish that have not eaten normally for a long time (more than a few days).



**Diagnosis**

- 1a. Liver: hydropic degeneration, diffuse, moderate (slide 3)
- 1b. Liver: hepatocellular karyomegaly, diffuse, mild (slide 3), moderate (slides 1, 2)
- 1c. Liver: hepatocellular single cell necrosis, diffuse, mild (slides 2, 3), moderate (slide 1)
- 1d. Liver: lipofuscin-pigmented sinusoidal macrophages, diffuse, mild (slide 3), moderate (slides 1, 2)
- 1e. Liver: hepatitis, lymphoplasmacytic, multifocal, mild (slides 2, 3), moderate (slide 1)
- 1f. Liver: peritonitis, lymphoplasmacytic, diffuse, moderate (slide 3)
2. Intestinal ceca and spleen: peritonitis, granulomatous, regionally diffuse, with fine fibrovascular fronds and intralesional vacuoles up to 300 µm in diameter, moderate (slide 2), severe (slide 1)
- 3a. Gill: lamellar epithelial separation, patchy to diffuse, mild to moderate (slide 1)
- 3b. Gill: branchitis, multifocal, pleocellular (macrophages, neutrophils, and eosinophilic granular leukocytes)
4. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 3)

**Final Comments**

Many features of these slides are consistent with netpen liver disease, most commonly associated with the algal toxin microcystin-LR in the water. Other toxins in the water or feed (e.g., aflatoxins) are less likely differentials. All liver sections have a fairly consistent pattern: a combination of single cell necrosis, hepatocellular karyomegaly, sinusoidal lipofuscin accumulation, and variable amounts of lymphoplasmacytic inflammation. Hepatic megalocytosis can result from exposure to several types of toxicants, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. Hydropic degeneration in slide 3 is probably a precursor to the more chronic lesions in the other fish. 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. 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.

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

Separation of gill lamellar epithelium from underlying pillar cells can be a result of edema and it is a common post mortem 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 post mortem artefact. Gill lesions are not described as a classic feature of net pen liver disease, but I don't know how often the gill has been examined under controlled exposure conditions.

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



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**Case Report**

**Submission** 2005-02322      **Date** 29-Jun-2005      **Report** 06-Jul-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14585 #5310, Marine Harvest (PO #636435)  
**Farm:**  
**Vet Clinic:**  
**Attending** Cilka LaTrace or 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



**History/Symptoms**

Log No. 5310.  
Species: Atlantic  
Sex: Regular  
Sample size: 2 histo cassettes  
Saltwater entry: 2003

Histo from 2 moribund Atlantics. Site has a plankton bloom/water quality event ongoing. Gill wet mount revealed no visible lesions. No bacterial growth resulted from culture onto Blood agar and TSA. Two viral samples (under separate cover) for IHNV-PCR and VHS-PCR and tissue culture.

Please quote PO #636435 for accounting purposes.

**Histopathology**

4 cassettes of preserved tissues were submitted for histopathology wrapped in moist towel.  
Slide 1 (5-5310-1, 6/23/05) - head kidney, trunk kidney, spleen, heart, liver, skeletal muscle, intestine, intestinal ceca and mesenteric fat  
Slide 2 (5-5310-1, 6/23/05) - gill  
Slide 3 (5-5310-2, 6/23/05) - gill  
Slide 4 (5-5310-2, 6/23/05) - head kidney, trunk kidney, spleen, heart, liver, skeletal muscle, intestine, intestinal ceca and mesenteric fat  
All organs on each slide were examined. Organs not listed below had no significant lesions.

Quality Control: Tissue preservation is good for most organs; some sections of intestine have mild autolysis. The margins of some organs have minimal evidence of dehydration after fixation; small foci in the affected organs have nuclei that stain dull blue, erythrocyte cytoplasm stains yellow instead of red, and cytoplasm of other cell types stains poorly or not at all). Tissues are free of acid hematin deposits.

Measures of physiologic condition  
Hepatocellular glycogen depletion, severe (slides 1, 4)  
Mesenteric adipose tissue depletion, none (slides 1, 4)

This pattern in the measures of physiologic condition is consistent with relatively healthy fish (abundant adipose tissue) that recently stopped eating normally (hepatocellular glycogen depletion).

**Diagnosis**

- 1a. Gill: lamellar edema and fusion, multifocal, acute, severe (slides 2 and 3)
- 1b. Gill: lamellar necrosis, acute, multifocal, moderate (slide 3)
- 2a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1, 4)
- 2b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 4)
3. Intestinal ceca: peritonitis, regionally diffuse, with fine fibrovascular fronds, mild (slide 4)



**Final Comments**

Both fish were moribund as a result of severe changes in the gill, consistent with the clinical history of plankton bloom/water quality event. Microscopic gill changes resulting from exposure of pen-raised fish to harmful algal blooms have not been well described, but acute necrosis has been observed (Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.). The changes in the gills in this case are otherwise fairly non-specific. 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). Separation of gill lamellar epithelium from underlying pillar cells can be a result of edema, but it is also a common post mortem 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.

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.

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

/bb



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**Case Report**

**Submission** 2005-02353      **Date** 30-Jun-2005      **Report** 12-Jul-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14600 #5310, Marine Harvest  
**Farm:**  
**Vet Clinic:**  
**Attending** Cilka LaTrace/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

**History/Symptoms**

Two viral samples for IHNV PCR and VHS - PCR and tissue culture.  
(2 histo. cassettes received June 29/05 - case 2322).

Species - Atlantic. Sex - Regular. Saltwater entry - 2003. Netpen - 4.

Histo. from 2 moribund Atlantics. Site has a plankton bloom/ water quality event ongoing. Gill wet mount revealed no visible lesions. No bacterial growth resulted from culture onto Blood agar and TSA.

Please quote PO #636935 for accounting purposes.

**Virology**

No virus isolated.  
2 samples inoculated onto tissue culture - both negative.

\* Results faxed on July 27/05.

**Molecular Diagnostics/PCR**

#5310-1,2: IHN and VHS Virus negative by PCR.

\* Results faxed July 12/05.

/bb



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**Case Report**

**Submission** 2005-02142      **Date** 16-Jun-2005      **Report** 23-Jun-2005

**Report**      **Copies**

**Submitter:** 12847      Heritage Salmon  
**Owner** 12847      Heritage Salmon  
**Farm:**  
**Vet Clinic:** 12847      Heritage Salmon  
**Attending**      Dr. P. McKenzie

**Specimen:** Other      **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



### **History/Symptoms**

Submitted 10 histology cassettes labeled 05-35-1 through 05-35-10 (gill, heart, ceca, liver, spleen, flesh, kidney, brain) for histology.

Fish have been in salt water for 3 months. Increased numbers of pinhead dropouts. Just finished SLICE treatment, number of mouth rot mortality increasing. High mortality.

\*\* Please use our case number 05-35. \*\*

\*\* PO #17204.

### **Histopathology**

10 cassettes of tissues preserved in 10% neutral buffered formalin were submitted immersed in ethanol for histopathology.

Slide 1 - skeletal muscle (and skin?), liver, intestinal cecum and mesenteric fat, trunk kidney, heart, gill, and spleen

Slide 2 - skeletal muscle and skin, liver, intestinal cecum and mesenteric fat, head kidney, trunk kidney, heart, and gill

Slide 3 - skeletal muscle and skin, liver, intestinal cecum and mesenteric fat, head kidney, trunk kidney, heart, brain, and gill

Slide 4 - skeletal muscle and skin, liver, intestinal cecum and mesenteric fat, trunk kidney, heart, spleen, brain, and gill

Slide 5 - skeletal muscle and skin, liver, intestinal cecum and mesenteric fat, trunk kidney, heart, spleen, brain, and gill

Slide 6 - skeletal muscle, liver, intestinal cecum and mesenteric fat, head kidney, heart, gill, and spleen

Slide 7 - skeletal muscle and skin, liver, intestinal cecum and mesenteric fat, head kidney, trunk kidney, heart, spleen, brain, and gill

Slide 8 - skeletal muscle and skin, liver, intestinal cecum and mesenteric fat, head kidney, trunk kidney, heart, spleen, brain, and gill

Slide 9 - skeletal muscle, liver, intestinal cecum and mesenteric fat, head kidney, heart, spleen, brain, and gill

Slide 10 - skeletal muscle, liver, intestinal cecum and mesenteric fat, trunk kidney, heart, spleen, brain, and gill

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

Quality Control: Tissue preservation is fair to poor for most organs. Autolysis is particularly severe in the gill and intestine. The margins of most organs have no evidence of dehydration after fixation; small foci in the few affected organs have nuclei that stain dull blue, erythrocyte cytoplasm stains yellow instead of red, and cytoplasm of other cell types stains poorly or not at all).

### **Diagnosis**

1. Liver: sinusoidal congestion, focal (600 µm in diameter), mild (slide 4)
2. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 8)
3. Spleen: splenitis, multifocal, granulomatous, mild (slide 8)



**Final Comments**

Moderate to severe autolysis in these tissues may have prevented the diagnosis of some lesions. Alternatively, the fish may be dying primarily from complications of mouth rot, described clinically.

The focal nature of sinusoidal congestion in the liver (slide 4) is consistent with local trauma. Sinusoidal congestion has also been associated with viral infections (ISA and VHSV), but I would expect changes from viral infection to be more widespread. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Granulomatous inflammation in the spleen (slide 8) is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* is the most common organism associated with these lesions. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.

/sr



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**Case Report**

**Submission** 2005-02227      **Date** 22-Jun-2005      **Report** 14-Jul-2005

**Report**      **Copies**

**Submitter:** 11899      Grieg Seafoods B.C. Ltd.

**Owner** 11899      Grieg Seafoods B.C. Ltd.

**Farm:**

**Vet Clinic:**

**Attending**      Dr. Milligan

**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**

From typed history:

Submitted samples from Campbell River

- 1) 5 histosettes multiple tissues for histology.
- 2) 3 bags of fish for virology and bacteriology (pen 12, pen 14, unidentified bag). I would like virology on a pool of 5 from each bag if possible (3 virology pools total) and individual bacteriology on 5 fish from each of the 3 bags (15 bacteriology total).
- 3) 5 bags of kidney tissue for BKD PCR

History of population is poor appetite, mortality elevated above background and poor weight gain for 3 months. The problem appears restricted to a population of approx. 200,000 fish with a separate origin. Of the population in question, there appears to be about 10 to 15% of the population affected. Bacterial stomatitis is occurring concurrently (fish have been treated with tribissen in the last week and those fish eating have apparently responded appropriately). On post mortem, there is decreased body condition and lack of feed in the digestive tracts with some fish exhibiting pale friable livers. See previous histo report on the fish in question (2005-00209). Please note that previous virology/bacteriology has not identified significant pathogens.

From handwritten history:

- 1) 5 histocassettes - multiple tissues from 10 fish
- 2) 5 pooled kidney tissues
- 3) fish, 5 from pen 12, 5 from pen 14, 5 from control pens. 3 bags.

Request:

- 1) histology X 5 histocassettes
- 2) PCR for BKD X 5 pools
- 3) virology pool from each bag (5 fish/pool) and 5 individual bacteriology/bag (3 virology pools and 15 bacteriology total)

2 mo. History of poor appetite. Elevated chronic mortality. Increased number of starve-outs. Poor body condition in approx. 10 - 15% of 2 pens (200,000) rest of population apparently healthy.

TX: mouth rot, tribissen last week

PM lesions: decreased body condition

Absence of feed in majority of fish. Concurrent bacterial stomatitis. Note previous bacteriology/virology did not find anything significant.



**Gross Pathology**

Three groups of salmon were received dead in three plastic bags labeled Pen, Pen 14, and Pen 12. The gel-ice packs included with the shipment were warm when received. Because fish tissues had significant postmortem autolysis, they were not cultured for bacteria. From each bag, kidney was removed from 5 fish for virus isolation. Spleen was removed from the five fish from the "Pen" bag but not from the other two bags (fish in those bags were too autolyzed to separate spleen tissue from intestine). Summary of tissues for virus isolation:

1. "Pen" - kidney and spleen pooled from 5 fish (8 extra fish were discarded)
2. "Pen 14" - kidney pooled from 5 fish
3. "Pen 12" - kidney pooled from 5 fish

Also submitted were 2 lock-seal plastic bags of kidney and 3 Whirl-pak bags of kidney. The five bags of kidney were analyzed for BKD by PCR.

**Histopathology**

- Slide 1A: heart, trunk kidney, gill (2 pieces), liver
- Slide 1B: heart, trunk kidney (2 pieces), gill, and intestinal cecum
- Slide 1C: liver, gill, and intestinal cecum (also stained with Schmorl's lipofuscin stain and an iron stain)
- Slide 2A: gill (2 pieces), heart, liver (small pieces), and intestinal cecum
- Slide 2B: trunk kidney, liver, gill, and intestinal cecum
- Slide 2C: liver, gill, and intestinal cecum (also stained with Schmorl's lipofuscin stain and an iron stain)
- Slide 3A: liver, spleen, trunk kidney, and intestinal ceca (2 pieces)
- Slide 3B: liver, heart, trunk kidney, and intestinal ceca
- Slide 3C: spleen, gill, trunk kidney, and intestinal ceca
- Slide 4A: trunk kidney, and heart (4 pieces)
- Slide 4B: eye, stomach, spleen, liver, and intestinal ceca
- Slide 4C: gill (2 pieces), trunk kidney (4 pieces), spleen, liver, intestinal ceca
- Slide 4D: gill and intestinal ceca
- Slide 5A: stomach, trunk kidney, liver, and intestinal ceca
- Slide 5B: spleen, liver, trunk kidney (2 pieces), heart, stomach, and intestinal ceca
- Slide 5C: gill (3 pieces)

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

Quality Control: Tissue preservation is fair to poor for most organs. Bacterial colonies in the heart (slide 1A) associated with no inflammation are probably a result of postmortem growth. Fungi on the margin of one piece of gill on slide 5C are probably also postmortem growth. Autolysis is not consistent. The liver in slide 1A is well preserved. Tissues have no evidence of dehydration after fixation or of acid hematin deposits.

Measures of physiologic condition:

Hepatocellular glycogen depletion: severe (slide 1A, 1B)

Mesenteric fat depletion: most samples lack good sections of mesenteric fat (either the fat is completely depleted, or mesenteric fat was not included in the sections); mild (slide 5A)

This pattern in the measures of physiologic condition in most fish is consistent with fish that have not been eating for at least several days (depletion of both hepatocellular glycogen and mesenteric fat).





**Virology**

3 samples inoculated onto tissue culture - all negative.

\* Results faxed on July 21/05.

**Molecular Diagnostics/PCR**

Addendum: July 27/05

Samples #1,3,5 Renibacterium salmoninarum Positive by PCR

Samples #2, 4 Renibacterium salmoninarum Negative by PCR

**Diagnosis**

- 1a. Liver: hydropic degeneration, diffuse, severe (slide 1A)
- 1b. Liver: hepatocellular karyomegaly, diffuse, mild (slides 1C, 2B, 2C, 3B, 4B, 5A, 5B), moderate (slide 1A)
- 1c. Liver: hepatitis, perivascular, lymphoplasmacytic, multifocal, mild (slide 1A, 1C, 2C, 5B)
- 1d. Liver: hepatocellular single cell necrosis, diffuse, mild (slide 2B, 2C, 3B, 4B, 5A, 5B), moderate (slide 4C), severe (slides 1C, 2A)
- 1e. Liver: lipofuscin-pigmented sinusoidal macrophages, diffuse, mild (slide 1C), moderate (slides 2B, 3A, 4B, 4C), severe (slides 2C, 3B, 5A, 5B)
- 1f. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 3A)
- 1g. Liver: pericholangitis, lymphoplasmacytic, multifocal, mild (slide 4B), moderate (slide 3B, 4C)
- 2. Intestinal cecum: peritonitis, regionally diffuse, granulomatous, with fine fibrovascular fronds, mild (slides 2C), moderate (slides 1B, 3A, 4C, 5A)
- 3. Trunk kidney: tubular epithelial cytoplasmic eosinophilic droplets, multifocal, mild (slide 3A, 5A, 5B)
- 4a. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages, multifocal, mild (slide 3B)
- 4b. Heart: mural thrombi, multifocal, mild (slide 4A)
- 5. Stomach: peritonitis, regionally diffuse, granulomatous, with fine fibrovascular fronds, moderate (slide 4B, 5B)
- 6. Spleen: peritonitis, regionally diffuse, granulomatous, with intralesional foreign material and fine fibrovascular fronds, mild (slide 5B), moderate (slide 4B, 5A)



**Final Comments**

Many features of these slides are consistent with netpen liver disease, most commonly associated with the algal toxin microcystin-LR in the water. Other toxins in the water or feed (e.g., aflatoxins) are less likely differentials. The liver in slide 1A has the most severe case of hydropic degeneration that I have ever seen.

The liver in slide 1C has the most severe case of single cell necrosis that I have ever seen. All liver sections other than 1A have a fairly consistent pattern similar to slide 1C: a combination of single cell necrosis, hepatocellular karyomegaly, sinusoidal lipofuscin accumulation, and variable amounts of lymphoplasmacytic inflammation. Hepatic megalocytosis can result from exposure to several types of toxicants, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. Hydropic degeneration in slide 1A might be a precursor to the more chronic lesions in the other fish. 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.

Peritonitis on the margin of several organs 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 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 (slide 3B) is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface throughout most of the ventricle are rarely more than 2 cell layers thick.

/sr/mm/sr



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**Case Report**

**Submission** 2005-02274      **Date** 24-Jun-2005      **Report** 18-Jul-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14563      A.3.3-37 (#1-6)

**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

**History/Symptoms**

Sample ID: A.3.3-37 (#1-6)

Provincial Surveillance Program samples. Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis.  
Culture any PCR positive samples.

ADDENDUM: (June 30, 2005)

Samples submitted for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the sections contain few foci of acid hematin. The margins of very few organs have evidence of dehydration after fixation (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.

Slide 1: autolysis, none (all organs)

- 1a. Liver: peritonitis, granulomatous, focal, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: epicarditis (severe, diffuse) and endocarditis (mild, multifocal), granulomatous
- 3a. Spleen: splenitis, granulomatous, multifocal, moderate
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, severe
- 4b. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild
- 5. Head kidney: not included on the slide

Comment: The most common organism associated with disseminated granulomas in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. 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. 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. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, mild (other organs) to severe (liver)

- 1. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2. Heart: no significant lesions
- 3a. Spleen: splenitis, granulomatous, multifocal, moderate
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, mild
- 5. Head kidney: nephritis, interstitial, granulomatous, multifocal, moderate

Comment: see comments on fish #1.

Slide 3: autolysis, none (other organs) to mild (trunk kidney and liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
  - 1b. Liver: peritonitis, chronic, focal, with fibrocellular fronds, mild
  - 2. Heart: no significant lesions
  - 3a. Spleen: splenitis, granulomatous, multifocal, mild
  - 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate
  - 4a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, moderate
  - 4b. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild
  - 5. Head kidney: nephritis, interstitial, granulomatous, multifocal, mild
- Comment: see comments on fish #1. Hepatic peritonitis is consistent with a vaccine reaction.

Slide 4: autolysis, mild (other organs) to moderate (liver)

- 1. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: not included on the slide
- 4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild





5. Head kidney: no significant lesions  
Comment: see comments on other fish.

Slide 5: autolysis, none (other organs) to mild (liver)

1a. Liver: yellow-brown pigmented macrophage aggregates, multifocal, mild

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: no significant lesions

3. Spleen: not included on the slide

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: 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.

Slide 6: autolysis, none (heart) to mild (other organs)

1. Liver: no significant lesions

2. Heart: no significant lesions

3. Spleen: not included on the slide

4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, mild

5. Head kidney: nephritis, interstitial, granulomatous, multifocal, moderate

Comment: see comments on other fish.

#### **Molecular Diagnostics/PCR**

Samples 1-3, 4-6: ISA, IHN, IPN, VHS, Piscirickettsia salmonis negative by PCR.

\* Results faxed July 19/05; corrected version faxed Aug. 8/05.

/bb



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**Case Report**

**Submission** 2005-02321      **Date** 29-Jun-2005      **Report** 06-Jul-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14584 #5304, Marine Harvest  
**Farm:**  
**Vet Clinic:**  
**Attending** Cilka LaTrace or 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

**History/Symptoms**

Submitted 3 fish histo. cassettes.

Log No. 5304

Species: Atlantic

Sex: Regular

Saltwater entry: 2005

Netpen/Tank ID: All

Suspect Netpen Liver Disease. TDX: NPLDz

**Histopathology**

3 cassettes of preserved tissues were submitted for histopathology wrapped in moist towel.

Slide 1 (5-5304-1, 6/21/05) - head kidney, trunk kidney, spleen, heart, liver, gill, skin with skeletal muscle, stomach, intestinal ceca and mesenteric fat

Slide 2 (5-5304-2, 6/21/05) - head kidney, trunk kidney, heart, liver, gill, skin with skeletal muscle, intestinal ceca and mesenteric fat

Slide 3 (5-5304-3, 6/21/05) - head kidney, trunk kidney, heart, liver, gill, skeletal muscle with skin, intestinal ceca and mesenteric fat

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

Quality Control: Tissue preservation is good for most organs, but the intestinal ceca and gill have mild autolysis. Intestinal ceca in slide 3 surrounded by granulomatous inflammation have severe autolysis. The margins of most organs have evidence of dehydration after fixation; small foci in the affected organs have nuclei that stain dull blue, erythrocyte cytoplasm stains yellow instead of red, and cytoplasm of other cell types stains poorly or not at all). Tissues are free of acid hematin deposits.

Measures of physiologic condition

Hepatocellular glycogen depletion, severe (slides 1, 2, and 3)

Mesenteric adipose tissue depletion, moderate (slide 3), severe (slides 1, 2)

This pattern in the measures of physiologic condition is consistent with fish that have not eaten normally for a long time (more than a few days).



**Diagnosis**

- 1a. Liver: hydropic degeneration, diffuse, moderate (slide 3)
- 1b. Liver: hepatocellular karyomegaly, diffuse, mild (slide 3), moderate (slides 1, 2)
- 1c. Liver: hepatocellular single cell necrosis, diffuse, mild (slides 2, 3), moderate (slide 1)
- 1d. Liver: lipofuscin-pigmented sinusoidal macrophages, diffuse, mild (slide 3), moderate (slides 1, 2)
- 1e. Liver: hepatitis, lymphoplasmacytic, multifocal, mild (slides 2, 3), moderate (slide 1)
- 1f. Liver: peritonitis, lymphoplasmacytic, diffuse, moderate (slide 3)
- 2. Intestinal ceca and spleen: peritonitis, granulomatous, regionally diffuse, with fine fibrovascular fronds and intralesional vacuoles up to 300 µm in diameter, moderate (slide 2), severe (slide 1)
- 3a. Gill: lamellar epithelial separation, patchy to diffuse, mild to moderate (slide 1)
- 3b. Gill: branchitis, multifocal, pleocellular (macrophages, neutrophils, and eosinophilic granular leukocytes)
- 4. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 3)

**Final Comments**

Many features of these slides are consistent with netpen liver disease, most commonly associated with the algal toxin microcystin-LR in the water. Other toxins in the water or feed (e.g., aflatoxins) are less likely differentials. All liver sections have a fairly consistent pattern: a combination of single cell necrosis, hepatocellular karyomegaly, sinusoidal lipofuscin accumulation, and variable amounts of lymphoplasmacytic inflammation. Hepatic megalocytosis can result from exposure to several types of toxicants, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. Hydropic degeneration in slide 3 is probably a precursor to the more chronic lesions in the other fish. 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. 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.

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

Separation of gill lamellar epithelium from underlying pillar cells can be a result of edema and it is a common post mortem 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 post mortem artefact. Gill lesions are not described as a classic feature of net pen liver disease, but I don't know how often the gill has been examined under controlled exposure conditions.

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





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**Case Report**

**Submission** 2005-02322      **Date** 29-Jun-2005      **Report** 06-Jul-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14585 #5310, Marine Harvest (PO #636435)  
**Farm:**  
**Vet Clinic:**  
**Attending** Cilka LaTrace or 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

**History/Symptoms**

Log No. 5310.  
Species: Atlantic  
Sex: Regular  
Sample size: 2 histo cassettes  
Saltwater entry: 2003

Histo from 2 moribund Atlantics. Site has a plankton bloom/water quality event ongoing. Gill wet mount revealed no visible lesions. No bacterial growth resulted from culture onto Blood agar and TSA. Two viral samples (under separate cover) for IHNV-PCR and VHS-PCR and tissue culture.

Please quote PO #636435 for accounting purposes.

**Histopathology**

4 cassettes of preserved tissues were submitted for histopathology wrapped in moist towel.  
Slide 1 (5-5310-1, 6/23/05) - head kidney, trunk kidney, spleen, heart, liver, skeletal muscle, intestine, intestinal ceca and mesenteric fat  
Slide 2 (5-5310-1, 6/23/05) - gill  
Slide 3 (5-5310-2, 6/23/05) - gill  
Slide 4 (5-5310-2, 6/23/05) - head kidney, trunk kidney, spleen, heart, liver, skeletal muscle, intestine, intestinal ceca and mesenteric fat  
All organs on each slide were examined. Organs not listed below had no significant lesions.

Quality Control: Tissue preservation is good for most organs; some sections of intestine have mild autolysis. The margins of some organs have minimal evidence of dehydration after fixation; small foci in the affected organs have nuclei that stain dull blue, erythrocyte cytoplasm stains yellow instead of red, and cytoplasm of other cell types stains poorly or not at all). Tissues are free of acid hematin deposits.

Measures of physiologic condition  
Hepatocellular glycogen depletion, severe (slides 1, 4)  
Mesenteric adipose tissue depletion, none (slides 1, 4)

This pattern in the measures of physiologic condition is consistent with relatively healthy fish (abundant adipose tissue) that recently stopped eating normally (hepatocellular glycogen depletion).

**Diagnosis**

- 1a. Gill: lamellar edema and fusion, multifocal, acute, severe (slides 2 and 3)
- 1b. Gill: lamellar necrosis, acute, multifocal, moderate (slide 3)
- 2a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1, 4)
- 2b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 4)
3. Intestinal ceca: peritonitis, regionally diffuse, with fine fibrovascular fronds, mild (slide 4)



**Final Comments**

Both fish were moribund as a result of severe changes in the gill, consistent with the clinical history of plankton bloom/water quality event. Microscopic gill changes resulting from exposure of pen-raised fish to harmful algal blooms have not been well described, but acute necrosis has been observed (Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.). The changes in the gills in this case are otherwise fairly non-specific. 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). Separation of gill lamellar epithelium from underlying pillar cells can be a result of edema, but it is also a common post mortem 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.

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.

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

/bb



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**Case Report**

**Submission** 2005-02353      **Date** 30-Jun-2005      **Report** 12-Jul-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14600 #5310, Marine Harvest  
**Farm:**  
**Vet Clinic:**  
**Attending** Cilka LaTrace/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

**History/Symptoms**

Two viral samples for IHNV PCR and VHS - PCR and tissue culture.  
(2 histo. cassettes received June 29/05 - case 2322).

Species - Atlantic. Sex - Regular. Saltwater entry - 2003. Netpen - 4.

Histo. from 2 moribund Atlantics. Site has a plankton bloom/ water quality event ongoing. Gill wet mount revealed no visible lesions. No bacterial growth resulted from culture onto Blood agar and TSA.

Please quote PO #636935 for accounting purposes.

**Virology**

No virus isolated.  
2 samples inoculated onto tissue culture - both negative.

\* Results faxed on July 27/05.

**Molecular Diagnostics/PCR**

#5310-1,2: IHN and VHS Virus negative by PCR.

\* Results faxed July 12/05.

/bb





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**Case Report**

**Submission** 2005-02408      **Date** 06-Jul-2005      **Report** 12-Jul-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 14632 #5312, 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**

Submitted two fresh tissue samples for virology - IHNV - PCR and VITSV - PCR. No IHNV isolated from this site in previous submissions. Some fish with hemorrhaging of pyloric ceca.

\*\* Please quote PO# 636440

**Molecular Diagnostics/PCR**

#5312 - 1,2: IHN and VHS Virus negative by PCR.

\* Results faxed July 12/05.

/bb

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**Case Report**

**Submission** 2005-02409      **Date** 06-Jul-2005      **Report** 12-Jul-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 14633 #5313, 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**

Submitted two fresh tissue samples for virology - IHNV - PCR and VITSV - PCR. No viruses isolated from this site in previous submissions. Sampled fish had hemorrhages in the liver and/or swim bladder.

\*\* Please quote PO# 636439.

**Molecular Diagnostics/PCR**

#5313 - 1,2: IHN and VHS Virus negative by PCR.

\* Results faxed July 12/05.

/bb

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**Case Report**

**Submission** 2005-02468      **Date** 11-Jul-2005      **Report** 18-Jul-2005

**Report**      **Copies**

**Submitter:** 12847      Heritage Salmon  
**Owner** 12847      Heritage Salmon  
**Farm:** 14666      #05-30  
**Vet Clinic:**  
**Attending**      Dr. Peter McKenzie

**Specimen:** Other      **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

**History/Symptoms**

Submitter - Tim Talbot. Case #05-30. PO # 17225.

Submitted 5 histo. cassettes (livers) fixed in formalin approx. 24 hrs. Shipped in Ethanol - 35%.

Approx. 4 kg fish. High number of silvers in mortalities. Recent mild bloom of Dictyocha speculum (reports of toxic production).

PM lesions - livers were shrunken, pale, with dark patches. They almost appeared "balled up" anterior to pylorus.

**Histopathology**

Five cassettes of formalin-fixed livers (1 piece of liver per cassette) were submitted for histopathology in a jar of 35% ethanol.

Quality Control: Tissue preservation is fair to poor for most livers; liver #4 is the best preserved. Sections have no significant dehydration after fixation and no significant acid hematin deposits.

**Diagnosis**

- 1a. Liver: hepatocellular cytosegresomes, mild (slide 1), moderate (slides 2, 5)
- 1b. Liver: hepatocellular karyomegaly, diffuse, mild (slide 2, 3, 4, 5)
- 1c. Liver: hepatocellular karyorrhexis or single cell necrosis, diffuse, mild (slides 1, 2, 4, 5)
- 1d. Liver: lipofuscin-pigmented sinusoidal macrophages, diffuse, moderate (slides 3), severe (slides 1, 2, 4, 5)
- 1e. Liver: hepatitis, lymphoplasmacytic, multifocal, mild (slides 2, 3, 4), moderate (slides 1, 5)
- 1f. Liver: peritonitis, lymphoplasmacytic, diffuse, mild (slide 1), with fibrous proliferation, moderate (slide 4)
- 1g. Liver: biliary hyperplasia, diffuse, mild (slides 2, 3, 4, 5), moderate (slide 1)

**Final Comments**

Most features of these slides are consistent with chronic exposure to a toxicant (e.g., algal toxin). The sections have a fairly consistent pattern dominated by lipofuscin accumulation, with variable amounts of single cell necrosis, hepatocellular karyomegaly, lymphoplasmacytic inflammation, cytosegresomes, and biliary hyperplasia. Several publications mention the potential for toxicity of *Dictyocha speculum* found in the area where these fish were sampled, but I was not able to find any studies that examined liver lesions in response to *D. speculum*-produced toxins. Other toxins in the water or feed (e.g., aflatoxins) are less likely differentials.

Hepatic megalocytosis can result from exposure to several types of toxicants, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR (thought to be the cause of "netpen liver disease"). Megalocytosis is not as prominent a feature in these slides as is often associated with literature descriptions of netpen liver disease.

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.

Cytosegresomes occur in liver cells that have been sublethally injured by a variety of insults, ranging from hypoxia through a variety of intoxications to malnutrition, specific deficiencies, and some viral infections. They may be formed when masses of cytoplasmic organelles are gathered and condensed, and are sequestered from remaining cytoplasm by membranes that fuse with lysosomes (autolysosomes).

/sr





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**Case Report**

**Submission** 2005-00247      **Date** 26-Jan-2005      **Report** 01-Feb-2005

**Report**      **Copies**

**Submitter:** 11036 Mainstream Canada (M)

**Owner** 11036 Mainstream Canada (M)

**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 a sample pool of Atlantic salmon from BA. Please do virology PCR (VHS, ISA), and viral culture.

Sample submitted by Sorka A Cerna Vera - Mainstream Canada.

**Virology**

VHS positive.

VHS-like CPE on tissue culture.

\* Results faxed Feb. 3/05.

**Molecular Diagnostics/PCR**

ISA Virus negative by PCR.

VHS Virus positive by PCR.

\* Results faxed Feb. 1/05.

/bb

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**Case Report**

**Submission** 2005-02509      **Date** 13-Jul-2005      **Report** 18-Jul-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14688      A3.4-72(1-4)

**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**

A3.4-72. Provincial Surveillance Program samples.

Request virology - PCR for IHN, ISA, IPN, VHS and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Note - Histo cassettes also included for Dr. Marty. The tissues are fresh so please fix with formalin before processing.

**Histopathology**

Quality control/quality assurance: the spleen sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation. The organs have no evidence of dehydration after fixation.

Slide 1: autolysis, mild (heart) to moderate (spleen, head kidney), to severe (trunk kidney and liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
- 3a. Spleen: leukocytic karyorrhexis, disseminated, moderate
- 3b. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. The presence of degenerating nuclei (karyorrhexis) in the spleen is evidence of increased cell turnover, possibly as part of an active inflammatory response. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, mild (heart) to moderate (spleen, kidney), to severe (intestinal cecum and liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: leukocytic karyorrhexis, disseminated, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions
6. Intestinal cecum and surrounding mesenteric fat: peritonitis, chronic, multifocal, with fibrocellular fronds, moderate

Comment: The pathogenesis of peritonitis around the intestinal ceca probably is the same as for splenic peritonitis.

Slide 3: autolysis, mild (heart) to moderate (spleen, kidney), to severe (gill and liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions
6. Gill: no significant lesions

Comment: Bacterial colonies on the margin of the heart probably are a result of post mortem growth.

Slide 4 (the overloaded cassette was split into A and B blocks for sectioning and staining: autolysis, mild (heart) to moderate (spleen, kidney), to severe (gill, intestinal cecum, and liver)

1. Liver (4A): no significant lesions
2. Heart (4A): no significant lesions
3. Spleen (4A): leukocytic karyorrhexis, disseminated, moderate
4. Trunk kidney (4B): no significant lesions
5. Head kidney (4B): no significant lesions
6. Intestinal cecum and surrounding mesenteric fat (4A and 4B): no significant lesions
7. Gill (4B): no significant lesions

Comment: none (autolyzed tissue).



**Molecular Diagnostics/PCR**

Samples 1-2, 3-4: ISA, IHN, IPN, VHS, Piscirickettsia salmonis negative by PCR.

\* Results faxed July 19/05; corrected version faxed Aug. 8/05.

/bb

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**Case Report**

**Submission** 2005-02511      **Date** 13-Jul-2005      **Report** 19-Jul-2005

**Report**      **Copies**

**Submitter:** 2007      Microtek International  
**Owner** 14690      Coble, Emily  
**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**

Atlantic salmon kidney. Please identify the enclosed bacteria labelled 5-1943-11.

**Bacteriology**

Bacterial isolate identified as *Shewanella putrefaciens*.

\* Results faxed July 19/05.

/bb

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**Case Report**

**Submission** 2005-02512      **Date** 13-Jul-2005      **Report** 18-Jul-2005

**Report**      **Copies**

**Submitter:** 12847      Heritage Salmon  
**Owner** 14691      Heritage Salmon, #05-31  
**Farm:**  
**Vet Clinic:**  
**Attending**      Dr. Peter McKenzie

**Specimen:** Other      **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

**History/Symptoms**

Submitted 5 histo. cassettes (liver, kidney, spleen). Please disregard "03-45" labelled on side cassettes. The case # for all 5 cassettes is 05-31.

Submitted by Tim Talbot. Case 05-31. Please quote PO #17228.

High mortality. High number of pin heads. Mouthrot mortalities ongoing.  
Recent "Slice" treatment.

Please report to Dr. Peter McKenzie by fax and email.

**Histopathology**

Five cassettes of formalin-fixed tissues (liver, kidney, and spleen) were submitted for histopathology in a plastic jar of 35% ethanol. The tops of the cassettes were all labeled "Simmonds Pt. July 06." The cassettes were arbitrarily labeled 1 through 5 for processing to slides 1 through 5. All organs on each slide were examined; organs not listed below have no significant lesions.

Quality Control: Tissue preservation is variable: poor (slide 4), fair (slide 2), to good (slides 1, 3, and 5). None of the slides has significant acid hematin deposits. Slide #s 1 and 2 have no significant dehydration after fixation. Slide #s 3 and 4 have foci of 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.

**Diagnosis**

- 1a. Liver: hepatocellular cytosegresomes, mild (slide 4)
- 1b. Liver: hepatocellular karyomegaly, diffuse, with some binucleate cells, moderate (slide 2)
- 1c. Liver: hepatocellular karyorrhexis or single cell necrosis, diffuse, mild (slide 2), moderate (slide 1)
- 1d. Liver: lipofuscin-pigmented sinusoidal macrophages, diffuse, mild (slide 4), moderate (slides 1, 2)
- 1e. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 4, 5), moderate (slide 1)
- 1f. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 3)
2. Spleen and surrounding fatty mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 3, 5), moderate (slides 1, 2, 4)



**Final Comments**

Features in three of these slides (1, 2, and 4) are consistent with chronic exposure to a toxicant (e.g., an algal toxin). The sections have a fairly consistent pattern dominated by lipofuscin accumulation, with variable amounts of single cell necrosis, hepatocellular karyomegaly, lymphoplasmacytic inflammation, cytosegresomes, and biliary hyperplasia. Other toxins in the water or feed (e.g., aflatoxins) are less likely differentials.

Hepatic megalocytosis can result from exposure to several types of toxicants, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR (thought to be the cause of "netpen liver disease"). Megalocytosis is not as prominent a feature in these slides as is often associated with literature descriptions of netpen liver disease.

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.

Cytosegresomes occur in liver cells that have been sublethally injured by a variety of insults, ranging from hypoxia through a variety of intoxications to malnutrition, specific deficiencies, and some viral infections. They may be formed when masses of cytoplasmic organelles are gathered and condensed, and are sequestered from remaining cytoplasm by membranes that fuse with lysosomes (autolysosomes).

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.

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

/sr



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**Case Report**

**Submission** 2005-02626      **Date** 22-Jul-2005      **Report** 01-Aug-2005

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14734 #5330, Marine Harvest  
**Farm:**  
**Vet Clinic:**  
**Attending**

**Report**      **Copies**  
 Stolt Sea Farm Inc.

**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

**History/Symptoms**

Submitted 5 pools of 3 for PCR (IHN and VHS) - spleen, head, kidney.

Seeing atypical A-Sal at this site, want to make sure nothing else is going on.

**Virology**

5 samples inoculated onto tissue culture - all negative.

\* Results faxed Aug. 17/05.

**Molecular Diagnostics/PCR**

Samples #1-3, 4-6, 7-9, 10-12, 13-15: IHN and VHS Virus negative by PCR.

\* Results faxed Aug. 8/05.

/bb



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## Case Report

**Submission** 2005-02627      **Date** 22-Jul-2005      **Report** 01-Aug-2005

<b>Submitter:</b> 9439	Marine Harvest Canada (M)	<b>Report</b>	<b>Copies</b>
<b>Owner</b> 14736	#5332, Marine Harvest		Stolt Sea Farm Inc.
<b>Farm:</b>			
<b>Vet Clinic:</b>			
<b>Attending</b>			

<b>Specimen:</b> Tissue - Fresh	<b>Count</b> 1	<b>Flock Herd Size:</b>
<b>Species:</b> Atlantic Salmon		<b>Age</b>
<b>Breed:</b>		<b>Sex:</b>
<b>Feed:</b>		<b>Feed</b>

**Vaccination**

**Treatmen**

**Diagnosis**

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

**History/Symptoms**

Submitted one fish for IHN and VHS PCR (spleen and kidney).

Petechial hemorrhage in liver.

**Virology**

No virus isolated.

\* Results faxed Aug. 17/05.

**Molecular Diagnostics/PCR**

Sample #5332: IHN and VHS Virus negative by PCR.

\* Results faxed Aug. 8/05.

/bb

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**Case Report**

**Submission** 2005-02628      **Date** 22-Jul-2005      **Report** 01-Aug-2005

**Submitter:** 9439 Marine Harvest Canada (M)      **Report**      **Copies**  
**Owner** 14737 #5331, Marine Harvest      Stolt Sea Farm Inc.  
**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 fish for IHN and VHS PCR (5 organs, 1 fish/bag).

Petechial hemorrhage in liver.

**Virology**

3 samples inoculated onto tissue culture - all negative.

\* Results faxed Aug. 17/05.

**Molecular Diagnostics/PCR**

Samples #1, 2 and 3: IHN and VHS Virus negative by PCR.

\* Results faxed Aug. 8/05.

/bb

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**Case Report**

**Submission** 2005-02629      **Date** 22-Jul-2005      **Report** 27-Jul-2005

<b>Submitter:</b> 9439	Marine Harvest Canada (M)	<b>Report</b>	<b>Copies</b>
<b>Owner</b> 14738	#5333, Marine Harvest		Stolt Sea Farm Inc.
<b>Farm:</b>			
<b>Vet Clinic:</b>			
<b>Attending</b>			

<b>Specimen:</b> Tissue - Formalized	<b>Count</b> 3	<b>Flock Herd Size:</b>
<b>Species:</b> Atlantic Salmon		<b>Age</b>
<b>Breed:</b>		<b>Sex:</b>
<b>Feed:</b>		<b>Feed</b>

**Vaccination****Treatmen****Diagnosis**

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

### **History/Symptoms**

Submitted 3 fish for histology.

Moribunds, anemic, visceral hemorrhage.

### **Histopathology**

Three cassettes of formalin-fixed tissues from moribund fish were submitted for histopathology wrapped in tissue. The cassettes were all labeled "G Lake, Pen 1." The cassettes were arbitrarily labeled 1 through 3 for processing to slides 1 through 3.

Slide 1: transverse body wedge with skeletal muscle and the trunk kidney/head kidney transition; trunk kidney alone, liver, spleen, heart; intestinal ceca and mesenteric fat.

Slide 2: transverse body wedge with trunk kidney, skeletal muscle, and skin; trunk kidney alone, liver, heart, gill, intestinal ceca and mesenteric fat.

Slide 3 (also, PAS stain and Twort's Gram stain): trunk kidney, head kidney, liver, heart, gill, stomach, intestinal ceca and mesenteric fat.

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

Quality Control: Tissue preservation is good for most organs except for the liver in slide 1 (mild autolysis), and intestinal ceca in all slides, which have mild autolysis on the tips of villi. The tissues have mild to moderate dehydration after fixation (i.e., 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.

Measures of physiologic condition

Hepatocellular glycogen depletion, severe (all slides)

Mesenteric adipose tissue depletion, none (slide 2), mild (slides 1 and 3)

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

### **Diagnosis**

- 1a. Liver: hepatic necrosis, acute, multifocal, moderate, with intralesional organisms consistent with *Sphaerothecum destruens* (formerly referred to as the Rosette Agent) (slide 3)
- 1b. Liver: hepatic necrosis, acute, multifocal, moderate (slide 2)
- 1c. Liver: biliary preductular and ductular cell hyperplasia, diffuse, moderate (slide 1)
- 1d. Liver: sinusoidal congestion, diffuse, moderate (slide 2)
2. Intestinal ceca: intraluminal adult cestodes, multifocal, small numbers (slide 3), abundant (slide 1)
- 3a. Trunk kidney: glomerular distortion with intraglomerular organisms consistent with *Sphaerothecum destruens* (slide 3)
- 3b. Trunk kidney: interstitial congestion and hemorrhage, diffuse, severe (slide 1)
4. Head kidney: interstitial congestion and hemorrhage, diffuse, moderate (slide 2)
5. Mesenteric fat: capillary congestion and hemorrhage, multifocal, moderate (slide 1)



**Final Comments**

*Sphaerothecum destruens*, formerly referred to as the Rosette Agent, is a primitive organism that is fairly common in some populations of Chinook salmon, but it has also been described in Atlantic salmon. Only one of three fish examined had evidence of *Sphaerothecum destruens*, and this is the only British Columbia case I have had in the last year. In this case, organisms range from 2 to 6 µm in diameter. Organisms in the liver are associated with foci of necrosis. Organisms in the glomeruli are associated with distortion of the glomerular tufts. Most of the organisms are Gram negative, but about 10% are Gram positive. Most of the organisms are weakly PAS positive.

Renal and hepatic congestion and hemorrhage are classic signs of infectious salmon anemia (ISA), but ISA has never been isolated from fish in BC, and it is rare during the freshwater phase of development. Renal congestion and hemorrhage has also been associated with VHSV, bacteria, and it may occur as a sampling artifact.

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*).

Biliary 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).

Intraluminal cestodes are common in wild salmonids, but this is the first time that I have seen them in cultured fish. These fish must be ingesting the infectious stage of the cestodes. The cestodes in the sections are up to 2 mm long and are about 250 µm in diameter.

Congestion and hemorrhage of mesenteric adipose tissue is a nonspecific clinical sign often associated with bacterial or viral infections. Consider virology if the problem persists.

/sr





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**Case Report**

**Submission** 2005-02669      **Date** 27-Jul-2005      **Report** 05-Aug-2005

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14762 #5330, Marine Harvest (Stolt PO  
#58948)

**Report** **Copies**  
Stolt Sea Farm Inc.

**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

**History/Symptoms**

Submitted three plates for bacteriology identification.

For invoice purposes, please invoice under Stolt and quote PO# 58948.

**Bacteriology**

Bact plate 1 - *Vibrio pomeroyi*

Bact plate 2 - *Aeromonas salmonicida*

Bact plate 3 - *Aeromonas salmonicida*

/sr

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**Case Report**

**Submission** 2005-02931      **Date** 17-Aug-2005      **Report** 23-Aug-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 14875 #5365, 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

**History/Symptoms**

Submitted two samples for virology (PCR for IHN and VHS) to rule out viral etiology. Gills taken from moribund fish and put into buffered formalin for 24 hours. Site has tested negative for VHS and IHN in previous samples.

Increase in mortality at sea site. Suspect plankton bloom.

PO# CL5310 - Marine Harvest.

**Histopathology**

One cassette of 3 formalin-fixed gills was received in a cassette wrapped in tissue in a plastic bag. The cassette was labeled 5365 91 11s.

Quality Control: Tissue preservation is good. The section has no dehydration after fixation or acid-hematin.

**Molecular Diagnostics/PCR**

Samples 1-3, 4-6 - IHN and VHS Virus negative by PCR.

\* Results faxed on Sept. 2/05.

**Diagnosis**

1. Gill: lamellar subepithelial edema, multifocal, mild (1 of 3 gills)
2. Gill: lamellar fusion, multifocal, mild (1 of 3 gills)

**Final Comments**

Despite widespread problems in marine net pens with algal toxins, histopathology of gill lesions after algal toxin exposure is poorly characterized. These gills did not have epithelial necrosis that has been associated with some types of algal blooms, and the sections did not include algae or diatoms. However, sections did have some nonspecific lesions that might have contributed to fish morbidity.

Separation of gill lamellar epithelium from underlying pillar cells can be a result of edema, but it is a common postmortem artifact. 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 artifact.

Gill lamellar fusion, without lamellar hypertrophy (as in this case), has been associated with exposure to heavy metals and with hypoxia (thought to be from fish gasping for oxygen at the water-air interface).

If elevated mortality continues, consider submission of other major organs for histopathology (e.g., liver, spleen, kidney, heart, brain, and intestine).

/sr/mm



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**Case Report**

**Submission** 2005-02932      **Date** 17-Aug-2005      **Report** 30-Aug-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 14876 #5353, 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 fresh fish tissue for virology - PCR for IHNV, and VHSV from 3 fresh dead silvers with no visible lesions.

PO# CL5311 - Marine Harvest.

**Molecular Diagnostics/PCR**

IHN and VHS virus negative by PCR.

/mb

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**Case Report**

**Submission** 2005-02933      **Date** 17-Aug-2005      **Report** 06-Sep-2005

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14877 #5359, Marine Harvest  
**Farm:**  
**Vet Clinic:**  
**Attending**

**Report**      **Copies**  
 Stolt Sea Farm Inc.

**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

**History/Symptoms**

Submitted 6 samples for virology labeled 1-5; 6-10; 11-15; 16-20; 21-25; 26-30 for PCR for IHN and VHS. This is a follow up to histology AHC 2005/02200. Results to r/o viral etiology.

Invoice Stolt - PO# 59296.

**Molecular Diagnostics/PCR**

Samples 1-5, 6-10, 11-15, 16-20, 21-25, 26-30: IHN and VHS virus Negative by PCR.

/mb

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**Case Report**

**Submission** 2005-02934      **Date** 17-Aug-2005      **Report** 06-Sep-2005

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14878 #5364, Maine Harvest  
**Farm:**  
**Vet Clinic:**  
**Attending**

**Report**      **Copies**  
 Stolt Sea Farm Inc.

**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 virology to rule out presence of IHN and VHS.

Increase in mortality at broodstock site. Gross lesions include ecchymotic hemorrhaging on swim bladder; petechial hemorrhages on liver; reddened anal fin.

A. salmonicida isolated from one fish. One Virology sample from fresh dead fish submitted to rule out presence of IHN or VHS. Fish transferred from sea site to fresh water site in July.

Invoice Stolt - PO# 59293.

**Molecular Diagnostics/PCR**

IHN and VHS virus Negative by PCR.

/mb

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**Case Report**

**Submission** 2005-02959      **Date** 18-Aug-2005      **Report** 31-Aug-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14888      A 3.3 - 63 (1-5)

**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

**History/Symptoms**

Submitted fish tissue as part of the Provincial Surveillance Program for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 3.3 - 63 (1-5).

ADDENDUM - Received 5 cassettes for routine histology processing and analysis on Aug. 25/05.

**Histopathology**

Quality control/quality assurance: this is the first of a group of monitoring cases that was preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Sections have no postfixation dehydration, and acid hematin is limited to foci of hepatic sinusoidal congestion in slides 3 and 4. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation; in these cases, sinusoidal congestion probably resulted in antemortem acidosis.

Slide 1: autolysis, none (other organs) to mild (trunk kidney)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

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

Slide 2: autolysis, none (all organs)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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).

Slide 3: autolysis, none (other organs) to mild (liver and kidney)

- 1a. Liver: hepatic necrosis, sinusoidal congestion, and intracytoplasmic amphophilic inclusions, acute, multifocal, moderate
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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*). Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Sinusoidal congestion (peliosis) is evidence of sinusoidal damage. In BC Atlantic salmon, peliosis is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Peliosis is one of the classic lesions associated with ISAV infections. Peliosis 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 peliosis in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The amphophilic cytoplasmic inclusions in hepatocytes are large, varying from the same size of nuclei to 50% larger than hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes or viral inclusions; however, no known salmon virus produces these types of inclusions.

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

Slide 4: autolysis, none (heart) to mild (other organs)





- 1a. Liver: hepatic necrosis, sinusoidal congestion, and intracytoplasmic amphophilic inclusions, acute, multifocal, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions
- Comment: see previous slides.

Slide 5: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, severe
- 1b. Liver: sinusoidal congestion, multifocal, mild
- 2. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: The pathogenesis of hepatic sinusoidal congestion in this fish is probably similar to the fish in slide 4, but this is a milder case.

#### **Molecular Diagnostics/PCR**

A3.3-63, 1-3, 4-5 IHN, VHS, IPN, ISA, Piscirickettsia salmonia negative by PCR.

\* Results faxed on Sept 19/05.

/bb/mm



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**Case Report**

**Submission** 2005-02960      **Date** 18-Aug-2005      **Report** 01-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14889      A 3.3 - 61 (1-5)

**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

**History/Symptoms**

Submitted fish tissue as part of the Provincial Surveillance Program for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonia*. Culture any PCR positive samples.

Sample ID: A 3.3 - 61 (1-5).

ADDENDUM - Received 5 cassettes for routine histology processing and analysis on Aug. 25/05.

**Histopathology**

Quality control/quality assurance: this is part of the first of a group of monitoring cases that was preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Sections have no postfixation dehydration, and acid hematin is limited to foci of hepatic sinusoidal congestion in slides 1, 2, and 3. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation; in these cases, sinusoidal congestion probably resulted in antemortem acidosis.

Slide 1: autolysis, none (all organs)

1a. Liver: sinusoidal congestion and intracytoplasmic amphophilic inclusions, multifocal, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

1c. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: epicarditis, multifocal, lymphoplasmacytic, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: nephritis, interstitial, granulomatous, focal, mild

Comment: Sinusoidal congestion (peliosis) is evidence of sinusoidal damage. In BC Atlantic salmon, peliosis is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*.

Peliosis is one of the classic lesions associated with ISAV infections. Peliosis 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 peliosis in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The amphophilic cytoplasmic inclusions in hepatocytes are large, varying from the same size of nuclei to 50% larger than hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes or viral inclusions; however, no known salmon virus produces these types of inclusions.

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).

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.

Slide 2: autolysis, none (all organs)

1a. Liver: sinusoidal congestion and intracytoplasmic amphophilic inclusions, multifocal, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

1c. Liver: hepatitis, perivascular and pericholangial, lymphocytic, multifocal, moderate

2. Heart: no significant lesions

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney (2 pieces): no significant lesions

Comment: Lymphocytic inflammation around vessels and 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.



Slide 3: autolysis, none (other organs) to mild (gill)

1a. Liver: sinusoidal congestion and intracytoplasmic amphophilic inclusions, multifocal, moderate

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: no significant lesions

3a. Spleen: splenitis, granulomatous, multifocal, moderate

3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: interstitial cell hyperplasia, diffuse, mild

6. Gill: abundant eosinophilic granular cells in the loose connective tissue of filaments

Comment: The most common organism associated with granulomatous splenitis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation. 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 this case, inflammation in the spleen might be the inciting cause. 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 was not included in the sections examined.

Slide 4: autolysis, none (all organs)

1a. Liver: intracytoplasmic amphophilic inclusions and scant sinusoidal congestion, multifocal, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

1c. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: no significant lesions

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: interstitial cell hyperplasia, diffuse, mild

Comment: see other slides.

Slide 5: autolysis, none (other organs) to mild (liver)

1a. Liver: pericholangitis, lymphocytic, multifocal, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

1c. Liver: hepatic necrosis and sinusoidal congestion, acute, multifocal, mild

2. Heart: no significant lesions

3a. Spleen: splenitis, granulomatous, focal, mild

3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: 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 lesions here seem to part of the same continuum of changes in the other slides: varying degrees of sinusoidal congestion, hepatocellular necrosis, and amphophilic intracytoplasmic inclusions.





**Molecular Diagnostics/PCR**

A3.3-61, 1-3, 4-5 IHN, VHS, IPN, ISA, Piscirickettsia salmonis negative by PCR.

\* Results faxed on Sept 19/05.

/bb/mm

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**Case Report**

**Submission** 2005-02961      **Date** 18-Aug-2005      **Report** 01-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14890      A 3.3 - 64 (1-6)

**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

**History/Symptoms**

Submitted fish tissue as part of the Provincial Surveillance Program for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 3.3 - 64 (1-6).

ADDENDUM - Received 6 cassettes for routine histology processing and analysis on Aug. 25/05.

**Histopathology**

Quality control/quality assurance: this case is part of the first of a group of monitoring cases that were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Sections have no postfixation dehydration or acid hematin.

Slide 1: autolysis, none (other organs) to mild (liver)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: 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 golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin 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. Small amounts of splenic lipofuscin are fairly common in pen-reared Chinook salmon. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Slide 2: autolysis, none (all organs)

- 1a. Liver: pericholangitis, lymphocytic, focal, mild
- 1b. Liver: hepatitis, granulomatous, lymphoplasmacytic, focal, mild
- 2. Heart: no significant lesions
- 3. Spleen: peritonitis, granulomatous, diffuse, with occasional vacuoles up to 250 µm in diameter, severe
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: 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. Granulomatous inflammation in the liver is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* is the most common organism associated with these lesions. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 3: autolysis, none (all organs)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

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

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, granulomatous, bifocal, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, severe
- 2. Heart: no significant lesions
- 3. Spleen: peritonitis, granulomatous, diffuse, with occasional vacuoles up to 250 µm in diameter, moderate



- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions
- Comment: see other slides.

Slide 5: autolysis, none (other organs) to mild (liver)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 2. Heart: no significant lesions
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: not included on the slide
- 5. Head kidney: no significant lesions
- Comment: see other slides.

Slide 6: autolysis, none (all organs)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 2. Heart: no significant lesions
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4a. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
- 4b. Trunk kidney: renal tubular epithelial necrosis, focal (one tubule), with regeneration, subacute, mild
- 5. Head kidney: no significant lesions

Comment: 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. 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). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as Gentamicin).

### **Molecular Diagnostics/PCR**

A3.3-64, 1-3, 4-6 IHN, VHS, IPN, ISA, Piscirickettsia salmonis negative by PCR.

\* Results faxed on Sept 19/05.

/bb/mm





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**Case Report**

**Submission** 2005-02962      **Date** 18-Aug-2005      **Report** 01-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14891      A 3.5 - 70 (1-6)

**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

**History/Symptoms**

Submitted fish tissue as part of the Provincial Surveillance Program for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 3.3 - 70 (1-6).

ADDENDUM - Received 5 cassettes for routine histology processing and analysis on Aug. 25/05.

**Histopathology**

Quality control/quality assurance: this case is part of the first of a group of monitoring cases that were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Sections have no postfixation dehydration or acid hematin.

Slide 1: autolysis, none (other organs) to mild (trunk kidney, liver)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1c. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: myocardial fibrosis, focal (80 x 50 µm), mild
3. Spleen: peritonitis, granulomatous, lymphoplasmacytic, regionally diffuse, with fine fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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. 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).

The focus of myocardial fibrosis probably is a site of resolving damage. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, mild (other organs) to severe (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: not included on the slide
4. Trunk kidney: interstitial congestion and hemorrhage, multifocal, mild
5. Head kidney: interstitial congestion and hemorrhage, diffuse, moderate

Comment: Renal congestion and hemorrhage is one of the classic signs of infectious salmon anemia (ISA), but ISA has never been isolated from fish in BC. Renal congestion and hemorrhage has also been associated with VHSV and bacterial infections.

Slide 3: autolysis, none (other organs) to mild (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: peritonitis, granulomatous, lymphoplasmacytic, regionally diffuse, with fine fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: see other slides.

Slide 4: autolysis, mild (other organs) to moderate (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: epicarditis, focal, lymphohistiocytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.



Slide 5: autolysis, none (all organs)

1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

1b. Liver: sinusoidal congestion, multifocal, moderate

2. Heart: no significant lesions

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Although about ½ of the liver section is involved, congestion of affected sinusoids is mild.

Slide 6: autolysis, mild (other organs) to moderate (liver)

1. Liver: no significant lesions

2. Heart: no significant lesions

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: interstitial congestion and hemorrhage, diffuse, moderate

5. Head kidney: no significant lesions

Comment: see other slides.

#### **Molecular Diagnostics/PCR**

A3.5-70, 1-3, 4-6 IHN, VHS, IPN, ISA, *Piscirickettsia salmonis* negative by PCR.

\* Results faxed on Sept 19/05.

/bb/mm



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**Case Report**

**Submission** 2005-02963      **Date** 18-Aug-2005      **Report** 01-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14892      A 3.2 - 60 (1-4)

**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 fish tissue as part of the Provincial Surveillance Program for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 3.2 - 60 (1-4).

ADDENDUM - Received 4 cassettes for routine histology processing and analysis on Aug. 25/05.



**Histopathology**

Quality control/quality assurance: this case is part of the first of a group of monitoring cases that were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Sections have no postfixation dehydration. Hepatocytes around large vessels in all slides have large deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 4 [note non-standard order]: autolysis, none (other organs) to mild (liver; one of the 2 pieces of liver has severe autolysis)

1. Liver (2 pieces): sinusoidal congestion, multifocal, moderate
2. Heart: no significant lesions
3. Spleen: not included on the slide
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: Hepatic sinusoidal congestion 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*. 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.

Slide 1: autolysis, mild (other organs), to moderate (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: peritonitis, lymphoplasmacytic, granulomatous, regionally diffuse, with fine fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

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

Slide 2: autolysis, mild (other organs), to moderate (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: peritonitis, lymphoplasmacytic, granulomatous, regionally diffuse, with fine fibrocellular fronds and intralesional vacuoles 30 - 80 µm in diameter, severe
4. Trunk kidney: interstitial congestion, diffuse, moderate
5. Head kidney: no significant lesions

Comment: Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Renal congestion and hemorrhage is one of the classic signs of infectious salmon anemia (ISA), but ISA has never been isolated from fish in BC. Renal congestion and hemorrhage has also been associated with VHSV and bacteria.

Slide 3: autolysis, mild (other organs), to severe (liver)

1. Liver: sinusoidal congestion, multifocal, moderate
  2. Heart: no significant lesions
  3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
  4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
  5. Head kidney: not included on the slide
- Comment: see other slides.



**Molecular Diagnostics/PCR**

A3.2-60, 1-2, 3-4 IHN, VHS, IPN, ISA, Piscirickettsia salmonis negative by PCR.

\* Results faxed on Sept 19/05.

/bb/mm

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**Case Report**

**Submission** 2005-02964      **Date** 18-Aug-2005      **Report** 01-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14893      A 2.3 - 48 (1-4)

**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 fish tissue as part of the Provincial Surveillance Program for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 2.3 - 48 (1-4).

ADDENDUM - Received 4 cassettes for routine histology processing and analysis on Aug. 25/05.

**Histopathology**

Quality control/quality assurance: this case is part of the first of a group of monitoring cases that were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Sections have no postfixation dehydration. Hepatocytes around large vessels in slide #1 have deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, moderate (other organs) to severe (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

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

Slide 2: autolysis, none (other organs) to mild (liver)

1. Liver: hepatic necrosis, acute, multifocal, mild
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart: epicarditis, multifocal, lymphoplasmacytic, mild
3. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: 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*). Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. 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.

Slide 3: autolysis, mild (other organs) to moderate (liver)

1. Liver: hepatic necrosis, acute, multifocal, moderate
2. Heart: epicarditis, multifocal, lymphoplasmacytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: see other slides.

Slide 4: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2a. Heart: epicarditis, multifocal, lymphoplasmacytic, mild
- 2b. Heart: endocarditis, multifocal, lymphoplasmacytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: not included on the slide

Comment: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Lymphoplasmacytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. 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.

**Molecular Diagnostics/PCR**

A2.3-46, 1-2, 3-4 IHN, VHS, IPN, ISA, Piscirickettsia salmonis negative by PCR.

\* Results faxed on Sept 19/05.

/bb/mm



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**Case Report**

**Submission** 2005-02976      **Date** 19-Aug-2005      **Report** 06-Sep-2005

**Submitter:** 9439 Marine Harvest Canada (M)      **Report**      **Copies**  
**Owner** 14900 #5367, Marine Harvest      Stolt Sea Farm Inc.  
**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

**History/Symptoms**

Submitted 5 samples for virology labeled 1-2, 4-6, 3-7, 8-9, 10. Please do PCR for IHNV and VHS.

Fish showing focal hemorrhaging of liver similar to cases 5355, and 5366.

PO# 59352 - Stolt.

**Molecular Diagnostics/PCR**

Samples #1, #2, #3, #7, #4-6, #8, #9, #10 - IHN and VHS virus Negative by PCR.

/mb

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**Case Report**

**Submission** 2005-02977      **Date** 19-Aug-2005      **Report** 06-Sep-2005

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14901 #5366, Marine Harvest  
**Farm:**  
**Vet Clinic:**  
**Attending**

**Report**      **Copies**  
 Stolt Sea Farm Inc.

**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 frozen sample for virology - PCR for IHNV and VHSV. Same site as case 5355. Liver lesions persisting in larger fish.

PO# 59351 - Stolt.

**Molecular Diagnostics/PCR**

Sample (Pen #9) - IHN and VHS virus Negative by PCR.

/mb

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**Case Report**

**Submission** 2005-02978      **Date** 19-Aug-2005      **Report** 23-Aug-2005

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 14902 #5355, Marine Harvest  
**Farm:**  
**Vet Clinic:**  
**Attending**

**Report**      **Copies**  
 Stolt Sea Farm Inc.

**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 histo taken from fish #2. Gross lesions were hemorrhagic liver. Approximately 75% of the mortality had liver lesions with no other visible lesions, there was some feed in lower intestine.

PO# 59350 - Stolt.

**Histopathology**

Two cassettes of formalin-fixed tissues were received wrapped in tissue in a plastic bag. Both cassettes were labeled 5355.

Slide 1: heart, liver, head kidney, trunk kidney, and spleen

Slide 2: gill

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

Quality Control: Tissue preservation is good. The sections have no dehydration after fixation or acid-hematin.

**Diagnosis**

- 1a. Liver: pericholangitis, lymphocytic, multifocal, moderate
- 1b. Liver: hepatitis, perivascular, lymphocytic, focal, moderate
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1d. Liver: sinusoidal congestion and hemorrhage around medium-sized blood vessels (peliosis), multifocal, with hepatocellular erythrophagocytosis, mild
2. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
3. Trunk kidney: renal tubular mineralization, multifocal, with dilated tubules and tubular epithelial hyperplasia, mild
4. Gill: Branchial epithelial karyorrhexis, multifocal, moderate

**Final Comments**

These tissues have a number of changes consistent with chronic exposure to bacterial or algal toxins.

Lymphocytic inflammation around bile ductules and vessels in the 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. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Hepatic peliosis is evidence of sinusoidal damage. In BC Atlantic salmon, peliosis is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Peliosis is also one of the classic lesions associated with ISAV infections. Consider bacteriology and virology and PCR for VHSV, IHNV, and ISAV (if not already done). Peliosis 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 peliosis in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data).

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

Renal mineralization is common in cultured fish species; when severe, the condition is termed nephrocalcinosis. The lesion is not considered fatal, although feed conversion may be adversely affected. The cause is unknown, 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.

The epithelium lining the interlamellar region of the filament contains several karyorrhectic nuclei per 40x objective-lens field. The karyorrhectic cells are probably leukocytes, but they might be epithelial cells. Turnover of leukocytes is usually associated with an active inflammatory response. Although the change is not specific, in this case karyorrhexis probably is part of a response to bacterial or algal toxins.

/sr





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**Case Report**

**Submission** 2005-00299      **Date** 01-Feb-2005      **Report** 16-Feb-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13379      A 3.3 - 17 (1-7)

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.3 - 17 (1-7).

**Histopathology**

Quality control/quality assurance: the spleen sections contain small foci 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 evidence of postfixation dehydration artifact.

Slide 1: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, multifocal, granulomatous, moderate
- 1b. Liver: pericholangitis, lymphocytic, multifocal, mild
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2a. Heart: myocarditis, focal, granulomatous, mild
- 2b. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: splenitis, multifocal, granulomatous, moderate
- 4a. Trunk kidney: nephritis, interstitial, multifocal, granulomatous, moderate
- 4b. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild
- 5. Head kidney: nephritis, interstitial, multifocal, granulomatous, mild

**COMMENT:**

Granulomatous inflammation in multiple organs is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* (bacterial kidney disease) is the most common organism associated with these lesions.

The cause and significance of myocardial karyomegaly 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).

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. 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.

Slide 2: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, multifocal, granulomatous, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), multifocal, mild
- 2. Heart: myocarditis, multifocal, granulomatous, mild
- 3. Spleen: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, moderate
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: Granulomatous inflammation in multiple organs is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* (bacterial kidney disease) is the most common organism associated with these lesions. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, multifocal, granulomatous, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1c. Liver: hepatocellular fatty change (lipidosis), multifocal, mild



- 1d liver: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 2a. Heart: myocarditis, multifocal, granulomatous, mild
- 2b. Heart: epicarditis, regionally diffuse, granulomatous, lymphoplasmacytic, moderate
- 2c. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: nephritis, interstitial, multifocal, granulomatous, mild
- 5. Head kidney: no significant lesions

**COMMENT:**

Granulomatous inflammation in multiple organs is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* (bacterial kidney disease) is the most common organism associated with these lesions. 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response. Epicarditis is evidence of chronic immune stimulation; it might be related to infection with *Renibacterium salmoninarum*. Splenic and hepatic peritonitis are consistent with a reaction to foreign material; peritonitis is common in fish that have been vaccinated.

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, multifocal, granulomatous, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), multifocal, mild
- 2. Heart: myocarditis, multifocal, granulomatous, mild
- 3a. Spleen: splenitis, multifocal, granulomatous, mild
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4a. Trunk kidney: nephritis, interstitial, multifocal, granulomatous, mild
- 4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate
- 5. Head kidney: not included on the slide

**COMMENT:**

See comments on slides 1-3.

Slide 5: autolysis, none (all organs)

- 1. Liver: hepatocellular fatty change (lipidosis), diffuse, severe
- 2. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: no significant lesions
- 4a. Trunk kidney: nephritis, interstitial, multifocal, granulomatous, mild
- 4b. Trunk kidney: peritubular fibrosis, with tubular epithelial basophilia, multifocal, moderate
- 5. Head kidney: no significant lesions

**COMMENT:**

Peritubular fibrosis with tubular epithelial basophilia is consistent with post-necrotic fibrosis and tubular regeneration. Causes of renal tubular necrosis in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamycin).

Slide 6: autolysis, none (all organs)

- 1a. Liver: hepatocellular fatty change (lipidosis), multifocal, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 2. Heart: mural thrombosis, multifocal, mild



- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

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

Slide 7: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), multifocal, moderate
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: not included on the slide
- 5. Head kidney: nephritis, interstitial, multifocal, granulomatous, mild

**COMMENT:**

See comments for similar lesions on other slides.

**Molecular Diagnostics/PCR**

Sample 1-4, 5-7, IHN, VHS, IPN, ISA, Piscirickettsia salmonis Negative by PCR.

\*Molecular Diagnostics results faxed on Feb. 16/05.

/sr





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

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**Case Report**

**Submission** 2005-00300      **Date** 01-Feb-2005      **Report** 17-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13380      A 3.2 - 12 (1-4)

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 3.2 - 12 (1-4).

Addendum: Mar 14/05 - submitted formalized tissue for histology.

**Histopathology**

Quality control/quality assurance: the sections contain moderate precipitates of acid hematin. Tissues might not have been fixed in neutral buffered formalin. Tissues received at the Animal Health Centre were processed directly to alcohol with no formalin intermediate. The margins of many organs have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cells types stains poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, none (other organs) to mild (liver, trunk kidney)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: peritonitis, lymphohistiocytic, multifocal, mild, with occasional fine fibrocellular fronds
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: 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 juvenile 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. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated (Mutoloki et al. 2004).

S. Mutoloki, S. Alexandersen, and Ø. Evensen. 2004. Sequential study of antigen persistence and concomitant inflammatory reactions relative to side-effects and growth of Atlantic salmon (*Salmo salar* L.) following intraperitoneal injection with oil-adjuvanted vaccines. *Fish & Shellfish Immunology* 16(5):633-644.

Slide 2: autolysis, none (other organs) to mild (liver)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: peritonitis, lymphohistiocytic, multifocal, moderate, with occasional fine fibrocellular fronds
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: see comments for fish #1.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1c. Liver: pericholangitis, lymphocytic, multifocal, mild
- 2. Heart: not included on the slide
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

Comment: 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.

Slide 4: autolysis, mild (other organs) to moderate (liver)

- 1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 2. Heart: no significant lesions
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions



5. Head kidney: no significant lesions

Comment: Hepatocyte cytoplasm tends to be partly hypereosinophilic. Hypereosinophilia is a feature of hepatocellular necrosis; however, the change is not discrete, and interpretation of its significance is difficult because of moderate autolysis.

**Molecular Diagnostics/PCR**

Samples 1-2, 3-4: IHN, VHS, IPN, ISA, Piscirickettsia salmonis negative by PCR.

\* Results faxed Feb. 16/05.

/bb

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1767 Angus Campbell Road

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

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

**Case Report**

**Submission** 2005-00301      **Date** 01-Feb-2005      **Report** 17-Feb-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13381      A 3.3 - 15 (1-8)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**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

**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.3 - 15 (1-8).

Addendum: February 3/05 - samples submitted for bacteriology; identification of isolates and culture and sensitivity. Samples 1, 6, and 7.

Addendum: Feb 9/05 - additional samples submitted for bacteriology; identification of isolates and culture and sensitivity. Samples 1 and 7.

Addendum: Mar 14/05 - submitted samples for histology.

**Bacteriology**

Bact. plates - 1, 6, 7: Photobacterium phosphoreum isolated from all samples.

\* Results faxed Feb. 15th and Mar. 7/05.





**Histopathology**

ADDENDUM (faxed - Mar. 18/05):

Quality control/quality assurance: the sections contain moderate precipitates of acid hematin. Tissues might not have been fixed in neutral buffered formalin. Tissues received at the Animal Health Centre were processed directly to alcohol with no formalin intermediate. The margins of many organs have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cells types stains poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, none (other organs) to mild (liver)

1a. Liver: hepatocellular degeneration and necrosis, acute, multifocal, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2a. Heart: myocardial karyomegaly, multifocal, mild

2b. Heart: endocarditis, multifocal, lymphoplasmacytic, mild

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections. 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.

Lymphoplasmacytic inflammation in the heart is evidence of chronic immune stimulation (e.g., low grade bacterial infection), but the cause is otherwise unknown. The cause and significance of myocardial karyomegaly 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).

Slide 2: autolysis, none (all organs)

1a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: epicarditis, regionally diffuse, lymphocytic, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response. Epicarditis is evidence of chronic immune stimulation, but the cause is otherwise unknown. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 3: autolysis, none (other organs) to mild (liver)

1a. Liver: parenchymal golden pigment, disseminated, intracellular, mild

1b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate

2. Heart: no significant lesions

3a. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

3b. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: The golden pigment in the spleen and liver most likely is lipofuscin. Accumulation of lipofuscin 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 have been associated with decreased growth and survival in several studies.

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: pericholangitis, lymphohistiocytic, multifocal, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 2. Heart: epicarditis, focal, lymphohistiocytic, mild
- 3. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: 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. Epicarditis is evidence of chronic immune stimulation, but the cause is otherwise unknown.

Slide 5: autolysis, none (all organs)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: endocarditis, multifocal, lymphohistiocytic, mild
- 3. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: none

Slide 6: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular single cell necrosis (apoptosis), disseminated, acute, moderate
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: mural thrombus, focal (70 x 50 µm), mild
- 3. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, moderate
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: Hepatocellular single cell necrosis (apoptosis) can occur 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). Exposure to toxins (endogenous or exogenous) might also increase the rate of apoptosis.

Slide 7: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 2. Heart: no significant lesions
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., *Piscirickettsia salmonis*). Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. One margin of the trunk kidney, about 1 mm in diameter, has severe autolysis.

Slide 8: autolysis, none (other organs) to mild (liver)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild



2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, mild
4. Trunk kidney: renal tubular dilation, regionally diffuse, with interstitial cell atrophy, moderate
5. Head kidney: no significant lesions

Comment: Renal tubular dilation is an uncommon lesion in Atlantic salmon. The change probably is a result of altered flow of filtrate/urine through the tubules. Because the lesion affects one section of kidney and not the other, the significance to the health of the fish is probably minimal.

**Molecular Diagnostics/PCR**

Samples 1-4, 5-8: IHN, VHS, IPN, ISA, Piscirickettsia salmonis negative by PCR.

\* Results faxed Feb. 16/05.

/bb

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

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**Case Report**

**Submission** 2005-03017      **Date** 24-Aug-2005      **Report** 26-Aug-2005

**Report**      **Copies**

**Submitter:** 11036 Mainstream Canada (M)  
**Owner** 14920 Mainstream 05-41 PO17912  
**Farm:**  
**Vet Clinic:**  
**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

**History/Symptoms**

High mortality with high rate of silvers. No visual signs of disease internal or external.

Specimens: 12 histo cassettes labelled 05-41-1 - 05-41-12. Contains: gill, heart, liver, pylorus, gut, spleen, kid, flesh. Please prepare for Dr. Gary Marty.

Please fax/email results to Dr. Peter McKenzie.

**Histopathology**

Twelve cassettes of formalin-fixed tissues were received in a jar of 35% ethanol. Tissues were processed routinely into paraffin with minimal re-exposure to formalin, sectioned, and stained with H&E. Cassettes labelled 05-41-1 through 05-41-12 yielded slides 1 through 12, respectively. Most slides contained gill, heart, skeletal muscle, spleen, stomach, intestinal ceca and mesenteric fat. All organs on each slide were examined. Organs not listed below had no significant lesions.

Quality Control: Autolysis was scored for liver on each slide as mild (slides 2, 6, 9, 10, 11), moderate (slides 1, 3, 4, 5, 8), or severe (slides 7, 12). Autolysis was scored for gill on each slide as mild (slides 6, 9), moderate (slides 1, 2, 5, 7, 8, 10), or severe (slides 3, 4, 11, 12). The margins of a few organs have evidence of dehydration after fixation (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. The cytoplasm of most erythrocytes has lost its normal orange staining; this occurs when tissues are transferred from formalin to ethanol, back to formalin, and then to ethanol again. I do not see the problem of lost erythrocyte staining when formalin-fixed tissues are transferred to water or physiologic saline before processing. The sections have no acid-hematin.

Measures of physiologic condition

Hepatocellular glycogen depletion, severe (all slides)

Mesenteric adipose tissue depletion, none (slides 4, 5, 6, 7, 8, 9, 11, 12), mild (slides 1, 2, 3, 10)

This pattern in the measures of physiologic condition is consistent with healthy growing fish (abundant mesenteric fat) that recently stopped feeding (severe glycogen depletion). Subtle differences in mesenteric fat among the fish might be a result of sectioning differences rather than real differences.

**Diagnosis**

- 1a. Gill: lamellar epithelial cell karyorrhexis, diffuse, mild (slides 2, 5, 6, 7, 8, 9), moderate (slides 1, 3, 4, 10, 11, 12)
- 1b. Gill: lamellar mucous cell hyperplasia, diffuse, mild (slide 6)
- 2a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 11)
- 2b. Liver: hepatitis, perivascular, lymphocytic, focal, mild (slide 8)
- 2c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 1, 5, 10), moderate (slides 2, 9)
- 3. Stomach: peritonitis, chronic, focal, with fibrocellular fronds, moderate (slide 10)
- 4. Mesenteric fat: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1), moderate (slides 2, 7)
- 5a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 3, 5)
- 5b. Spleen: intrahistiocytic protein droplets 15 to 30 µm in diameter, mild (slide 3)





**Final Comments**

Interpretation of these slides is limited by autolysis, particularly in the gills, but many of the changes are consistent with exposure to a toxin.

Karyorrhexis is one of the phases of nuclear degeneration that can occur as part of apoptosis or necrosis. Because karyorrhexis is widespread in these gills, necrosis is more likely than apoptosis. Lamellar epithelial necrosis is most often associated with exposure to toxins. The toxins may come from infectious organisms, especially bacteria, or from the water. In pen-reared salmon, algal toxins are the most likely waterborne toxin. My confidence in the significance of karyorrhexis in these gills is limited by the degree of autolysis, but I don't normally associate karyorrhexis with autolysis. Hyperplasia of the mucous epithelium lining gill lamellae is a non-specific response to irritation. Inciting causes include parasites, bacteria, and waterborne toxins.

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). Lymphocytic inflammation around bile ductules and vessels in the 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. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

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

/bb



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**Case Report**

**Submission** 2005-00302      **Date** 01-Feb-2005      **Report** 17-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13382      A 3.2 - 10 (1-3)

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.2 - 10 (1-3).

Addendum - Mar 14/05: Submitted formalized samples for histology.

### **Histopathology**

Quality control/quality assurance: the sections contain moderate precipitates of acid hematin. Tissues might not have been fixed in neutral buffered formalin. Tissues received at the Animal Health Centre were processed directly to alcohol with no formalin intermediate.

Slide 1: autolysis, mild (other organs) to moderate (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

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

Slide 2 (fish #3): autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild
3. Spleen: no significant lesions
4. Trunk kidney: renal tubular dilation, regionally diffuse, mild
5. Head kidney: nephritis, interstitial, histiocytic, diffuse, with eosinophilic granular cells, moderate

Comment: Renal tubular dilation is an uncommon lesion in Atlantic salmon. The change probably is a result of altered flow of filtrate/urine through the tubules. The significance to the health of the fish is probably minimal. Histiocytic inflammation in the head kidney sometimes forms aggregates up to 500 µm in diameter, but inflammatory cells never form discrete granulomas; this lesion is consistent with chronic immune stimulation, but the specific cause is unknown. Epicarditis also provides evidence of chronic immune stimulation, but the cause is unknown.

Slide 3 (fish #2): autolysis, mild (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: epicarditis, focal, lymphohistiocytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4a. Trunk kidney: interstitial cell hyperplasia, diffuse, moderate
- 4b. Trunk kidney: renal tubular dilation, regionally diffuse, mild
5. Head kidney: interstitial cell hyperplasia, diffuse, moderate

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



**Molecular Diagnostics/PCR**

Samples 1-3: IHN, VHS, IPN, ISA, *Piscirickettsia salmonis* negative by PCR.

\* Results faxed Feb. 16/05.

/bb

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

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**Case Report**

**Submission** 2005-00303      **Date** 01-Feb-2005      **Report** 16-Feb-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13383      A 3.4 - 20 (1-3)

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.4 - 20 (1-3).

ADDENDUM: March 24, 2005 - samples submitted for routine histology processing and analysis.



**Histopathology**

Quality control/quality assurance: the spleen sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. Tissues have mild postfixation dehydration artifact. This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, none (all organs)

- 1a. Liver: hepatitis, multifocal, granulomatous, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 2. Heart: endocarditis, focal, lymphohistiocytic, mild
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: nephritis, interstitial, granulomatous, multifocal, mild

**COMMENT:**

The most common organism associated with granulomatous inflammation in several organs is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation (e.g., low grade bacterial infection); it might be related to *Renibacterium salmoninarum* 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 juvenile 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.

Slide 2: autolysis, none (all organs)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: peritonitis, granulomatous, focal, with occasional fine fibrocellular fronds, mild
- 4. Trunk kidney: renal tubular epithelial necrosis, focal (1 tubule), acute, mild
- 5. Head kidney: not included in the section

Comment: Splenic 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 (as in this case). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamycin).

Slide 3: autolysis, mild (heart) to moderate (other organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 2. Heart: endocarditis, focal, lymphoplasmacytic, mild
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

**COMMENT:**

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).



Lymphoplasmacytic inflammation in the heart is evidence of chronic immune stimulation (e.g., low grade bacterial infection), but the cause is otherwise unknown. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 4: autolysis, none (all organs)

1a. Liver: hepatic necrosis, acute, multifocal, moderate

1b. Liver: biliary preductular cell hyperplasia, diffuse, moderate

1c. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: endocarditis, multifocal, with endothelial cell hypertrophy and a thin layer of macrophages, mild

3. Spleen: leukocytic karyorrhexis, disseminated, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicaemia virus, *Piscirickettsia salmonis*; note that PCR results rule out active infections with these common causes of hepatic necrosis). Proliferative biliary preductular cells are evidence of a chronic toxic cause for the hepatic necrosis.

The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface of foci in the ventricle are rarely more than 2 cell layers thick.

The presence of degenerating nuclei (karyorrhexis) in the spleen is evidence of increased cell turnover, possibly as part of an active inflammatory response.

#### **Molecular Diagnostics/PCR**

Samples 1-3 IHN, VHS, IPN, ISA, *Piscirickettsia salmonis* Negative by PCR.

\*Molecular Diagnostics results faxed on Feb. 16/05.

/sr



**ANIMAL HEALTH CENTRE**

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Ministry of  
Agriculture, Food and Fisheries  
1767 Angus Campbell Road

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

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

**Case Report**

**Submission** 2005-00304      **Date** 01-Feb-2005      **Report** 16-Feb-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13384      A 3. 4 - 21 (1)

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.4 - 21 (1).

ADDENDUM: March 24, 2005. Samples submitted for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the spleen sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. Tissues have mild postfixation dehydration artifact. This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, none (all organs)

- 1a. Liver: pericholangitis, lymphocytic, multifocal, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1d. Liver: hepatic necrosis, acute, multifocal, mild
- 2. Heart: epicarditis, regionally diffuse, lymphoplasmacytic, mild
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

**COMMENT:**

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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response. 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. 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 septicaemia virus, Piscirickettsia salmonis). Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Epicarditis is evidence of chronic immune stimulation, but the cause is otherwise unknown.

**Molecular Diagnostics/PCR**

Sample 1: IHN, VHS, IPN, ISA, Piscirickettsia salmonis Negative by PCR.

\*Molecular Diagnostics results faxed on Feb. 16/05.

/sr



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

**Case Report**

**Submission** 2005-00305      **Date** 01-Feb-2005      **Report** 18-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13385      A 3.3 - 19 (1-6)

**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



**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 3.3 - 19 (1-6).

Addendum: Mar 14/05 - submitted formalized tissue for histology.

**Histopathology**

Quality control/quality assurance: the sections contain moderate precipitates of acid hematin. Tissues might not have been fixed in neutral buffered formalin. Tissues received at the Animal Health Centre were processed directly to alcohol with no formalin intermediate.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: no significant lesions
3. Spleen (very small piece): peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, mild (other organs) to moderate (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, moderate
4. Trunk kidney: interstitial fibrosis, multifocal, with tubular dysplasia and multiple foci of intralesional golden pigment, moderate
5. Head kidney: no significant lesions

Comment: Foci of renal interstitial fibrosis with golden pigment (probably lipofuscin) are each about 500 µm in diameter. They are indicative of chronic membrane damage, but the specific cause is unknown. 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).

Slide 3: autolysis, mild (other organs) to moderate (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: none

Slide 4: autolysis, mild (other organs) to severe (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate
5. Head kidney: not included on the section

Comment: 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.

Slide 5: autolysis, none (other organs) to mild (liver)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, severe
4. Trunk kidney: no significant lesions



5. Head kidney: not included on the section

Comment: none

Slide 6: autolysis, none (other organs) to mild (liver)

1a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate

1b. Liver: biliary preductular cell hyperplasia, multifocal, mild

2. Heart: no significant lesions

3. Spleen (and liver): peritonitis, chronic, focal, with fibrocellular fronds, moderate

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: none

**Molecular Diagnostics/PCR**

Sample 1-3, 4-6: IHN, VHS, IPN, ISA, Piscirickettsia salmonis negative by PCR.

\* Results faxed Feb. 16/05.

/bb



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1767 Angus Campbell Road

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

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

**Case Report**

**Submission** 2005-03063      **Date** 26-Aug-2005      **Report** 02-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14936      A.2.3-47 (1-5)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Formalized

**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

**History/Symptoms**

Submitted 5 cassettes for routine histology processing and analysis.

Provincial Surveillance Program.

Sample ID - A.2.3-47 (1-5).

**Histopathology**

Quality control/quality assurance: this case is part of the first of a group of monitoring cases that were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Sections have no postfixation dehydration. Large vessels in the heart of slide #2, spleen of slide #4, and liver of slide #5 have deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, mild (all organs)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

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

Slide 2: autolysis, none (other organs) to mild (liver and intestinal cecum)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: no significant lesions
3. Spleen and intestinal cecum: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: not included on the slide

Comment: 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. 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.

Slide 3: autolysis, moderate (other organs) to severe (liver and intestinal ceca)

1. Liver: pericholangitis, lymphocytic, multifocal, mild
2. Heart: epicarditis, multifocal, lymphoplasmacytic, mild
3. Spleen and intestinal ceca: peritonitis, granulomatous, regionally diffuse, with intralesional foreign material and vacuoles up to 100 µm in diameter, severe
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: 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. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 4: autolysis, mild (other organs) to severe (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
- 3a. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate
- 3b. Spleen: foreign body granuloma, focal, ~500 µm in diameter, mild
4. Trunk kidney: no significant lesions





5. Head kidney: not included on the slide

Comment: The vacuole in the centre of the foreign body granuloma of the spleen is surrounded by a rim of mixed inflammatory cells and fibroblasts that is about 60 µm thick.

Slide 5: autolysis, none (other organs) to mild (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: no significant lesions

3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

Comment: 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).



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

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

**Case Report**

**Submission** 2005-03064      **Date** 26-Aug-2005      **Report** 08-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14937      A.2.4-53 (1-7)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Multiple Specimens

**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**

Provincial Surveillance Program. Submitted 7 fresh tissues for Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis. Culture any PCR positive samples.

Submitted 7 cassettes for routine histology processing and analysis.

Sample ID - A.2.4-53 (1-7).

**Histopathology**

Quality control/quality assurance: this case is part of the first of a group of monitoring cases that were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Sections have no postfixation dehydration. Slide #s1, 3, 4 have deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, mild (other organs) to none (heart)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: parenchymal golden pigment, disseminated, intracellular, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney and head kidney: moderate numbers of eosinophilic granular cells in interstitial tissue
5. Head kidney: no significant lesions

Comment: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. 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). The golden pigment in the liver and 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. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies. Increased numbers of eosinophilic granular cells in the kidney is a distinctive finding in Atlantic salmon. 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*. Increased numbers of eosinophilic granular cells are sometimes associated with chronic parasitic infections, but again, the inciting cause was not included in the sections examined.

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.

Slide 2: autolysis, mild (other organs) to moderate (liver)

- 1a. Liver: parenchymal golden pigment, disseminated, intracellular, mild
- 1b. Liver: hepatic necrosis, acute, multifocal, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: 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*). Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis.

Slide 3: autolysis, mild (other organs) to moderate (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: sinusoidal congestion, acute, multifocal, moderate
- 2a. Heart: endocarditis, multifocal, lymphoplasmacytic, mild
- 2b. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney and head kidney: nephritis, interstitial, granulomatous, multifocal, mild
5. Head kidney: no significant lesions



Comment: 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). Sinusiodal congestion is evidence of sinusoidal damage. In BC Atlantic salmon, peliosis is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Sinusiodal congestion is one of the classic lesions associated with ISAV infections. Consider bacteriology and virology and PCR for VHSV, IHNV, and ISAV (if not already done). Sinusiodal 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 Sinusiodal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data).

Lymphoplasmacytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: parenchymal golden pigment, disseminated, intracellular, mild
- 1c. Liver: hepatic necrosis, acute, multifocal, mild

2. Heart: no significant lesions

3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

4a. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate

4b. Trunk kidney and head kidney: small numbers of eosinophilic granular cells in interstitial tissue

Comment: 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.

Slide 5: autolysis, none (other organs) to mild (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: no significant lesions

4. Trunk kidney: renal tubular epithelial necrosis, focal (1 tubule), acute, mild

5. Head kidney: not included on the slide

Comment: 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). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin).

Slide 6: autolysis, none (all organs)

1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

1b. Liver: parenchymal golden pigment, disseminated, intracellular, mild

1c. Liver: hepatitis, pericholangial, perivascular, lymphoplasmacytic, multifocal, mild

1d. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions





5. Head kidney: not included on the slide

Comment: Lymphocytic inflammation around vessels and bile ductules in the 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.

Slide 7: autolysis, none (other organs) to mild (liver and trunk kidney)

1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2a. Heart: myocardial karyomegaly, multifocal, mild

2b. Heart: mural thrombosis, focal (700 x 500 µm), moderate

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

Comment: Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial

### **Molecular Diagnostics/PCR**

Samples 1-4, 5-7 IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* negative by PCR.

\* Results faxed on Sept 28/05.

/sr/mm



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Agriculture, Food and Fisheries  
1767 Angus Campbell Road

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

Facsimile: (604) 556-3010  
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**Case Report**

**Submission** 2005-03067      **Date** 26-Aug-2005      **Report** 08-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14938      A.3.2-55 (1-11)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Formalized

**Count** 11

**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 11 cassettes for routine histology processing and analysis.

Provincial Surveillance Program.

Sample ID - A.3.2-55 (1-11).

**Histopathology**

Quality control/quality assurance: this case is part of the first of a group of monitoring cases that were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Sections have no postfixation dehydration. Hepatocytes around large vessels in slide #11 have deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: parenchymal golden pigment, disseminated, intracellular, mild
- 1c. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1d. Liver: sinusoidal congestion with intracytoplasmic amphophilic inclusions, acute, multifocal, mild
- 2. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. The golden pigment in the liver 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. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies. 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). Sinusoidal congestion 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*. The amphophilic cytoplasmic inclusions in hepatocytes are large, varying from the same size of nuclei to 50% larger than hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes or viral inclusions; however, no known salmon virus produces these types of inclusions.

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

Slide 2: autolysis, mild (other organs) to moderate (trunk kidney and liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: parenchymal golden pigment, disseminated, intracellular, mild
- 1c. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1d. Liver: sinusoidal congestion with intracytoplasmic amphophilic inclusions, acute, multifocal, mild
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart: epicarditis, multifocal, lymphoplasmacytic, mild
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

Comment: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 3: autolysis, none (all organs)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: parenchymal golden pigment, disseminated, intracellular, mild
- 1c. Liver: biliary preductular cell hyperplasia, diffuse, mild



2. Heart: myocardial karyomegaly, multifocal, mild
  3. Spleen: no significant lesions
  4. Trunk kidney: no significant lesions
  5. Head kidney: not included on the slide
- Comment: see other slides.

Slide 4: autolysis, none (other organs) to mild (trunk kidney, liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
  - 1b. Liver: parenchymal golden pigment, disseminated, intracellular, mild
  - 1c. Liver: biliary preductular cell hyperplasia, diffuse, moderate
  2. Heart: not included on the slide
  3. Spleen: no significant lesions
  4. Trunk kidney: no significant lesions
  5. Head kidney: no significant lesions
- Comment: see other slides.

Slide 5: autolysis, none (heart) to mild (other organs)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: parenchymal golden pigment, disseminated, intracellular, moderate
- 1c. Liver: biliary preductular cell hyperplasia, diffuse, moderate
- 1d. Liver: sinusoidal congestion with intracytoplasmic amphophilic inclusions, acute, focal, mild
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart, ventricle: endocardial hyperplasia, multifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney and head kidney: small numbers of eosinophilic granular cells in interstitial tissue, diffuse

Comment: Endothelial cell hyperplasia is an unusual lesion in the heart, probably in response to local physical or chemical damage (e.g., bacterial toxins). Increased numbers of eosinophilic granular cells in the kidney is a distinctive finding in Atlantic salmon. 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*. Increased numbers of eosinophilic granular cells are sometimes associated with chronic parasitic infections, but again, the inciting cause was not included in the sections examined.

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.

Slide 6: autolysis, none (heart) to mild (other organs)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
  - 1b. Liver: parenchymal golden pigment, disseminated, intracellular, mild
  - 1c. Liver: biliary preductular cell hyperplasia, diffuse, moderate
  - 1d. Liver: sinusoidal congestion with intracytoplasmic amphophilic inclusions, acute, focal, mild
  2. Heart: myocardial karyomegaly, multifocal, mild
  3. Spleen: not included on the slide
  4. Trunk kidney: dilation of renal tubules and urinary spaces, diffuse, mild
  5. Head kidney: not included on the slide
- Comment: Dilation of renal components is evidence of impaired renal function, but the cause is unknown.

Slide 7: autolysis, none (heart) to mild (other organs)

- 1a. Liver: parenchymal golden pigment, disseminated, intracellular, moderate
- 1b. Liver: hepatitis, pericholangial, perivascular, lymphoplasmacytic, multifocal, mild





- 1c. Liver: biliary preductular cell hyperplasia, diffuse, moderate
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart: endocarditis, focal (100 µm in diameter), lymphoplasmacytic, mild
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

Comment: Lymphocytic inflammation around vessels and bile ductules in the 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. Lymphoplasmacytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 8: autolysis, mild (other organs) to moderate (trunk kidney and liver)

- 1a. Liver: parenchymal golden pigment, disseminated, intracellular, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, severe
- 2. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

Comment: This is the only case I have ever seen in which I scored biliary preductular cell hyperplasia as severe.

Slide 9: autolysis, none (other organs) to mild (liver)

- 1a. Liver: pericholangitis, lymphocytic, multifocal, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, moderate
- 1c. Liver: parenchymal golden pigment, disseminated, intracellular, mild
- 1d. Liver: sinusoidal congestion with intracytoplasmic amphophilic inclusions, acute, focal, mild
- 1e. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 2. Heart: no significant lesions
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

Comment: 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.

Slide 10: autolysis, mild (other organs) to moderate (liver)

- 1b. Liver: biliary preductular cell hyperplasia, diffuse, moderate
- 1c. Liver: parenchymal golden pigment, disseminated, intracellular, mild
- 1d. Liver: sinusoidal congestion with intracytoplasmic amphophilic inclusions, acute, bifocal, mild
- 1e. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 2a. Heart: myocardial karyomegaly, multifocal, moderate
- 2b. Heart: endocarditis, multifocal, lymphoplasmacytic, mild
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: see other slides.

Slide 11: autolysis, none (other organs) to mild (trunk kidney and liver)

- 1a. Liver: pericholangitis, lymphocytic, multifocal, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, moderate
- 1c. Liver: sinusoidal congestion with intracytoplasmic amphophilic inclusions, acute, multifocal, mild



2. Heart: myocardial karyomegaly, multifocal, moderate
  3. Spleen: no significant lesions
  4. Trunk kidney:
  5. Head kidney: no significant lesions
- Comment: see other slides.

/sr

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**Case Report**

**Submission** 2005-03068      **Date** 26-Aug-2005      **Report** 08-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14939      A.3.2-56 (1-3)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Formalized

**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 three cassettes for routine histology processing and analysis.

Provincial Surveillance Program.

Sample ID - A.3.2-56 (1-3).

**Histopathology**

Quality control/quality assurance: this case is part of the first of a group of monitoring cases that were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Sections have no postfixation dehydration. Large foci of erythrocytes in slide #1 have deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

1a. Liver: pericholangitis, lymphocytic, multifocal, mild

1b. Liver: hepatic necrosis, acute, multifocal, mild

1c. Liver: hepatitis, granulomatous, lymphoplasmacytic, focal, mild

1d. Liver: yellow-brown pigmented macrophage aggregate, multifocal

2a. Heart: endocarditis, multifocal, with endothelial cell hypertrophy and a thin layer of macrophages, moderate

2b. Heart: epicarditis, multifocal, lymphohistiocytic, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, mild

4b. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: no significant lesions

Comment: 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, *Renibacterium salmoninarum*). Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Granulomatous inflammation in the liver is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* is the most common organism associated with these lesions. 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.

The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface are rarely more than 2 cell layers thick. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation. 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.

Slide 2: autolysis, none (heart) to mild (other organs)

1a. Liver: pericholangitis, lymphocytic, eosinophilic, multifocal, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions





5. Head kidney: no significant lesions

Comment: Pericholangitis is common in farmed Atlantic salmon, but eosinophilic granular cells are an unusual component of this inflammation. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. 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).

Slide 3: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2a. Heart: myocardial karyomegaly, multifocal, mild

2b. Heart: epicarditis, multifocal, lymphoplasmacytic, mild

2c. Heart, coronary vessels at junction of spongy and compact layers of ventricle: endothelial cell hypertrophy and hyperplasia, multifocal, moderate

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Hypertrophy and hyperplasia of coronary endothelial cells in the heart is consistent with a systemic immune stimulation, probably a bacterial infection; alternatively, features of the coronary vessels might be a result of a developmental abnormality.

/sr



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**Case Report**

**Submission** 2005-03069      **Date** 26-Aug-2005      **Report** 08-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14940      A.3.2-59 (1-6)

**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

**History/Symptoms**

Submitted 7 cassettes for routine histology processing and analysis.

Provincial Surveillance Program.

Sample ID - A.3.2-59 (1-7).

Please refer to virology case 2005-3098.

**Histopathology**

Quality control/quality assurance: this case is part of the first of a group of monitoring cases that were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Sections have no postfixation dehydration. Large foci of erythrocytes in most slides have deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: no significant lesions
3. Spleen: peritonitis, granulomatous, diffuse, with occasional fine fibrocellular fronds, severe
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

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

Slide 2: autolysis, none (all organs)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, moderate
4. Trunk kidney: dilation of renal tubules and urinary spaces, diffuse, mild
5. Head kidney: not included on the slide

Comment: 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. Dilation of renal components is evidence of impaired renal function, but the cause is unknown.

Slide 3: autolysis, mild (other organs) to severe (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: see other slides

Slide 4: autolysis, mild (other organs) to moderate (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1c. Liver: sinusoidal congestion, acute, multifocal, moderate
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: Sinusoidal congestion 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 infections. 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).

Slide 5: autolysis, none (other organs) to mild (liver)

1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

1b. Liver: sinusoidal congestion, acute, multifocal, moderate

2. Heart:

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

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

Slide 6: autolysis, mild (other organs) to moderate (liver)

1. Liver: sinusoidal congestion, acute, multifocal, moderate

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: peritonitis, granulomatous, diffuse, with occasional fine fibrocellular fronds, moderate

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

Comment: 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).

Slide 7: autolysis, none (other organs) to mild (liver)

1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

1b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate

1c. Liver: hepatitis, granulomatous, multifocal, mild

1d. Liver: hepatitis, pericholangial, perivascular, lymphoplasmacytic, multifocal, mild

2a. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages, moderate

2b. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild

3. Spleen: peritonitis, granulomatous, diffuse, with occasional fine fibrocellular fronds, moderate

4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, moderate

5. Head kidney: nephritis, interstitial, granulomatous, multifocal, moderate

Comment: The most common organism associated with granulomatous nephritis and hepatitis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation. Lymphocytic inflammation around vessels and bile ductules in the 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.

The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface throughout most of the ventricle are rarely more than 2 cell layers thick. In this case, the reaction might be related to infection with *Renibacterium salmoninarum*. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

/sr







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**Case Report**

**Submission** 2005-03070      **Date** 26-Aug-2005      **Report** 08-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14941      A.3.4-67 (1-6)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Formalized

**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 6 cassettes for routine histology processing and analysis.

Provincial Surveillance Program.

Sample ID - A.3.4-67 (1-6).

**Histopathology**

Quality control/quality assurance: this case is part of the first of a group of monitoring cases that were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Sections have no postfixation dehydration. Large foci of erythrocytes in slide #2 have deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

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

Slide 2: autolysis, none (all organs)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart: endocarditis, focal, lymphohistiocytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. 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). Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: pericholangitis, lymphohistiocytic, multifocal, mild
2. Heart: epicarditis, multifocal, lymphoplasmacytic, mild
3. Spleen: no significant lesions
4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, mild
5. Head kidney: nephritis, interstitial, granulomatous, multifocal, mild

Comment: Lymphohistiocytic 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. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: hepatitis, pericholangial, perivascular, lymphohistiocytic, multifocal, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions



5. Head kidney: nephritis, interstitial, granulomatous, multifocal, mild

Comment: Lymphohistiocytic inflammation in the 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. It might also be related to infection with *Renibacterium salmoninarum*.

Slide 5: autolysis, none (other organs) to mild (liver)

1a. Liver: hepatic necrosis, acute, multifocal, moderate

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, severe

2. Heart: no significant lesions

3. Spleen: no significant lesions

4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, mild

5. Head kidney: nephritis, interstitial, granulomatous, multifocal, mild

Comment: 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.

Slide 6: autolysis, none (other organs) to mild (liver)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate

2. Heart: no significant lesions

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: 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.

/sr



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**Case Report**

**Submission** 2005-03071      **Date** 26-Aug-2005      **Report** 08-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14942      A.3.4-69 (1)

**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



**History/Symptoms**

Submitted 1 cassette for routine histology processing and analysis.

Provincial Surveillance Program.

Sample ID - A.3.4-69 (1).

**Histopathology**

Quality control/quality assurance: this case is part of the first of a group of monitoring cases that were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Sections have no postfixation dehydration and no acid hematin deposits.

Slide 1: autolysis, none (heart) to mild (other organs)

1a. Liver: hepatic necrosis and sinusoidal congestion, acute, multifocal, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: endothelial cell hypertrophy and hyperplasia, focal, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: 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. Hypertrophy and hyperplasia of endothelial cells in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

/sr



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**Case Report**

**Submission** 2005-03077      **Date** 26-Aug-2005      **Report** 30-Aug-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 14947 #5376, Marine Harvest

**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

**History/Symptoms**

Submitted formalized fish tissue for histopathology.

Log #5376.

Unusual heart lesions - tumour? Sample fixed in buffered formalin then transported in tap water as per Dr. G. Marty instruction.

Species - Atlantic. Sex - regular. Saltwater entry - 2005.

Please invoice Stolt Sea Farm. Please quote PO # 59458 for invoice purposes.

**Histopathology**

Formalin-fixed tissues were received in water and processed into 3 slides.

Slide 1 - trunk kidney, bulbus arteriosus, liver

Slide 2 - spleen, intestine, and fatty mesenteries

Slide 3 - heart

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

Quality Control: Tissue preservation is good for all organs. The sections have no dehydration after fixation, no acid-hematin, and no adverse affects of shipment in water.

Measures of physiologic condition

Hepatocellular glycogen depletion: severe (slides 1).

Mesenteric adipose tissue depletion: none (slide 2)

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

**Diagnosis**

1. Heart, atrium: mural thrombosis, acute, focal, moderate (~3 mm in diameter, slide 3)

2a. Liver: hepatic necrosis, acute, multifocal, mild (slide 1)

2b. Liver: biliary preductular cell hyperplasia, diffuse, moderate (slide 1)

2c. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slide 1)

2d. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 1)

3. Spleen, intestine, and surrounding fatty mesenteries: peritonitis, granulomatous, multifocal, coalescing, with occasional fine fibrocellular fronds and intralesional vacuoles and foreign material (foci 40-100 µm in diameter), moderate (slide 2)



**Final Comments**

The unusual gross heart lesion is the result of a large thrombus in the atrium. This thrombus is not associated with significant inflammation: evidence that the thrombus is sterile. Necrosis of cells in the thrombus is evidence that the thrombus was present for a few hours before the fish died. In general, thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection. The pathogenesis of the endocardial thrombus probably is the same as the liver lesions.

Hepatic necrosis can be caused by inadequate vascular perfusion (e.g., the atrial thrombus) or direct cytotoxicity from viral or bacterial infections. 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). Proliferative lesions in the biliary system are evidence for a chronic toxic cause for the hepatic necrosis; the toxin could be from the water or from bacteria in the fish. 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.

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

/bb



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**Case Report**

**Submission** 2005-03078      **Date** 26-Aug-2005      **Report** 08-Sep-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 14948 #5379, 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



**History/Symptoms**

Submitted 10 (pooled) samples for virology - PCR for IHNV and VHSV.

Log No. 5379.

Species - Atlantic. Sex - regular. Saltwater entry - 2006. Average weight - 700g. Mortality - 0.05%/week.

Please invoice Stolt Sea Farm. Please quote PO # 59450 for invoice purposes.

**Molecular Diagnostics/PCR**

Samples 1-2, 3-4, 5-7, 9-10, 11-14, 15-16, 17-19, 20-22, 24-25, 26-28 Tested.

Results: Sample 1

Infectious Hematopoietic Necrosis virus Negative

Viral Hemorrhagic Septicemia virus Negative

/sr



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**Case Report**

**Submission** 2005-03098      **Date** 30-Aug-2005      **Report** 22-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14957      A 3.2 - 59

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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 as part of the Provincial Surveillance Program for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Histo. submission - 2005-3069.

Sample ID: A 3.2 - 59.

**Molecular Diagnostics/PCR**

Samples 1-4, 5-7: IHN, VHS, ISA, IPN, Piscirickettsia salmonis negative by PCR.

\* Results faxed Sept. 28/05.

/bb

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## Case Report

**Submission** 2005-03099      **Date** 30-Aug-2005      **Report** 22-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14958      A 3.2 - 55 (1-11)

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**Specimen:** Tissue - Fresh

**Count** 11

**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 as part of the Provincial Surveillance Program for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Histo. submission - 2005-3067.

Sample ID: A 3.2 - 55 (1-11).

**Molecular Diagnostics/PCR**

Samples 1-4, 5-8, 9-11: IHN, VHS, ISA, IPN, Piscirickettsia salmonis negative by PCR.

\* Results faxed Sept. 28/05.

/bb

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**Case Report**

**Submission** 2005-03100      **Date** 30-Aug-2005      **Report** 22-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14959      A 2.3 - 47

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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

**History/Symptoms**

Submitted fresh tissue samples as part of the Provincial Surveillance Program for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Histo. submission - 2005-3063.

Sample ID: A 2.3 - 47.

**Molecular Diagnostics/PCR**

Samples 1-3, 4-5: IHN, VHS, ISA, IPN, Piscirickettsia salmonis negative by PCR.

\* Results faxed Sept. 28/05.

/bb



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**Case Report**

**Submission** 2005-03101      **Date** 30-Aug-2005      **Report** 15-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14960      A 2.4 - 51

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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

**History/Symptoms**

Submitted fresh tissue samples as part of the Provincial Surveillance Program for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 2.4 - 51.

ADDENDUM: Sept 8, 2005 - samples received for histology.

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. The margins of some organs (e.g., spleen in slide 4) have evidence of dehydration after fixation (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. Large foci of erythrocytes have deposits of acid hematin (e.g., spleen in slide 5). Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
- 3a. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
- 3b. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

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

Small amounts of splenic lipofuscin are fairly common in pen-reared Chinook salmon, but less common in Atlantic salmon. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies. 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.

Slide 2: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: endocarditis, multifocal, lymphoplasmacytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate
5. Head kidney: no significant lesions

Comment: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Lymphoplasmacytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 3: autolysis, none (all organs)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: endocarditis, multifocal, lymphoplasmacytic, mild
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: see other slides

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, severe
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: no significant lesions



3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: 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.

Slide 5: autolysis, none (heart) to mild (liver and spleen)

1. Liver: no significant lesions
2. Heart: epicarditis and endocarditis, multifocal, lymphoplasmacytic, moderate
3. Spleen and surrounding mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: not included on the slide
5. Head kidney: not included on the slide

Comment: see other slides.

#### **Molecular Diagnostics/PCR**

Samples 1-3, 4-5 IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* negative by PCR.

\* Results faxed on Sept 28/05.

/sr/mm



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**Case Report**

**Submission** 2005-03102      **Date** 30-Aug-2005      **Report** 15-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14961      A 2.3 - 50

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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

**History/Symptoms**

Submitted fresh tissue samples as part of the Provincial Surveillance Program for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 2.3 - 50.

ADDENDUM: Sept 8, 2005 - samples received for histology.



**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. The margins of some organs have evidence of dehydration after fixation (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. Large foci of erythrocytes have deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: parenchymal yellow-green pigment, disseminated, intracellular, mild
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart: epicarditis, multifocal, lymphoplasmacytic, mild
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: The yellow-green pigment in the liver and 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.

Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies. 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.

Slide 2: autolysis, none (other organs) to mild (liver)

- 1a. Liver: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

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

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: sinusoidal congestion, acute, multifocal, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2a. Heart: mural thrombosis, focal, mild
- 2b. Heart: endocarditis, multifocal, lymphoplasmacytic, mild
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney: not included on the slide
5. Head kidney: no significant lesions

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

Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection. Lymphoplasmacytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 4: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
  2. Heart: no significant lesions
  3. Spleen: no significant lesions
  4. Trunk kidney: no significant lesions
  5. Head kidney: not included on the slide
- Comment: see slide #2 comments.

Slide 5: autolysis, none (other organs), mild (liver), or moderate (intestinal cecum)

1. Liver: hepatic necrosis, acute, multifocal, moderate
2. Heart: no significant lesions
3. Spleen and intestinal cecum: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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.

### **Molecular Diagnostics/PCR**

Samples 1-3, 4-5 IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* negative by PCR.

\* Results faxed on Sept 28/05.

sr/mm



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**Case Report**

**Submission** 2005-03103      **Date** 30-Aug-2005      **Report** 22-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14962      A 3.2 - 56

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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 fresh tissue samples as part of the Provincial Surveillance Program for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Histo. submission - 2005-3068.

Sample ID: A 3.2 - 56.

**Molecular Diagnostics/PCR**

Samples 1-3: IHN, VHS, ISA, IPN, Piscirickettsia salmonis negative by PCR.

\* Results faxed Sept. 28/05.

/bb

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**Case Report**

**Submission** 2005-03104      **Date** 30-Aug-2005      **Report** 22-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14963      A 3.4 - 69

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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 samples as part of the Provincial Surveillance Program for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Histo. submission - 2005-3071.

Sample ID: A 3.4 - 69.

**Molecular Diagnostics/PCR**

Sample #1 - IHN, VHS, ISA, IPN, Piscirickettsia salmonis negative by PCR.

\* Results faxed Sept. 28/05.

/bb



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**Case Report**

**Submission** 2005-03105      **Date** 30-Aug-2005      **Report** 22-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 14964      A 3.4 - 67

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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

**History/Symptoms**

Submitted fresh tissue samples as part of the Provincial Surveillance Program for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Histo. submission - 2005-3070.

Sample ID: A 3.4 - 57.

**Molecular Diagnostics/PCR**

Samples 1-3, 4-6: IHN, VHS, ISA, IPN, Piscirickettsia salmonis negative by PCR.

\* Results faxed Sept. 28/05.

/bb

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**Case Report**

**Submission** 2005-03191      **Date** 08-Sep-2005      **Report** 15-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15001      A 2.4 - 52 (1)

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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

**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples. Also submitted samples for histology.

Sample ID: A 2.4 - 52 (1).

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. The margins of some organs (e.g., spleen) have evidence of dehydration after fixation (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. Large foci of erythrocytes in the spleen have deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (all organs)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: interstitial cell hyperplasia, diffuse, mild
5. Head kidney: no significant lesions

Comment: 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."

**Molecular Diagnostics/PCR**

Sample 1, IHN, VHS, IPN, ISA, Piscirickettsia salmonis negative by PCR.

\* Results faxed on Oct 5/05.



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**Case Report**

**Submission** 2005-03193      **Date** 08-Sep-2005      **Report** 15-Sep-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15003      A 3.2 - 57 (1-4)

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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

**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples. Also submitted samples for histology.

Sample ID: A 3.2 - 57 (1-4).

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. The margins of some organs have evidence of dehydration after fixation (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. Organs have no deposits of acid hematin.

Slide 1: autolysis, none (other organs) to mild (liver)

1a. Liver: hepatic necrosis, acute, multifocal, mild

1b. Liver: hematoma, focal, 1.5 mm in diameter, surrounded by a rim of necrotic hepatocytes, acute, moderate

1c. Liver: hepatitis, granulomatous, focal (300 x 150 µm), mild

2. Heart: epicarditis, multifocal, lymphoplasmacytic, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: 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. The hematoma might be a result of trauma. Necrosis at the margin of the hematoma probably resulted from inadequate vascular perfusion. Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Granulomatous inflammation in the liver is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* is the most common organism associated with these lesions. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, none (other organs) to mild (liver)

1a. Liver: parenchymal golden pigment, disseminated, intracellular, mild

1b. Liver: hepatitis, pericholangial, perivascular, lymphoplasmacytic, focal, mild

2. Heart: no significant lesions

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: no significant lesions

Comment: The golden pigment in the liver 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. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies. Lymphocytic inflammation around vessels and bile ductules in the 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.

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.

Slide 3: autolysis, none (other organs) to mild (liver and trunk kidney)

1. Liver: no significant lesions





- 2. Heart: no significant lesions
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate
- 4a. Trunk kidney, archinephric duct: intratubular necrotic cells, focal, acute, with epithelial cell regeneration, moderate
- 4b. Trunk kidney, distal tubules (adjacent to archinephric duct in 4a): intraluminal yellow-green to golden pigment
- 5. Head kidney: no significant lesions

Comment: 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). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin). In this fish, the affected archinephric duct is lined by attenuated epithelium; the lumen of the archinephric duct is about ½ filled with necrotic cells characterized by nuclear pyknosis or karyorrhexis, and cytoplasmic hypereosinophilia. Pigment in the adjacent distal tubules is probably a result of the same process as the necrotic cells in the archinephric duct.

Slide 4: autolysis, mild (other organs) to moderate (liver)

- 1. Liver: no significant lesions
- 2a. Heart: endothelial cell hypertrophy and hyperplasia, multifocal, mild
- 2b. Heart: epicarditis, focal, lymphoplasmacytic, mild
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: Hypertrophy and hyperplasia of endothelial cells in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

### **Molecular Diagnostics/PCR**

Samples 1-2, 3-4, IHN, VHS, IPN, ISA, Piscirickettsia salmonis negative by PCR.

\* Results faxed on Oct 5/05.

sr/mm



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**Case Report**

**Submission** 2005-03285      **Date** 15-Sep-2005      **Report** 23-Sep-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 15033 #5406, 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**

Atlantic salmon (4) fresh tissues and (4) histo submitted for diagnostic work.  
Histo. taken from 4 moribund fish. Mortality up slightly. Fish #2 with white fibrinous membrane over heart(bac-T pending). Four pooled samples for virology and PCR for IHN and VHS.  
Saltwater entry: 2005.  
For billing purposes please quote PO #CL5324. Also histo cassettes submitted

**Histopathology**

Formalin-fixed tissues were received in 4 cassettes and processed routinely to 4 slides.  
Slide 1 (5406-1) - trunk kidney, head kidney, liver, heart, gill, intestine, intestinal ceca, spleen, and mesenteric fat  
Slide 2 (5406-4; plus Twort's Gram stain) - trunk kidney, head kidney, liver, heart, gill, intestine, intestinal ceca, spleen, and mesenteric fat  
Slide 3 (5406-7) - trunk kidney, head kidney, liver, heart, intestine, intestinal ceca, spleen, and mesenteric fat  
Slide 4 (5406-8) - trunk kidney, head kidney, liver, heart, intestine, intestinal ceca, spleen, and mesenteric fat  
All organs on each slide were examined. Organs not listed below had no significant lesions.

Quality Control: Tissue preservation is excellent for all organs except the intestine, which sometimes has mild autolysis. Erythrocytes in slide 3 have evidence of dehydration after fixation (e.g., erythrocyte cytoplasm stains yellow instead of red); this most commonly results when preserved tissues are removed from liquid for more than a few minutes. The sections rarely have acid-hematin deposits (e.g., heart in slide 3).

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

**Virology**

Fish Viruses Negative  
Tissue culture = negative.

\* Results faxed on Oct 11/05.

**Molecular Diagnostics/PCR**

Samples 1-3, 4-6, 7-9, 10-12, IHN, VHS negative by PCR.

\* Results faxed on Sept 28/05.



**Diagnosis**

- 1a. Gill: interlamellar Gram-negative filamentous bacteria, multifocal, mild (slides 1, 2)
- 1b. Gill: lamellar epithelial hyperplasia and fusion, chronic, multifocal, with foci of Gram-negative filamentous bacteria between adjacent gill filaments, moderate (slide 2)
- 1c. Gill filament, tip: necrosis, acute, focal, with abundant Gram-negative filamentous bacteria, severe (slide 2)
- 1d. Gill: lamellar subepithelial edema, multifocal, mild (slide 1)
- 2a. Trunk kidney: renal tubular dilation, multifocal, mild (slide 1)
- 2b. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, mild (slide 1)
- 2c. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 1)
- 2d. Trunk Kidney: glomerulonephritis, membranous, focal, mild
- 3a. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slide 1)
- 3b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1, 2, 4)
- 3c. Liver: hepatic necrosis, acute, multifocal, mild (slide 2)
- 4a. Heart: epicarditis, multifocal, lymphoplasmacytic, mild (slide 1)
- 4b. Heart: epicardial pseudocyst, 3 mm wide, 1 mm high, and filled with protein and small numbers of histiocytes (slide 4)
- 5. Spleen, intestinal ceca, and mesenteric fat: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, moderate (slides 1, 2, 3, 4)

**Final Comments**

Many of the lesions in these fish probably are associated with Gram-negative filamentous bacteria in the gills. Thin rod-shaped to Gram-negative filamentous bacteria are common on the gills of debilitated juvenile salmonids. Although bacterial culture or PCR is required for a definitive diagnosis, common species in the gill include *Flavobacterium columnare* (the cause of columnaris disease), *F. psychrophilum* (the cause of coldwater disease), and *F. branchiophilum* (the cause of bacterial gill disease). Infections are usually associated with crowding or poor water quality. Hyperplasia of the epithelium lining gill lamellae is a nonspecific response to irritation. In this case, filamentous bacteria are the most likely inciting cause. Fusion of adjacent lamellae is evidence of chronicity.

Separation of gill lamellar epithelium from underlying pillar cells can be a result of edema, and it also is a common postmortem artifact. 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. Because these fish were moribund when sampled, lamellar edema is probably a real change.

Dilation of renal components is evidence of impaired renal function, but the cause is unknown. In this case, tubular dilation is sometimes associated with necrosis of renal tubular epithelial 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). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin). 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.

Membranous glomerulonephritis is fairly uncommon in Atlantic salmon; it may be 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.

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.

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.

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 in the liver, but hepatic necrosis might be related to filamentous bacteria in the gill. Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis.

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. The epicardial pseudocyst might be a result of focal epicardial trauma.

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





Slide #4 includes several multinucleate giant cells that surround mineral and poorly defined, eosinophilic, radially oriented, fibrillar material (probably vaccine material).

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**Case Report**

**Submission** 2005-03422      **Date** 26-Sep-2005      **Report** 29-Sep-2005

**Report**      **Copies**

**Submitter:** 11036 Mainstream Canada (M)

**Owner** 15102 Mainstream 05-42

**Farm:**

**Vet Clinic:**

**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

**History/Symptoms**

Submitted 5 histology cassettes - livers, please prepare for histo exam.

Recent BKD - treated with OTC. Recent toxic algae blooms. Chronic low or fluctuating dissolved O2.

Livers contained large cratered "doughnut" shaped lesions.

Internal reference: 05-42.  
PO# 17942.

**Histopathology**

Formalin-fixed livers were received in 5 cassettes. Slides 1 through 6 correspond to cassettes labeled 05-42-1 through 05-42-6.

Quality Control: Autolysis in the liver is either mild (slides 4, 5) or moderate (slides 1, 2, 3). The sections have no significant postfixation dehydration and no acid-hematin deposits.

Measures of physiologic condition  
Hepatocellular glycogen depletion: severe (all slides).

**Diagnosis**

1. Liver: vasculitis, subacute, lymphoplasmacytic, necrotizing, with fibrinocellular thrombi, multifocal, moderate (slides 1, 2, 4, 5), severe (slide 3)
2. Liver: hepatitis, acute, multifocal, neutrophilic, with necrosis and intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), moderate (slide 2)
3. Liver: hepatitis, subacute, multifocal, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), severe (slides 1, 5)
4. Liver: hepatitis, acute, multifocal, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), severe (slides 3, 4)
5. Liver: hepatic fibrosis, diffuse, with parenchymal atrophy, moderate (slide 5), severe (slide 1)
6. Liver: biliary preductular cell hyperplasia, diffuse, moderate (slide 1)
7. Liver: hepatocellular karyomegaly and megalocytosis, multifocal, with nuclear syncytia, moderate (slide 1)
8. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 5)
9. Liver: peritonitis, lymphoplasmacytic, histiocytic, regionally diffuse, with fine fibrocellular fronds, moderate (slide 1)



**Final Comments**

The gross finding of "large cratered doughnut-shaped lesions" is consistent with the microscopic diagnosis of *Piscirickettsia salmonis*. The gross findings are a result of necrosis and parenchymal collapse, followed by variable amounts of neutrophilic inflammation, fibrosis, and regeneration.

In slide 1, the extent and severity of fibrosis is greater than I have ever seen in a fish liver. This fish probably is the most chronic of the 5 cases, with replacement of necrotic hepatocytes by fibrosis. 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 megalocytosis can result from exposure to several types of toxicants, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. Syncytial cell formation is more often associated with viral infections than with toxicant exposure. However, the changes are not characteristic of any common salmon virus. Consider virus culture, if not already done. Vasculitis is consistent with *Piscirickettsia salmonis*. Also, consider the possibility that exposure to algal toxins might have exacerbated lesions associated with *Piscirickettsia salmonis* 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 salmon it might be related to increased protein needed as part of an inflammatory response. Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

/sr



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**Case Report**

**Submission** 2005-03498      **Date** 04-Oct-2005      **Report** 13-Oct-2005

**Submitter:** 11036 Mainstream Canada (M)      **Report**      **Copies**  
**Owner** 15136 Mainstream 05-45 POLBBH      McKenzie, Peter (Fax: 250-286-0042)  
**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**

Submitter: Tim Talbot. PO#LBBH 05-45. Please report to Dr. Peter McKenzie.

Specimens: 4 pools of kidney tissue labelled P24, P13, P58, P87. Please run PCR on each pool for BKD.

**Molecular Diagnostics/PCR**

Samples P24, P13, P58 and P87: all negative for Renibacterium salmoninarum by PCR.

\* Results faxed Oct. 13/05.

/bb

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**Case Report**

**Submission** 2005-03527      **Date** 06-Oct-2005      **Report** 18-Oct-2005

**Report**      **Copies**

**Submitter:** 11036 Mainstream Canada (M)

**Owner** 15149 San Mated #05-46

**Farm:**

**Vet Clinic:**

**Attending** Dr. P. McKenzie

**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 fresh fish tissue; 4 pools of kidney tissues - 05-46-P10, 05-46-P9, 05-46-P3, 05-46-P4. Please run each pool for PCR - BKD.

Internal reference: 05-46. Copy of report to Dr. P. McKenzie (Mainstream).

**Molecular Diagnostics/PCR**

Samples P10, P3, P9 and P4: *Renibacterium salmoninarum* negative by PCR.

\* Results faxed Oct. 18/05.

/bb

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**Case Report**

**Submission** 2005-03577      **Date** 12-Oct-2005      **Report** 24-Oct-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15169      A 3.2 -78 (6)

**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 Provincial Surveillance Program samples for bacteriology - identification of isolates and culture and sensitivity.

Sample ID; A 3.2 - 78 (6).

**Bacteriology**

Bact. plate - *Vibrio logei*.

Sensitive to: Florfenicol, Sulfa-methox-trimeth. and Tetracycline.

\* Results faxed Oct. 24/05.

/bb

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**Case Report**

**Submission** 2005-03578      **Date** 12-Oct-2005      **Report** 19-Oct-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15170      A 3.3 - 66 (1-6)

**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

**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples. Sample ID: A 3.3 -66 (1-6).

ADDENDUM: Oct 14, 2005 - samples submitted for routine histology and analysis.

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. The margins of some organs (e.g. spleen in slide 2) have evidence of dehydration after fixation (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. Tissues have no deposits of acid hematin.

Slide 1: autolysis, none (all organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: no significant lesions
- 3a. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: 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.

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 fairly common in pen-reared Chinook salmon. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). 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.

Slide 2: autolysis, none (all organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatitis, granulomatous, multifocal, mild
- 1c. Liver: pericholangitis, histiocytic, lymphocytic, multifocal, mild
- 2. Heart: no significant lesions
- 3a. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate
- 4. Trunk kidney: focal granuloma (~60 µm in diameter), with a central vacuole
- 5. Head kidney: no significant lesions

Comment: Granulomatous inflammation in the liver is consistent with a chronic bacterial infection; differentials include *Renibacterium salmoninarum* or other bacteria. 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. The granuloma in the kidney is either a reaction to foreign material (e.g. vaccine material) or an early response in infection with *Renibacterium salmoninarum*.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1. Liver: no significant lesions
- 2. Heart: no significant lesions
- 3a. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions





Comment: see above slides.

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart: mural thrombosis, bifocal, mild
- 2c. Heart: endothelial cell hypertrophy and hyperplasia, multifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions
6. Dumbbell-shaped focus of granulomatous inflammation, focal, moderate

Comment: 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). Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection. Hypertrophy and hyperplasia of endothelial cells in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Each end of the dumbbell-shaped focus of granulomatous inflammation is about 400 µm in diameter; the inflammation is more consistent with a vaccine reaction than with *Renibacterium salmoninarum* infection (i.e., the focus of inflammation probably was from the mesenteries around the spleen).

Slide 5: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: no significant lesions
3. Spleen: splenitis, multifocal, granulomatous, mild
4. Trunk kidney: no significant lesions
5. Head kidney: bifocal (100-200 µm in diameter), granulomatous, mild

Comment: Hepatic lipidosis in Atlantic salmon usually occurs as one to a few cytoplasmic vacuoles that range from 2 to 10 µm in diameter. By comparison, most of the hepatocytes on this slide are slightly distended by uniform small cytoplasmic vacuoles, most of which are less than 2 µm in diameter. This pattern of small lipid vacuoles is common in northern fishes, and is considered a normal form of lipid storage in healthy fish. The liver also has several small foci (30 - 100 µm in diameter) of hepatocytes that are severely distended by lipid vacuoles; the largest of these foci are often near bile ducts and they often contain melanomacrophages.

Granulomatous inflammation in the spleen is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* is the most common organism associated with these lesions. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.



Slide 6: autolysis, none (other organs) to mild (liver and head kidney)

1a. Liver: hepatitis, granulomatous, multifocal, mild

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: endocarditis, focal (300 x 100 µm), lymphocytic, histiocytic, mild

3a. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: Inflammation in the heart is evidence of chronic immune stimulation (e.g., low grade bacterial

### **Molecular Diagnostics/PCR**

Infectious Hematopoietic Necrosis virus, Infectious Pancreatic Necrosis virus, Infectious Salmon Anemia virus, *Piscirickettsia salmonis* and Viral Haemorrhagic Septicemia virus - Negative by PCR.

Samples 1-3, 4-6.

\* Results faxed on Jan 3/06.

sr/mm



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**Case Report**

**Submission** 2005-03579      **Date** 12-Oct-2005      **Report** 12-Oct-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15171      A 3.3 - 62 (1-9)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**Count** 9

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.3 -62 (1-9)

ADDENDUM: Oct 14, 2005 - samples submitted for routine histology and analysis.

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no significant dehydration after fixation. The spleen in slide 2 has deposits of acid hematin primarily in the connective tissue surrounding blood vessels. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: pale yellow pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4a. Trunk kidney: renal tubular epithelial necrosis, focal (one tubule), acute, mild
- 4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: 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. Splenic 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 (as in this case). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin). The only affected tubule in this fish is adjacent to the edge of the section, so other tubules not included in the sample might have been affected. 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.

Slide 2: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: tubular hyperplasia with peritubular fibrosis, focal (300 x 200  $\mu$ m), mild
5. Head kidney: eosinophilic granular cells in interstitial tissue, multifocal, mild

Comment: 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. Proliferative lesions in the biliary system are evidence for a chronic toxic cause for the hepatic necrosis. 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.

The focus of renal tubular hyperplasia with peritubular fibrosis might be a site of previous renal tubular necrosis (described for slide 1).

Increased numbers of eosinophilic granular cells in the kidney is a distinctive finding in Atlantic salmon.





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*. Increased numbers of eosinophilic granular cells are sometimes associated with chronic parasitic infections, but again, the inciting cause was not included in the section.

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.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, granulomatous, multifocal, moderate
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, moderate
2. Heart: endocarditis, granulomatous, diffuse, mild
3. Spleen: splenitis, granulomatous, multifocal, moderate
- 4a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, severe
- 4b. Trunk kidney: interstitial cell hyperplasia, diffuse, moderate
5. Head kidney: nephritis, interstitial, granulomatous, multifocal, severe

Comment: The most common organism associated with disseminated granulomas in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. Inflammation in the heart uniformly lines the endocardium, in most cases only one to three cells thick. 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 this case it is associated with disseminated granulomatous inflammation.

Slide 4: autolysis, none (other organs) to mild (trunk kidney and liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, mild
5. Head kidney: nephritis, interstitial, granulomatous, multifocal, mild

Comment: The kidney sections have early lesions that provide evidence of infection with *Renibacterium salmoninarum*.

Slide 5: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic abscess, focal (3 mm diameter), chronic, with peripheral fibrosis and intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), moderate
- 1b. Liver: hepatitis, granulomatous, multifocal to diffuse, moderate
- 1c. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 2a. Heart: epicarditis, multifocal, lymphoplasmacytic, moderate
- 2b. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages, moderate
3. Spleen: splenitis, histiocytic, multifocal to diffuse, with edema, moderate
4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), moderate
5. Head kidney: nephritis, interstitial, granulomatous, multifocal to diffuse, moderate

Comment: Most of the lesions in this fish are consistent with *Piscirickettsia salmonis* infection; granulomatous inflammation in this case is more poorly defined than it is with the primary differential--*Renibacterium salmoninarum*. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface throughout most of the ventricle are rarely more than 2 cell layers thick.



Most references say that fish do not form abscesses or pus, but the lesion in the liver has all the classic features of a chronic abscess: a margin of dissecting fibrosis and necrosis, with a central necrotic region filled with abundant degenerating fibrin and neutrophils. 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.

Slide 6: autolysis, none (other organs) to mild (liver)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: endocarditis, focal (700 x 200 µm), with endothelial cell hypertrophy and a thin layer of macrophages, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4a. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate
- 4b. Trunk kidney: renal tubular mineralization, multifocal, mild
5. Head kidney: no significant lesions

Comment: Renal mineralization is common in cultured fish species; when severe, the condition is termed nephrocalcinosis. The lesion is not considered fatal, although feed conversion may be adversely affected. The cause is unknown, 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.

Slide 7: autolysis, mild (other organs) to moderate (liver)

1. Liver: hepatic necrosis, acute, multifocal, moderate
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: renal tubular mineralization, focal, mild
5. Head kidney: no significant lesions

Comment: 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).

Slide 8: autolysis, none (heart) to mild (other organs)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: no significant lesions
- 4a. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate
- 4b. Trunk kidney: eosinophilic granular cells in interstitial tissue, diffuse, mild
5. Head kidney: eosinophilic granular cells in interstitial tissue, diffuse, mild

Comment: see other slides.

Slide 9: autolysis, mild (heart) to moderate (kidney and spleen) or severe (liver)

1. Liver: hepatic necrosis, acute, multifocal, severe, with hepatocellular karyorrhexis and intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*)
- 2a. Heart: epicarditis, multifocal, lymphoplasmacytic, moderate
- 2b. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages, moderate
3. Spleen: no significant lesions
- 4a. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
- 4b. Trunk kidney: eosinophilic granular cells in interstitial tissue, diffuse, mild
- 4c. Trunk kidney: interstitial cell hyperplasia, diffuse, mild



5. Head kidney: eosinophilic granular cells in interstitial tissue, diffuse, moderate

Comment: This fish probably died of complications related to *Piscirickettsia salmonis* infection. Examples of

**Molecular Diagnostics/PCR**

Samples 1-3, 4-6, 7-9 - Infectious Hematopoietic Necrosis virus, Infectious Pancreatic Necrosis virus, Infectious Salmon Anemia virus, and Viral Haemorrhagic Septicemia virus - Negative by PCR.

Samples 4-6, 7-9 - *Piscirickettsia salmonis* Positive.

Samples 1-3 - *Piscirickettsia salmonis* Negative.

\* Results faxed on Jan 3/06.

sr/mm

**ANIMAL HEALTH CENTRE**

AAVLD - Accredited Laboratory

Ministry of  
Agriculture, Food and Fisheries  
1767 Angus Campbell Road

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

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

**Case Report**

**Submission** 2005-03580      **Date** 12-Oct-2005      **Report** 25-Oct-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15172      A 3.4 - 68 (1-10)

**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

**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.4 -68 (1-10)

ADDENDUM: Oct 14, 2005 - samples submitted for routine histology and analysis.



**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Sections have no significant postfixation dehydration. The spleen in slides 4 and 7 has deposits of acid hematin primarily in the connective tissue surrounding blood vessels; the liver in slide 10 has acid hematin around blood vessels. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (all organs)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: no significant lesions
3. Spleen: peritonitis, granulomatous, regionally diffuse, with an intralesional vacuole about 150 µm in diameter, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

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

Slide 2: autolysis, none (heart) to mild (other organs)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2a. Heart, compact layer of ventricle: myofibre degeneration, with lymphocytic and histiocytic inflammation, multifocal, coalescing, moderate
- 2b. Heart: epicarditis, multifocal, lymphoplasmacytic, with activated fibroblasts, moderate
3. Spleen: peritonitis, granulomatous, multifocal, mild
4. Trunk kidney: interstitial cell hyperplasia, diffuse, mild
5. Head kidney: not included on the slide

Comment: The compact layer of the ventricle has an unusual lesion. Affected cardiac myofibres lack the eosinophilic cytoplasm and cross striations of adjacent normal fibres; instead, cytoplasm in pale eosinophilic with poorly defined vacuoles. Nuclear morphology is more normal. Inflammation (lymphocytes and histiocytes) is limited to areas in contact with overlying epicarditis. This change might be congenital or degenerative, and it might have contributed to the death of this fish. Epicarditis 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). 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).

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: see other slides.

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2a. Heart: epicarditis, multifocal, lymphoplasmacytic, regionally diffuse, mild



2b. Heart: endocarditis, focal (400 x 150 µm), with endothelial cell hypertrophy and a thin layer of macrophages and lymphocytes, mild

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

Comment: The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface throughout the lesion are rarely more than 2 cell layers thick.

Slide 5: autolysis, none (heart) to mild (other organs)

1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild

1c. Liver: pericholangitis, lymphocytic, focal, mild

1d. Liver: sinusoidal congestion, acute, multifocal, mild

2. Heart: endocarditis, focal (200 x 150 µm), with endothelial cell hypertrophy and a thin layer of macrophages and lymphocytes, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

Comment: 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 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 infections. 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).

Slide 6: autolysis, none (heart) to mild (other organs) and moderate (intestinal ceca)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2. Heart: no significant lesions

3. Spleen and mesenteric fat: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 100 µm in diameter, moderate

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

6. Intestinal ceca: no significant lesions

Comment: see other slides.

Slide 7: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: no significant lesions

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

Comment: see other slides.

Slide 8: autolysis, none (other organs) to mild (liver)

1. Liver: no significant lesions

2. Heart: no significant lesions

3. Spleen and mesenteric fat: peritonitis, granulomatous, multifocal, mild



- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide
- Comment: see other slides.

Slide 9: autolysis, none (all organs)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen and mesenteric fat: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions
- Comment: see other slides.

Slide 10: autolysis, mild (other organs) to moderate (liver)

- 1. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: no significant lesions
- 4. Trunk kidney: interstitial congestion, diffuse, moderate
- 5. Head kidney: not included on the slide

Comment: Renal congestion and hemorrhage is one of the classic signs of infectious salmon anemia (ISA), but ISA has never been isolated from fish in BC. Renal congestion has also been associated with VHSV, bacteria, and postmortem pooling.

### **Molecular Diagnostics/PCR**

Infectious Hematopoietic Necrosis virus, Infectious Pancreatic Necrosis virus, Infectious Salmon Anemia virus, *Piscirickettsia salmonis* and Viral Haemorrhagic Septicemia virus - Negative by PCR.

Sample 1-5, 6-10.

\* Results faxed on Dec 22/05.

sr/mm



**ANIMAL HEALTH CENTRE**

AAVLD - Accredited Laboratory

Ministry of  
Agriculture, Food and Fisheries  
1767 Angus Campbell Road

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

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

**Case Report**

**Submission** 2005-03581      **Date** 12-Oct-2005      **Report** 03-Nov-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15173      A 3.2-58 (1-13)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**Count** 13

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.2 -58 (1-13)

ADDENDUM: Oct 14, 2005 - samples submitted for routine histology and analysis.



**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no significant postfixation dehydration. Some large foci of erythrocytes have deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, mild (other organs) to moderate (liver)

1a. Liver: pericholangitis, lymphocytic, multifocal, mild

1b. Liver: yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild

2. Heart: epicarditis, multifocal, lymphoplasmacytic, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: not included on the slide

Comment: 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. 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.

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

Slide 2: autolysis, mild (other organs) to moderate (liver)

Special stains: lipofuscin (Schmorl's), iron

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild

1b. Liver: hepatitis, granulomatous, focal (400 µm in diameter), mild

1c. Liver: yellow-green pigmented (iron and lipofuscin) macrophage aggregates and sinusoidal macrophages, disseminated, mild

2. Heart: epicarditis, multifocal, lymphoplasmacytic, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: renal tubular casts of protein and yellow-brown pigment (lipofuscin), with tubular epithelial degeneration and regeneration, multifocal, mild

5. Head kidney: not included on the slide

Comment: 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). The most common organism associated with disseminated granulomas in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease; consider *Yersinia ruckeri* as a differential.

Pigment in the liver includes lipofuscin and iron (probably hemosiderin), but pigments in the spleen and renal tubules include lipofuscin but not iron (all confirmed with special stains). 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. Hemosiderin accumulation in the liver is evidence of increased turnover of red blood cells.

Slide 3: autolysis, mild (other organs) to severe (liver)

1. Liver: yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild
  2. Heart: no significant lesions
  3. Spleen: no significant lesions
  4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate
  5. Head kidney: not included on the slide
- Comment: see other slides.

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1c. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1d. Liver: yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild

2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate
5. Head kidney: not included on the slide

Comment: 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.

Slide 5: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*) and endothelial cell hypertrophy, severe
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1c. Liver: biliary preductular cell hyperplasia, multifocal (most prominent around larger bile ductules), mild
2. Heart: endocarditis, multifocal, lymphohistiocytic, with plump endothelial cells and intracellular basophilic structures consistent with *Piscirickettsia salmonis*, mild
3. Spleen: no significant lesions
- 4a. Trunk kidney: nephritis, interstitial, histiocytic, diffuse, with multifocal interstitial cell hyperplasia and intracellular bacteria (consistent with *Piscirickettsia salmonis*), moderate
- 4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: not included on the slide

Comment: This fish probably died of complications related to *Piscirickettsia salmonis* infection.

Slide 6: autolysis, mild (other organs) to moderate (liver)

- 1a. Liver: yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 2a. Heart: epicarditis, multifocal, lymphoplasmacytic, mild
- 2b. Heart: endocarditis, multifocal, lymphocytic, mild
3. Spleen: no significant lesions
- 4a. Trunk kidney: renal tubular casts of yellow-green pigment, focal, mild
- 4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: Lymphocytic inflammation in the heart is evidence of chronic immune stimulation; differentials



include a low grade bacterial infection and reaction to a vaccine.

Slide 7: autolysis, none (other organs) to mild (liver)

1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

1c. Liver: biliary preductular cell hyperplasia, diffuse, mild

1d. Liver: yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: no significant lesions

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate

5. Head kidney: not included on the slide

Comment: 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).

Slide 8: autolysis, none (other organs) to mild (liver)

Special stains: Congo Red (for amyloid), PAS, and lipofuscin

1a. Liver: intrahistiocytic protein, subacute, multifocal, with occasional multinucleate giant cells, moderate

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

1c. Liver: biliary preductular cell hyperplasia, diffuse, mild

1d. Liver: yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild

2. Heart: no significant lesions

3a. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

3c. Spleen: hyalinization of vessel walls with PAS-positive material, diffuse, moderate

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: no significant lesions

Comment: The liver has an unusual pattern of intrahistiocytic protein that I have not previously seen in Atlantic salmon. Protein-laden macrophages tend to line blood vessels. A few of the affected macrophages have multiple nuclei, consistent with persistent foreign material, but the cells contain no obvious organisms. The material is not amyloid or lipofuscin, but it is strongly PAS positive: evidence that it contains glycoproteins. This might be an unusual reaction to a vaccine, or it might be a precursor to the more common accumulation of golden pigment (lipofuscin), which is also present in small intracellular foci. The golden pigment in the spleen and liver 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. Hyalinization of the spleen with PAS positive material probably has the same cause as the PAS positive material in liver.

Slide 9: autolysis, mild (other organs) to severe (liver)

1a. Liver: fibrosis, focal (0.5 x 1.5 mm), with peripheral congested sinusoids, moderate

1b. Liver: yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild

2. Heart: no significant lesions

3. Spleen: not included on the slide

4. Trunk kidney (and Corpuscle of Stannius): tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: not included on the slide

Comment: This fish has an unusual focus of immature fibrosis. Congested sinusoids at the margin of the fibrosis are continuous with the sinusoids of the more normal parenchyma; this provides evidence that the



focus is a post-necrotic scar. Lack of nuclear atypia or mitotic figures is evidence against neoplasia.

Slide 10: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), vasculitis, and endothelial cell hypertrophy, severe
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1c. Liver: biliary preductular cell hyperplasia, multifocal (most prominent around larger bile ductules), mild
- 1d. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2a. Heart: endocarditis and epicarditis, multifocal, lymphohistiocytic, with plump endothelial cells, mild
- 2b. Heart: mural thrombosis, multifocal, mild
- 3a. Spleen: splenitis, histiocytic, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), moderate
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 3c. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
- 4a. Trunk kidney: nephritis, interstitial, histiocytic, diffuse, with diffuse interstitial cell hyperplasia and intracellular bacteria (consistent with *Piscirickettsia salmonis*), severe
- 4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: nephritis, interstitial, histiocytic, diffuse, with diffuse interstitial cell hyperplasia and intracellular bacteria (consistent with *Piscirickettsia salmonis*), moderate

Comment: Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection; in this case, it probably is a result of infection with *Piscirickettsia salmonis*. Inflammation in the heart probably is also related to infection with *Piscirickettsia salmonis*, but the section contains no organisms.

Slide 11: autolysis, mild (other organs) to severe (liver and gill)

1. Liver: yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate
5. Head kidney: not included on the slide
6. Gill: filament necrosis (2 adjacent filaments), with abundant filamentous bacteria, focal, severe

Comment: Thin rod-shaped to filamentous bacteria are common on the gills of debilitated juvenile salmonids. Although bacterial culture or PCR is required for a definitive diagnosis, the common species in the gill include *Flavobacterium columnare* (the cause of columnaris disease), *F. psychrophilum* (the cause of coldwater disease), and *F. branchiophilum* (the cause of bacterial gill disease). Infections are usually associated with crowding or poor water quality. The affected gill probably had a couple discolored filaments.

Slide 12: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), vasculitis, and endothelial cell hypertrophy, severe
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: not included on the slide
3. Spleen: splenitis, histiocytic, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), moderate
- 4a. Trunk kidney: nephritis, interstitial, histiocytic, diffuse, with diffuse interstitial cell hyperplasia, eosinophilic granular cells, intracellular bacteria (consistent with *Piscirickettsia salmonis*), vasculitis, and endothelial cell hypertrophy, severe
- 4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: nephritis, interstitial, histiocytic, diffuse, with diffuse interstitial cell hyperplasia and intracellular bacteria (consistent with *Piscirickettsia salmonis*), moderate





Comment: see other slides

**Molecular Diagnostics/PCR**

Samples 1-4, 5-8, 9-13 - Infectious Hematopoietic Necrosis virus, Infectious Pancreatic Necrosis virus, Infectious Salmon Anemia virus, and Viral Haemorrhagic Septicemia virus - Negative by PCR.

Samples 5-8, 9-13 - *Piscirickettsia salmonis* Positive.

Samples 1-3 - *Piscirickettsia salmonis* Negative.

\* Results faxed on Dec 22/05.

sr/mm

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1767 Angus Campbell Road

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

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

**Case Report**

**Submission** 2005-03583      **Date** 12-Oct-2005      **Report** 03-Nov-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15174      A 3.2-78 (1-8)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**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

**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 3.2 -78 (1-8)

ADDENDUM: Oct 14, 2005 - samples submitted for routine histology and analysis.

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs in all slides except #8 have no dehydration after fixation and no deposits of acid hematin. Erythrocytes in slide #8 have evidence of dehydration after fixation (i.e., erythrocyte cytoplasm stains yellow instead of red). This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, none (other organs) to mild (liver)

- 1a. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, moderate
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
- 4. Trunk kidney: renal tubular epithelial necrosis with interstitial golden pigment, focal (~1 mm in diameter), mild
- 5. Head kidney: not included on the slide

Comment: 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. Pigment in the spleen probably is lipofuscin (not iron). 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.

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).

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; in this kidney, only 1-2 cells/tubule are affected. Damage is reversible if the basement membrane is spared (as in this case). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin). Accumulation of interstitial golden pigment (probably lipofuscin) in the same focus probably is related to the same underlying cause.

Slide 2: autolysis, none (other organs) to mild (liver)

- 1a. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
- 4. Trunk kidney: renal tubular protein casts, multifocal, mild
- 5. Head kidney: no significant lesions

Comment: Renal tubular protein casts result from glomerular or tubular dysfunction; excess protein leaks through glomeruli, or tubules are unable to reabsorb protein.

Slide 3: autolysis, none (all organs)

- 1a. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages,



disseminated, mild

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment:

Slide 4: autolysis, none (all organs)

1a. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, moderate

1b. Liver: biliary preductular cell hyperplasia, diffuse, moderate

2. Heart: no significant lesions

3a. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

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

Slide 5: autolysis, none (all organs)

1a. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild

1b. Liver: biliary preductular cell hyperplasia, diffuse, moderate

1c. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: no significant lesions

3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: see other slides.

Slide 6: autolysis, none (other organs) to mild (liver)

1a. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild

1b. Liver: biliary preductular cell hyperplasia, diffuse, moderate

2. Heart: no significant lesions

3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

4. Trunk kidney: not included on the slide

5. Head kidney: moderate numbers of eosinophilic granular cells in interstitial tissue, diffuse

Comment: Increased numbers of eosinophilic granular cells in the head kidney is a distinctive finding in Atlantic salmon. 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*. Increased numbers of eosinophilic granular cells are sometimes associated with chronic parasitic infections, but again, the inciting cause was not included in the sections examined.

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.



Slide 7: autolysis, none (other organs) to mild (trunk kidney) and moderate (liver)

1a. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild

1b. Liver: biliary preductular cell hyperplasia, diffuse, moderate

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: no significant lesions

4. Trunk kidney: not included on the slide

5. Head kidney: not included on the slide

Comment: see other slides.

Slide 8: autolysis, none (other organs) to mild (liver)

1a. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild

1c. Liver: pericholangitis, lymphocytic, multifocal, mild

1d. Liver: sinusoidal congestion, bifocal (each ~400 µm in diameter), mild

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

4. Trunk kidney: nephritis, interstitial, granulomatous, focal, mild

5. Head kidney: nephritis, interstitial, granulomatous, focal, mild

Comment: The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation. 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. The two foci of sinusoidal congestion probably are a result of trauma.

### **Molecular Diagnostics/PCR**

Infectious Hematopoietic Necrosis virus, Infectious Pancreatic Necrosis virus, Infectious Salmon Anemia virus, *Piscirickettsia salmonis* and Viral Haemorrhagic Septicemia virus - Negative by PCR.

Samples 1-4, 5-8.

\* Results faxed on Dec 22/05.

sr/mm





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

Facsimile: (604) 556-3010  
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**Case Report**

**Submission** 2005-03584      **Date** 12-Oct-2005      **Report** 03-Nov-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15175      A 3.2-80 (1-5)

**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

**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.2 -80 (1-5)

ADDENDUM: Oct 14, 2005 - samples submitted for routine histology and analysis.

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Swelling of hepatocyte nuclei in slide 2 probably is an artefact resulting from transfer to water before full penetration of the formalin. Organs have no postfixation dehydration. The spleen in slide 4 has small deposits of acid hematin primarily in the connective tissue surrounding blood vessels. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: 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). 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).

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 fairly common in pen-reared Chinook salmon. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Slide 2: autolysis, mild (other organs) to moderate (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: see other slides.

Slide 3: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, severe
2. Heart: not included on the slide
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4a. Trunk kidney: renal tubular epithelial necrosis, multifocal, subacute, with fibrosis and regeneration, mild
- 4b. Trunk kidney: small numbers of eosinophilic granular cells in interstitial tissue, diffuse
5. Head kidney: small numbers of eosinophilic granular cells in interstitial tissue, diffuse

Comment: Splenic 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 (as in this case). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin). Increased numbers of eosinophilic granular cells in the heart, kidney, and spleen is a distinctive finding in Atlantic salmon. 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*. Increased numbers of eosinophilic granular cells are sometimes associated with chronic parasitic infections, but again, the inciting cause was not included in the sections examined.

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.

Slide 4: autolysis, none (other organs) to mild (liver)

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, severe

2. Heart: not included on the slide

3a. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: see other slides

Slide 5: autolysis, none (heart) to mild (other organs)

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

1c. Liver: sinusoidal congestion, focal (~500 µm in diameter), mild

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

Comment: The focus of sinusoidal congestion might be a result of trauma.

### **Molecular Diagnostics/PCR**

Infectious Hematopoietic Necrosis virus, Infectious Pancreatic Necrosis virus, Infectious Salmon Anemia virus, *Piscirickettsia salmonis* and Viral Haemorrhagic Septicemia virus - Negative by PCR.

Samples 1-3, 4-5.

\* Results faxed on Dec 22/05.

sr/mm



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1767 Angus Campbell Road

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

Facsimile: (604) 556-3010  
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**Case Report**

**Submission** 2005-03585      **Date** 12-Oct-2005      **Report** 09-Nov-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15176      A 3.1-54 (1-2)

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.1 -54 (1-2)

ADDENDUM: Oct 14, 2005 - samples submitted for routine histology and analysis.

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no postfixation dehydration and no deposits of acid hematin.

Slide 1: autolysis, mild (other organs) to moderate (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: not included on the slide

Comment: 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). 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). 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 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.

Slide 2: autolysis, mild (other organs) to moderate (liver)

- 1a. Liver: hepatitis, granulomatous, multifocal, with multinucleate giant cells surrounding radiating globular green-red material that is consistent with vaccine material, moderate
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1c. Liver: small numbers of parenchymal macrophages with intracellular pale basophilic round structures immunohistochemically positive for *Piscirickettsia salmonis*
- 2a. Heart: endocarditis, multifocal, lymphohistiocytic, with intracellular pale basophilic round structures immunohistochemically positive for *Piscirickettsia salmonis*, mild
- 2b. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: small numbers of parenchymal macrophages with intracellular pale basophilic round structures immunohistochemically positive for *Piscirickettsia salmonis*
- 4a. Trunk kidney: nephritis, interstitial, diffuse, granulomatous, with multinucleate giant cells surrounding radiating globular green-red material that is consistent with vaccine material, severe
- 4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
- 4c. Trunk kidney: small numbers of interstitial macrophages with intracellular pale basophilic round structures immunohistochemically positive for *Piscirickettsia salmonis*
5. Head kidney: not included on the slide

Comment: Vaccine material does not commonly occur in the liver and kidney. It may be that the vaccine was injected directly into the liver of this fish instead of the peritoneal cavity. Presence of vaccine material in the kidney is evidence that at some point the material was in the circulatory system (lymphatics?). 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. The immunohistochemical stain clearly highlights several *Piscirickettsia salmonis* organisms in the liver, spleen, and kidney that were not seen on H&E; organisms are more obvious in the heart H&E section.



**Molecular Diagnostics/PCR**

Infectious Hematopoietic Necrosis virus, Infectious Pancreatic Necrosis virus, Infectious Salmon Anemia virus, *Piscirickettsia salmonis* and Viral Haemorrhagic Septicemia virus - Negative by PCR.

Samples 1-2.

\* Results faxed on Dec 22/05.

sr/mm

**Final Comments**

See histopathology comments.

/sr/mm

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1767 Angus Campbell Road

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

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**Case Report**

**Submission** 2005-03665      **Date** 18-Oct-2005      **Report** 24-Oct-2005

**Report**      **Copies**

**Submitter:** 11036 Mainstream Canada (M)  
**Owner** 15203 Mainstream 05-47 POLBBH  
**Farm:**  
**Vet Clinic:** 11036 Mainstream Canada (M)  
**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

**History/Symptoms**

Submitted one culture plate TSA - 2 cultures - 90-5B, 81-10B. Please do culture and sensitivity testing on both samples.

Internal hemorrhage.

\* Please use our internal reference 05-47. Report to Dr. Peter McKenzie.

**Bacteriology**

Isolate 90-5b: *Pseudomonas fluorescens*.

Isolated 60-10b: *Pseudomonas fluorescens*.

See attached sheet for sensitivities.

\* Results faxed Oct. 24/05.

/bb



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**Case Report**

**Submission** 2005-03666      **Date** 18-Oct-2005      **Report** 31-Oct-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15204      A 2.4-75 (3)

**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 Provincial Surveillance Program samples for routine bacteriology - identification of isolates and culture and sensitivity.

Sample ID: A 2.4-75 (3) subculture.

**Bacteriology**

Isolate A2.4-75: *Vibrio tasmaniensis* isolated.

Sensitive to: Florfenicol, Romet 30, Tri-sulfas, Sulfa-methox-trimeth. and Tetracycline.

\* Results faxed Oct. 31/05.

/bb

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Facsimile: (604) 556-3010  
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**Case Report**

**Submission** 2005-00378      **Date** 04-Feb-2005      **Report** 07-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13438      A 3.3 - 18 (3, 5)

**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 Provincial Surveillance Program samples for routine bacteriology - identification of isolates and culture and sensitivity.

Sample ID: A 3.3 -18 (3,5).

Lab Note: Virology samples for PCR is on case #2005-626.

**Bacteriology**

Both plates isolated growth of Photobacterium phosphoreum.

\* Due to the poor growth of these organisms, antibiotic sensitivities were unable to be performed.

\* Results faxed Mar. 7/05.

/bb

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

Facsimile: (604) 556-3010  
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**Case Report**

**Submission** 2005-03787      **Date** 26-Oct-2005      **Report** 31-Oct-2005

**Report**      **Copies**

**Submitter:** 11036 Mainstream Canada (M)

**Owner** 15244 Penny Creek

**Farm:**

**Vet Clinic:**

**Attending** Dr. P. 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

**History/Symptoms**

Submitted one TSA (5% sheep blood) plate labelled 05-48 for bacteriology - ID and sensitivities.

Please report to Dr. P. McKenzie.

Internal Reference: 05-48.

**Bacteriology**

Plate - Vibrio sp. isolated.

Sensitive to: Florfenicol, Romet 30, Sulfa-methox-trimeth. and Tetracycline.

\* Results faxed Oct. 31/05.

/bb

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1767 Angus Campbell Road

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

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

**Case Report**

**Submission** 2005-03788      **Date** 26-Oct-2005      **Report** 15-Nov-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15245      A 2.4 - 75 (1-6)

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Also submitted samples for routine histology.

Sample ID: A 2.4-75 (1-6).

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no dehydration after fixation. The spleen in slide 4 has small deposits of acid hematin primarily in the connective tissue surrounding blood vessels. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (heart) to mild (other organs)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, severe
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

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

Slide 2: autolysis, none (other organs) to mild (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: myocardial karyomegaly, multifocal, mild
- 3a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: 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). 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. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Slide 3: autolysis, mild (other organs) to moderate (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: see other slides

Slide 4: autolysis, none (other organs) to mild (liver)

- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions





Comment: see other slides

Slide 5: autolysis, none (other organs) to mild (liver)

1a. Liver: hepatitis, granulomatous, multifocal, with multinucleate giant cells surrounding globular brown-yellow material that is consistent with vaccine material, severe

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: epicarditis and endocarditis, granulomatous, multifocal, moderate

3a. Spleen: splenitis, granulomatous, multifocal, with multinucleate giant cells, moderate

3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, with multinucleate giant cells surrounding globular brown-yellow material that is consistent with vaccine material, severe

5. Head kidney: not included on the slide

6. Gill: branchitis, granulomatous, multifocal, coalescing, with multinucleate giant cells surrounding globular brown-yellow material that is consistent with vaccine material, severe

Comment: Vaccine material is uncommon in the parenchyma of several organs. The vaccine might have been injected directly into a vascular organ (liver?) of this fish instead of the peritoneal cavity. Because foreign material is not included in all foci of granulomatous inflammation, this fish might also have had a concurrent infection with *Renibacterium salmoninarum*.

Slide 6: autolysis, none (other organs) to mild (liver)

1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: no significant lesions

3a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

3b. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: 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.

### **Molecular Diagnostics/PCR**

Samples 1-3, 4-6 IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* Negative by PCR.

\* Results faxed on Jan. 12/06.

sr/mm



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Facsimile: (604) 556-3010  
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**Case Report**

**Submission** 2005-03789      **Date** 26-Oct-2005      **Report** 15-Nov-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15246      A 3.3 - 84 (1-4)

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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

**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Also submitted samples for routine histology.

Sample ID: A 3.3-84 (1-4).

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Large foci of erythrocytes have deposits of acid hematin (e.g., liver in slide 3). Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. Organs have no postfixation dehydration.

Slide 1: autolysis, none (all organs)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

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

Slide 2: autolysis, mild (all organs)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: see other slides

Slide 3: autolysis, mild (all organs)

1. Liver: sinusoidal congestion, acute, multifocal, mild
2. Heart: endocarditis, multifocal, lymphohistiocytic, with endocardial hypertrophy, mild
3. Spleen and intestinal cecum: peritonitis, pleocellular, regionally diffuse, with abundant fine fibrocellular fronds, severe
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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 infections. 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). Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, but this reaction is more severe than in most vaccinated fish.

Slide 4: autolysis, none (other organs) to mild (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: epicarditis, multifocal, lymphoplasmacytic, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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). Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

**Molecular Diagnostics/PCR**

Samples 1-2, 3-4 IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* Negative by PCR.

\* Results faxed on Jan. 12/06.

sr/mm



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

Facsimile: (604) 556-3010  
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## Case Report

**Submission** 2005-03790      **Date** 26-Oct-2005      **Report** 15-Nov-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15247      A 3.3 - 89 (1-2)

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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

### **History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Also submitted samples for routine histology.

Sample ID: A 3.3-89 (1-2).

### **Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no postfixation dehydration and no large deposits of acid hematin.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: epicarditis, multifocal, lymphoplasmacytic, moderate
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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). Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 2: autolysis, none (all organs)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: no significant lesions
3. Spleen: no significant lesions
- 4a. Trunk kidney: small numbers of eosinophilic granular cells in interstitial tissue, diffuse
- 4b. Trunk kidney: renal tubular dilation, diffuse, mild
5. Head kidney: no significant lesions

Comment: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Increased numbers of eosinophilic granular cells in the kidney is a distinctive finding in Atlantic salmon. 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*. Increased numbers of eosinophilic granular cells are sometimes associated with chronic parasitic infections, but again, the inciting cause is not included in the sections examined. Dilation of renal tubules is evidence of impaired renal function, but the cause is unknown.

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.



**Molecular Diagnostics/PCR**

Samples 1-2 IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* Negative by PCR.

\* Results faxed on Jan. 12/06.

sr/mm

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

**Case Report**

**Submission** 2005-03791      **Date** 26-Oct-2005      **Report** 15-Nov-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15248      A 3.3 - 85 (1-4)

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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

**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Also submitted samples for routine histology.

Sample ID: A 3.3-85 (1-4).

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no postfixation dehydration and no significant deposits of acid hematin.

Slide 1: autolysis, none (heart) to mild (other organs)

1a. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, with intracellular hemosiderin and lipofuscin, mild

1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild

1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2. Heart: epicarditis, multifocal, lymphoplasmacytic, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: interstitial cell hyperplasia, diffuse, moderate

5. Head kidney: interstitial cell hyperplasia, diffuse, moderate

Comment: 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. 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.

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. 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).

Slide 2: autolysis, none (other organs) to mild (liver)

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: no significant lesions

3. Spleen: no significant lesions

4. Trunk kidney:

5. Head kidney: small numbers of eosinophilic granular cells in interstitial tissue, diffuse

Comment: 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). Increased numbers of eosinophilic granular cells in the kidney is a distinctive finding in Atlantic salmon. 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*. Increased numbers of eosinophilic granular cells are sometimes associated with chronic parasitic infections, but again, the inciting cause is not included in the sections examined.

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.

Slide 3: autolysis, none (heart) to mild (other organs) or moderate (trunk kidney)





1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart: endocarditis, focal, with endothelial cell hypertrophy and a thin layer of macrophages, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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). The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface in foci of inflammation are rarely more than 2 cell layers thick.

Slide 4: autolysis, none (other organs) to mild (liver, trunk kidney)

1. Liver: no significant lesions
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: see other slides.

#### **Molecular Diagnostics/PCR**

Samples 1-2, 3-4 IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* Negative by PCR.

\* Results faxed on Jan. 12/06.

sr/mm



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

**Case Report**

**Submission** 2005-03792      **Date** 26-Oct-2005      **Report** 15-Nov-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15249      A 3.2 - 83 (1-4)

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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

**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Also submitted samples for routine histology.

Sample ID: A 3.2-83 (1-4).

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no postfixation dehydration. Connective tissue and hepatocytes around large foci of erythrocytes have deposits of acid hematin (e.g., liver and spleen in slide 3). Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, severe
2. Heart: no significant lesions
3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. (Mutoloki et al. 2004).

S. Mutoloki, S. Alexandersen, and Ø. Evensen. 2004. Sequential study of antigen persistence and concomitant inflammatory reactions relative to side-effects and growth of Atlantic salmon (*Salmo salar* L.) following intraperitoneal injection with oil-adjuvanted vaccines. *Fish & Shellfish Immunology* 16(5):633-644. 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.

Slide 2: autolysis, mild (other organs) to severe (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: This is a rare case with no significant lesions in any of the organs examined.

Slide 3: autolysis, mild (other organs) to severe (liver)

1. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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). 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.

Slide 4: autolysis, none (other organs) to mild (liver, trunk kidney)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild



2. Heart: epicarditis, multifocal, lymphoplasmacytic, mild
3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: 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. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

#### **Molecular Diagnostics/PCR**

Samples 1-3, 4-5 IHN, VHS, ISA, IPN, Piscirickettsia salmonis Negative by PCR.

\* Results faxed on Jan. 12/06.

sr/mm

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

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

## Case Report

**Submission** 2005-03794      **Date** 26-Oct-2005      **Report** 15-Nov-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15251      A 3.3 - 87 (1-5)

**Farm:**

**Vet Clinic:**

**Attending**      Dr. J. Constantine

**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



**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Also submitted samples for routine histology.

Sample ID: A 3.3-87 (1-5).

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no postfixation dehydration. Connective tissue and hepatocytes around large foci of erythrocytes have deposits of acid hematin (e.g., liver in slide 1). Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: 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. 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 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.

Slide 2: autolysis, mild (other organs) to moderate (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: see slide #1.

Slide 3: autolysis, none (other organs) to mild (liver, trunk kidney)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: see slide #1.

Slide 4: autolysis, none (spleen and heart), mild (head and trunk kidney) to moderate (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate
5. Head kidney: no significant lesions

Comment: see slide #1.

Slide 5: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, moderate
- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild



- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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. Proliferation in the biliary system is evidence for a toxic cause for the hepatic necrosis.

#### **Molecular Diagnostics/PCR**

Samples 1-3, 4-5 IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* Negative by PCR.

\* Results faxed on Jan. 12/06.

sr/mm

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Facsimile: (604) 556-3010  
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**Case Report**

**Submission** 2005-03924      **Date** 04-Nov-2005      **Report** 15-Nov-2005

**Report**      **Copies**

**Submitter:** 11036 Mainstream Canada (M)  
**Owner** 15310 Cliff Bay, 05-52 PCR  
**Farm:**  
**Vet Clinic:** 15092 McKenzie, Peter (Fax: 250-286-0042)  
**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**

Recent entries into saltwater. Possible contact with BKD in freshwater.  
Submitted 3 pools of kidney tissue labelled: 05-52-2, 05-52-4, 05-52-5.

Please run PCR for BKD on all 3 pools.

Please report to Tim Talbot and Dr. Peter McKenzie

**Molecular Diagnostics/PCR**

Samples 52-2 and 52-4: negative for Renibacterium salmoninarum.  
Sample 52-2: positive for Renibacterium salmoninarum.

\* Results faxed Nov. 15/05.

/bb

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**Case Report**

**Submission** 2005-03927      **Date** 04-Nov-2005      **Report** 14-Nov-2005

**Report**      **Copies**

**Submitter:** 11036 Mainstream Canada (M)  
**Owner** 15312 Cliff Bay, #05-52B  
**Farm:**  
**Vet Clinic:** 15092 McKenzie, Peter (Fax: 250-286-0042)  
**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**

Submitter: Tim Talbot.

Recent entries into saltwater. History or possible exposure to BKD. Internal hemorrhage on flesh and pyoris. Submitted one culture plate with 2 cases 05-52B-2-1, 05-52B-5-1. Please do culture and sensitivity on both cultures.

**Bacteriology**

Plate 05-52B-2-1 and 05-52B-5-1: *Yersinia ruckeri*.

Sensitive to: Florfenicol, Romet 30, Sulfa-methox-trimeth. and Tetracycline.

\* Results faxed Nov. 14/05.

/bb



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## Case Report

**Submission** 2005-03928      **Date** 04-Nov-2005      **Report** 10-Nov-2005

**Report**      **Copies**

**Submitter:** 11036      Mainstream Canada (M)  
**Owner** 15313      Maude, #05-50  
**Farm:**  
**Vet Clinic:** 15092      McKenzie, Peter (Fax: 250-286-0042)  
**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

**History/Symptoms**

Submitter: Tim Talbot.

History of BKD. Recent OTC treatment. PM lesions: large cratered granulomas in liver. Submitted 7 histo cassettes labelled 05-50-1-1, 05-50-1-2, 05-50-3-1, 05-50-3-2, 05-50-4-1, 05-50-5-1, 05-50-6-1.

Please prepare for histo exam for Dr. Gary Marty.

**Histopathology**

Six cassettes of formalin-fixed tissues were received in a plastic bottle containing ethanol.

Slide 1 (05-50-1-1) - head kidney, spleen, liver

Slide 2 (05-50-3-1) - head kidney, spleen, liver

Slide 3 (05-50-4-1) - head kidney, spleen, liver

Slide 4 (05-50-5-1) - trunk kidney, spleen, liver

Slide 5 (05-50-6-1) - trunk kidney, spleen, liver

Slide 6 (05-50-1-2) - trunk kidney, spleen, liver

Slide 6 (05-50-3-2) - trunk kidney, spleen, liver

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

Quality Control: Autolysis is variable of the different slides. For liver, autolysis is mild (slides 3, 4, 6, 7), moderate (slides 1, 2, 5). Organs have no postfixation dehydration and no acid-hematin deposits. The cytoplasm of most of the erythrocytes in these slides fails for stain, and the margins of some organs stain poorly (e.g., spleen in slide 2); this might be related to transfer of tissues to ethanol for shipment (I never see this problem when fixed tissues are left in formalin or are transferred to water for shipment).

Liver (slide 6) - The liver contains multiple foci, each 200-400 µm in diameter, composed of degenerating spherical hepatocytes that seem to be detached from adjacent cells. Nuclei of affected hepatocytes vary from pyknotic to karyorrhectic; cytoplasm is globular and orange.



**Diagnosis**

- 1a. Liver: hepatic necrosis, acute, multifocal, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), moderate (slide 5), severe (slides 2, 4)
- 1b. Liver: vasculitis, multifocal, severe (slide 2), with thrombosis, moderate (slides 4, 5)
- 1c. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slide 4)
- 1d. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 3, 7)
- 1e. Liver: hepatic necrosis, acute, multifocal, moderate (slide 3)
- 1f. Liver: hepatocellular degeneration, multifocal, with globular eosinophilic cytoplasm, moderate (slide 6)
- 1g. Liver: hepatitis, perivascular, lymphocytic, focal, mild (slide 7)
- 1h. Liver: hepatitis, granulomatous, multifocal, with multinucleate giant cells surrounding globular green-yellow material that is consistent with vaccine material, mild (slide 7)
- 1i. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, with intracellular hemosiderin and lipofuscin, mild (slide 1)
- 2a. Spleen: splenitis, histiocytic, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), moderate (slide 2)
- 2b. Spleen: splenitis, granulomatous, multifocal, with multinucleate giant cells surrounding globular green-yellow material that is consistent with vaccine material, moderate (slide 7)
- 2c. Spleen: parenchymal golden pigment, disseminated, intracellular, mild (slide 6)
- 2d. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 3a. Trunk kidney: nephritis, granulomatous, multifocal, with multinucleate giant cells surrounding globular green-yellow material that is consistent with vaccine material, mild (slide 7), moderate (slide 5)
- 3b. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 5, 6, 7)
- 3c. Trunk kidney: interstitial cell hyperplasia, diffuse, moderate (slides 4, 5)
- 4a. Head kidney: moderate numbers of eosinophilic granular cells in interstitial tissue, diffuse (slides 2, 3)



**Final Comments**

: The fish in slides 2, 4, and 5 probably died of complications related to *Piscirickettsia salmonis* infection. Vasculitis with thrombosis in the liver of some of these fish probably is related to *P. salmonis* 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 salmon it might be related to increased protein needed as part of an inflammatory response.

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*). Slide #3 has no obvious organisms. Proliferative lesions in the biliary system are evidence for a toxic cause for the hepatic necrosis.

Some features of the degenerating hepatocytes in slide 6 can occur as a result of autolysis (e.g., dark contracted nuclei, and dissociation of cell-cell adhesion). Features not consistent with autolysis include the globular hypereosinophilic cytoplasm and the fairly discrete margins. The changes are probably a result of exposure to foreign substances, including algal or bacterial toxins.

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

Vaccine material is uncommon in the hepatic, splenic, and renal parenchyma. The vaccine might have been injected directly into a vascular organ (liver? kidney?) of affected fish instead of the peritoneal cavity. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Pigment in the liver could be lipofuscin, hemosiderin, or both. The golden pigment in the spleen most likely is lipofuscin, but probably not hemosiderin. 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 fairly common in pen-reared Chinook salmon. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). Conditions that lead to moderate to abundant 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.

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).

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.

Increased numbers of eosinophilic granular cells in the head kidney is a distinctive finding in Atlantic salmon. 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*. Increased numbers of eosinophilic granular cells are sometimes associated with chronic parasitic infections, but again, the inciting cause was not included in the sections examined.



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.



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**Case Report**

**Submission** 2005-04043      **Date** 17-Nov-2005      **Report** 08-Dec-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15379      A 3.3 - 88 (1-8)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**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

**History/Symptoms**

Submitted Provincial Surveillance program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.3 - 88 (1-8).

Addendum Dec. 2/05 : samples received for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Connective tissue around large foci of erythrocytes has deposits of acid hematin (e.g., spleen in slide 5). Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. Organs have no postfixation dehydration.

Slide 1: autolysis, none (all organs)

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: 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. 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.

Slide 2: autolysis, none (other organs) to mild (liver)

- 1a. Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, focal, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: moderate numbers of eosinophilic granular cells in interstitial tissue, diffuse
5. Head kidney: moderate numbers of eosinophilic granular cells in interstitial tissue, diffuse

Comment: Sinusoidal congestion (sometimes called "peliosis") is evidence of sinusoidal damage. In BC Atlantic salmon, peliosis 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 infections. 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). 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.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Increased numbers of eosinophilic granular cells in the kidney is a distinctive finding in Atlantic salmon. 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*. Increased numbers of eosinophilic granular cells are sometimes associated with chronic parasitic infections, but the inciting cause was not included in the sections examined.

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.

Slide 3: autolysis, none (all organs)

1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: endocarditis, multifocal, lymphohistiocytic, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

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

Slide 4: autolysis, none (other organs) to mild (liver)

1. Liver: hepatic necrosis, acute, multifocal, mild

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate

5. Head kidney: no significant lesions

Comment: 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. 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).

Slide 5: autolysis, none (all organs)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: no significant lesions

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: see other slides

Slide 6: autolysis, none (other organs) to mild (liver)

1a. Liver: hepatic necrosis, acute, multifocal, moderate

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild

1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild

3. Spleen: no significant lesions

4. Trunk kidney: small numbers of eosinophilic granular cells in interstitial tissue, diffuse

5. Head kidney: moderate numbers of eosinophilic granular cells in interstitial tissue, diffuse

Comment: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 7: autolysis, mild (other organs) to severe (liver)



1. Liver: no significant lesions
2. Heart: endocarditis, multifocal, lymphohistiocytic, mild
3. Spleen: splenitis, granulomatous, focal (1 x 1.5 m), moderate
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: The most common organism associated with granulomatous inflammation in cultured salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease.

Slide 8: autolysis, none (other organs) to mild (liver)

- 1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1c. Liver: hepatitis, perivascular, lymphoplasmacytic, multifocal, mild
- 2a. Heart: endocarditis, focal, lymphohistiocytic, mild
- 2b. Heart: epicarditis, multifocal, lymphohistiocytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

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

#### **Molecular Diagnostics/PCR**

Samples 1-4, 5-8 IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* Negative by PCR.

\* Results faxed on Jan. 12/06.

sr/mm





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

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**Case Report**

**Submission** 2005-04044      **Date** 17-Nov-2005      **Report** 09-Dec-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15380      A 3.3 - 86 (1-9)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**Count** 9

**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 Provincial Surveillance program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.3 - 86 (1-9).

Addendum Dec. 2/05 : samples received for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no postfixation dehydration and no acid hematin deposits.

Slide 1: autolysis, none (other organs) to mild (liver)

1a. Liver: peritonitis, diffuse, chronic, with granulation tissue and superficial fibrin, severe

1b. Liver: vasculitis and pericholangitis, diffuse, histiocytic, with occasional multinucleate giant cells, moderate

1c. Liver: hepatic necrosis, acute, multifocal, mild

1d. Liver: basophilic hepatocellular cytoplasm, diffuse, mild

1e. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2. Heart: no significant lesions

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4a. Trunk kidney: nephritis, interstitial, granulomatous, with intralesional amorphous pale staining material, multifocal, mild

4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: not included on the slide

Comment: Most of the liver is covered by a band of granulation tissue admixed with fibrin; the band is 200-300 µm thick. The external tunic of medium-sized vessels in the liver is expanded by macrophages, some of which are multinucleate. I have not seen this pattern of hepatic vasculitis and peritonitis in Atlantic salmon. It probably is a response to chronic immune stimulation; differentials include an unusual vaccine reaction or a bacterial infection. 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 section has no obvious organisms, and an immunohistochemical stain for *P. salmonis* is negative. 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 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.

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

Slide 2: autolysis, mild (other organs) to moderate (liver)

1a. Liver: vasculitis, lymphohistiocytic, with karyorrhexis, multifocal, mild

1b. Liver: hepatic necrosis, acute, multifocal, mild

2. Heart: epicarditis, multifocal, lymphohistiocytic, mild

3. Spleen: no significant lesions

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: no significant lesions

Comment: Vasculitis in the liver is consistent with an active bacterial infection. This section has no obvious organisms, and an immunohistochemical stain for *P. salmonis* is negative. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 3: autolysis, none (other organs) to mild (liver)

1a. Liver: hepatocellular eosinophilic cytoplasmic inclusions, multifocal, mild (slide 1)



- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: 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. The inclusions might be phagocytosed cellular debris or plasma protein, or accumulation of protein synthesized in 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 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. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, moderate
- 1b. Liver: hepatic fibrosis, bifocal (each about 1 mm in, diameter), moderate
- 1c. Liver: peritonitis, diffuse, chronic, with granulation tissue and superficial fibrin, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: not included on the slide

Comment: Foci of hepatic necrosis have some features of infection with *Piscirickettsia salmonis*, but immunohistochemistry is negative for *P. salmonis*. Hepatic fibrosis is an uncommon lesion; it might be a postnecrotic scar.

Slide 5: autolysis, none (other organs) to mild (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: see other slides.

Slide 6: autolysis, none (other organs) to mild (kidney)

- 1a. Liver: vasculitis and hepatic necrosis, acute, multifocal, with abundant intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), severe
- 1b. Liver: peritonitis, diffuse, chronic, with granulation tissue and superficial fibrin, moderate
- 1c. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1d. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1e. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, with intracellular hemosiderin and lipofuscin, mild
2. Heart: endocarditis, multifocal, with endothelial cell hypertrophy, moderate
- 3a. Spleen: peritonitis, diffuse, chronic, with granulation tissue, fibrin, and intrahistiocytic bacteria consistent with *Piscirickettsia salmonis*, moderate
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4a. Trunk kidney and head kidney: moderate numbers of eosinophilic granular cells in interstitial tissue, diffuse
- 4b. Trunk kidney and head kidney: interstitial cell hyperplasia, diffuse, moderate

Comment: This fish probably died of complications related to *Piscirickettsia salmonis* infection. 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). 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.

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.

Increased numbers of eosinophilic granular cells in the kidney is a distinctive finding in Atlantic salmon. Eosinophilic granular cells have been associated with experimental infection of rainbow trout with *Listonella anguillarum*, but I have not seen this pattern of inflammation described in Atlantic salmon exposed to *Listonella anguillarum*. In this case, it probably is related to infection with *P. salmonis*. 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).

Slide 7: autolysis, mild (other organs) to moderate (liver)

1. Liver: yellow to yellow-red pigmented sinusoidal macrophages, disseminated, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: not included on the slide

Comment: Pigment in the liver 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.

Slide 8: autolysis, none (heart) to mild (other organs)

1. Liver: yellow-pigmented sinusoidal macrophages, focal, mild
2. Heart:
3. Spleen:
4. Trunk kidney: renal tubular mineralization, focal, mild
5. Head kidney: not included on the slide

Comment: Pigmented macrophages are in foci 50 - 80 µm in diameter, and nearly all pigmented macrophages are within a focus ~2 mm in diameter. This might represent a resolved focus of hemorrhage or hepatic necrosis. Renal mineralization is common in cultured fish species; when severe, the condition is termed nephrocalcinosis. The lesion is not considered fatal, although feed conversion may be adversely affected. The cause is unknown, 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.

Slide 9: autolysis, none (other organs) to mild (liver)

- 1d. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
- 1e. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- tubular intracytoplasmic protein droplets, multifocal, mild
2. Heart: epicarditis, neutrophilic, histiocytic, diffuse, with abundant intrahistiocytic bacteria (consistent with





*Piscirickettsia salmonis*), severe

3. Spleen, small vessels: endothelial cell hypertrophy, with granular eosinophilic cytoplasm, diffuse, moderate

4a. Trunk kidney, small vessels: endothelial cell hypertrophy, with granular eosinophilic cytoplasm, diffuse, moderate

4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: not included on the slide

Comment: Endothelial cell hypertrophy with granular eosinophilic cytoplasm is a distinctive finding in the

### **Molecular Diagnostics/PCR**

Samples 1-5, 6-9 IHN, VHS, ISA, IPN, Negative by PCR.

Samples 6-9 *Piscirickettsia salmonia* Positive by PCR.

\* Results faxed on Jan. 12/06.

sr/mm

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Ministry of  
Agriculture, Food and Fisheries  
1767 Angus Campbell Road

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

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

**Case Report**

**Submission** 2005-04045      **Date** 17-Nov-2005      **Report** 09-Dec-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15381      A 2.3 - 71 (1-2)

**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 Provincial Surveillance program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 2.3 - 71 (1-2).

Addendum Dec. 2/05 : samples received for routine histology processing and analysis.

**Bacteriology**

n/a

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no postfixation dehydration and no acid hematin deposits.

Slide 1: autolysis, none (other organs) to mild (liver)

1a. Liver: vasculitis and pericholangitis, diffuse, histiocytic, with occasional karyorrhexis, moderate

1b. Liver: hepatic necrosis, acute, multifocal, with abundant intrahistiocytic and intrahepatocellular bacteria consistent with *Piscirickettsia salmonis*, moderate

1c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild

1d. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2a. Heart: epicarditis, neutrophilic, histiocytic, multifocal, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), mild

2b. Heart: endothelial cell hypertrophy and hyperplasia, multifocal, mild

3a. Spleen: small vessels: endothelial cell hypertrophy, with granular eosinophilic cytoplasm, diffuse, severe

3b. Spleen, medium-sized vessels: vasculitis, multifocal, acute, with karyorrhexis, moderate

4a. Trunk kidney: small vessels: endothelial cell hypertrophy, with granular eosinophilic cytoplasm, diffuse, moderate

4b. Trunk kidney: peritonitis, diffuse, with edema and histiocytes with abundant intracellular bacteria consistent with *Piscirickettsia salmonis*, moderate

4c. Trunk kidney, medium-sized vessels: vasculitis, multifocal, acute, with karyorrhexis, mild

5. Head kidney: small vessels: endothelial cell hypertrophy, with granular eosinophilic cytoplasm, diffuse, moderate

Comment:

This fish probably died of complications related to infection with *Piscirickettsia salmonis*. 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 (basophilic cytoplasm) in the heart is consistent with a systemic immune stimulation, probably a bacterial infection.

Endothelial cell hypertrophy with granular eosinophilic cytoplasm is a distinctive finding in the kidney and spleen of Atlantic salmon. Endothelial cell hypertrophy probably is related to systemic immune stimulation (e.g., a bacterial infection). Here, endothelial cell hypertrophy in small vessels and vasculitis of medium-sized vessels probably is a result of infection with *Piscirickettsia salmonis*.

Slide 2: autolysis, mild (other organs) to moderate (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2. Heart: no significant lesions

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

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



**Molecular Diagnostics/PCR**

Samples 1-2 IHN, VHS, ISA, IPN, Negative by PCR.

Samples 1-2 *Piscirickettsia salmonia* Suspect by PCR.

\* Results faxed on Jan. 12/06.

sr/mm

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Agriculture, Food and Fisheries  
1767 Angus Campbell Road

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

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

**Case Report**

**Submission** 2005-04046      **Date** 17-Nov-2005      **Report** 13-Dec-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15382      A 3.3 - 92 (1-12)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**Count** 12

**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 Provincial Surveillance program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.3 - 92 (1-12).

Addendum Dec. 2/05 : samples received for routine histology processing and analysis.



**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no postfixation dehydration. Large foci of erythrocytes have deposits of acid hematin (e.g., liver in slide 9). Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (all organs)

1. Liver: not included on the slide
2. Heart: mural thrombosis, bifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, none (other organs) to mild (liver)

1. Liver: 1. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2a. Heart: mural thrombosis, focal, mild
- 2b. Heart: endocarditis, multifocal, lymphohistiocytic, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

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

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: epicarditis, focal, lymphoplasmacytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: 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). Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, moderate
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: 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. 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. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Slide 5: autolysis, none (all organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
  - 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
  2. Heart: no significant lesions
  3. Spleen: no significant lesions
  4. Trunk kidney: no significant lesions
  5. Head kidney: not included on the slide
- Comment: see other slides

Slide 6: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
  2. Heart: no significant lesions
  3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
  4. Trunk kidney: no significant lesions
  5. Head kidney: not included on the slide
- Comment: see other slides

Slide 7: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
  2. Heart: no significant lesions
  3. Spleen: peritonitis, granulomatous, regionally diffuse, with neutrophils, occasional fine fibrocellular fronds, with fibrocellular fronds, moderate
  4. Trunk kidney: no significant lesions
  5. Head kidney: not included on the slide
- Comment: Splenic peritonitis is consistent with a vaccine reaction.

Slide 8: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
  - 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
  2. Heart: no significant lesions
  - 3a. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
  - 3b. Spleen: peritonitis, granulomatous, regionally diffuse, with neutrophils, occasional fine fibrocellular fronds, with fibrocellular fronds, moderate
  4. Trunk kidney: no significant lesions
  5. Head kidney: not included on the slide
- Comment: see other slides

Slide 9: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
  2. Heart: no significant lesions
  3. Spleen: no significant lesions
  4. Trunk kidney: no significant lesions
  5. Head kidney: no significant lesions
- Comment: see other slides



Slide 10: autolysis, none (other organs) to mild (liver and trunk kidney)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide
6. Mesenteric fat: peritonitis, chronic, focal, with fibrocellular fronds, mild

Comment: Peritonitis of mesenteric fat is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 11: autolysis, none (other organs) to mild (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: see other slides

Slide 12: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: see other slides

### **Molecular Diagnostics/PCR**

Samples 1-4, 5-8, 9-12 IHN, VHS, ISA, IPN, Piscirickettsia salmonis Negative by PCR.

\* Results faxed on Jan. 12/06.

sr/mm



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Ministry of  
Agriculture, Food and Fisheries  
1767 Angus Campbell Road

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

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

**Case Report**

**Submission** 2005-04047      **Date** 17-Nov-2005      **Report** 13-Dec-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15383      A 2.3 - 72 (1-5)

**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

**History/Symptoms**

Submitted Provincial Surveillance program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 2.3 - 72 (1-5).

Addendum Dec. 2/05 : samples received for routine histology processing and analysis.



**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no postfixation dehydration and no acid hematin deposits.

Slide 1: autolysis, none (other organs) to mild (liver)

1a. Liver: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: no significant lesions

3. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate

4. Trunk kidney: not included on the slide

5. Head kidney: no significant lesions

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

Slide 2: autolysis, none (other organs) to mild (liver)

1a. Liver: hepatitis, granulomatous, focal (~200 µm in diameter), with multinucleate giant cells, mild

1b. Liver: hepatitis, perivascular, pericholangial, lymphocytic, multifocal, mild

1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2. Heart: no significant lesions

3a. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate

3b. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: no significant lesions

Comment: Multinucleate giant cells in the focus of granulomatous hepatitis are consistent with reaction to foreign material (e.g., vaccine material). 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.

Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Lymphocytic inflammation around bile ductules and vessels in the 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 or vascular system. 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.

Slide 3: autolysis, none (other organs) to mild (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild

2. Heart: no significant lesions

3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide



Comment: see other slides

Slide 4: autolysis, none (other organs) to mild (liver)

1a. Liver: haematopoiesis, perivascular, focal (~500 µm in diameter), mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

1c. Liver: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, mild

2. Heart: no significant lesions

3a. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate

3b. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: not included on the slide

Comment: The focus of haematopoiesis in the liver is uncommon, but not necessarily a lesion. Other haematopoietic organs (kidney and spleen) are morphologically within normal limits; therefore, the single focus of haematopoiesis in liver might be a case of normal tissue in an abnormal spot. Species like sturgeon normally have haematopoiesis in the liver.

Slide 5: autolysis, none (other organs) to mild (liver)

1a. Liver: pericholangial fibrosis, with eosinophilic granular cells, focal (~2 x 0.5 mm), moderate

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2. Heart: endocarditis, multifocal, lymphohistiocytic, mild

3a. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate

3b. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: no significant lesions

Comment: Pericholangial fibrosis is an unusual finding. Fibrosis usually follows some type of tissue damage, and eosinophilic granular cells are sometimes associated with bacterial infections.

### **Molecular Diagnostics/PCR**

Samples 1-3, 4-5 IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* Negative by PCR.

\* Results faxed on Jan. 12/06.

sr/mm



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

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

**Case Report**

**Submission** 2005-04048      **Date** 17-Nov-2005      **Report** 17-Nov-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15384      A 3.4 - 90 (1-7)

**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 Provincial Surveillance program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.4 - 90 (1-7).

Addendum Dec. 2/05: samples received for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. The margins of some organs (e.g., spleen on slide 1) have evidence of dehydration after fixation (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. Tissues have no deposits of acid hematin.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

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

Slide 2: autolysis, none (all organs)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: 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).

Slide 3: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: mural thrombosis, bifocal, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

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

Slide 4: autolysis, none (other organs) to mild (liver, kidney)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: endocarditis, multifocal, with endothelial cell hypertrophy and a thin layer of lymphocytes, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface in foci of inflammation are rarely more than 2 cell layers thick.

Slide 5: autolysis, none (other organs) to mild (liver)

- 1a. Liver: pericholangitis, lymphocytic, multifocal, with karyorrhexis, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: not included on the slide
- 3a. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate





4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

Comment: 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. 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. 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.

Slide 6: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2. Heart: no significant lesions

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

Comment: see other slides

Slide 7: autolysis, none (all organs)

1a. Liver: hepatitis, focal (400 µm in diameter), with neutrophils, lymphocytes, and pale golden pigment, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2. Heart: epicarditis and endocarditis, granulomatous, diffuse, moderate

3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: no significant lesions

Comment: Focal pigment and mild inflammation in the liver might be site of previous necrosis that is now resolving, with accumulation of lipofuscin pigment. *Renibacterium salmoninarum*, the cause of bacterial kidney disease, is the most common organism associated with granulomatous inflammation in the heart of per-reared salmon. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.

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.

### **Molecular Diagnostics/PCR**

Samples 1-4, 5-7 IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* Negative by PCR.

\* Results faxed on Jan. 12/06.

sr/mm



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**Case Report**

**Submission** 2005-04101      **Date** 24-Nov-2005      **Report** 30-Nov-2005

**Report**      **Copies**

**Submitter:** 11036 Mainstream Canada (M)

**Owner** 11036 Mainstream Canada (M)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Whole Animal

**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

**History/Symptoms**

Submitted 5 fish. Please do bacteriology on two fish and virology - 1 pool from 5 fish (PCR and cell culture).

This site has Atlantic smolts of 160 g, Sea entry in September. The water temperature is around 8.0 C.

Francisco.miranda.morales@mainstreamcanada.com

**Gross Pathology**

Five Atlantic salmon (each 18 - 20 cm long) were received in a lock-seal plastic bag and surrounded by 2 packs of gel ice. Samples of kidney and spleen were removed and pooled from all 5 fish for virology (cell culture) and IHN PCR. The kidney of the two freshest fish was swabbed for bacteriology. The left eye of one fish had been enucleated. The left pectoral girdle and heart had been excised from another fish; lack of hemorrhage at the margins is evidence that the tissues were removed after death (by birds?).

**Bacteriology**

See attached report.

**Virology**

Fish viruses negative - by culture.

\* Results faxed on Dec 21/05.

**Molecular Diagnostics/PCR**

IHN Virus Negative by PCR.

**Final Comments**

*Hafnia alvei* is a Gram-negative, facultative, anaerobic bacillus of the family Enterobacteriaceae that may occur as a gastrointestinal commensal. It has been described from natural environments such as soil, sewage and water, and it has also been isolated from different kinds of foodstuffs. In freshwater aquaculture, it has been associated with epizootic haemorrhagic septicaemia in rainbow trout, kidney lesions in cherry salmon (*Oncorhynchus masou*), and as the causal agent of mortalities in brown trout after intraperitoneal injection. This is the first time I have seen *Hafnia alvei* cultured from Atlantic salmon. Because this organism is a common contaminant, I recommend bacterial culture from freshly dead fish to better determine the significance of this isolate. Source:

Padilla, D., F. Real, V. Gómez, E. Sierra, B. Acosta, S. Déniz, and F. Acosta. 2005. Virulence factors and pathogenicity of *Hafnia alvei* for gilthead seabream, *Sparus aurata* L. J. Fish Dis. 28(7):411-417.

/bb/mm

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**Case Report**

**Submission** 2005-04146      **Date** 29-Nov-2005      **Report** 01-Dec-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 15443 #5474, Marine Harvest

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Multiple Specimens

**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 4 samples for virology tissue culture and PCR for iHNV and VHSV labelled 1-3, 4-6, 7-9, 10-12, 1 plate for ID/confirmation of Yersinia ruckeri and 3 histo cassettes - suspect ERM.

\*\* PO# CL5355.

### **Bacteriology**

See attached sheet.

### **Histopathology**

3 cassettes of preserved tissues were submitted for histopathology in formalin. After processing routinely into paraffin, the gills and one or two other organs were removed from each cassette and embedded separately for sectioning.

Slide 1A (Thorpe 10) - gill, trunk kidney, and spleen

Slide 1B (Thorpe 10) - liver, heart, head kidney, intestine, stomach, intestinal ceca and mesenteric fat

Slide 2A (Thorpe 11) - gill, intestinal ceca and mesenteric fat

Slide 2B (Thorpe 11) - liver, heart, trunk kidney, and spleen

Slide 3A - gill, trunk kidney, and mesenteric fat

Slide 3B - liver, heart, head kidney, spleen, 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 for most organs is excellent; intestinal villi sometimes have mild autolysis. Organs have no postfixation dehydration and no acid hematin deposits.

Measures of physiologic condition:

Hepatocellular glycogen depletion, severe (slides 1B, 2B)

Mesenteric adipose tissue depletion: none (slides 1B, 2A, 3B)

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 in the past few days (severe hepatocellular glycogen depletion).

### **Virology**

4 samples inoculated onto tissue culture - all negative.

\* Results faxed on Jan 3/06.





**Molecular Diagnostics/PCR**

IHN virus and VHS virus negative by PCR.

\* Results faxed on Dec 5/05.

**Diagnosis**

1. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages, moderate (slides 1B, 2B, 3B)
2. Head kidney: interstitial cell hyperplasia, diffuse, moderate (slides 1B, 3B)
- 3a. Mesenteric fat and exocrine pancreas: vasculitis, focal, neutrophilic, mild (slides 1B, 2A)
- 3b. Mesenteric fat and exocrine pancreas: steatitis and pancreatitis, focal, granulomatous, mild (slide 2A), moderate (slide 3A); multifocal, moderate (slide 3B)
- 4a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slides 1B, 2B, 3B)
- 4b. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1B, 2B, 3B)
- 5a. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (slides 2B, 3A)
- 5b. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 1A, 2B)
6. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate (slides 2B, 3B)

**Final Comments**

Several features of this fish are consistent with a systemic bacterial infection (e.g., with *Yersinia ruckeri*). Inflammatory cells lining the endocardial surface throughout most of the ventricle are rarely more than 2 cell layers thick. 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). Other potentially related lesions include vasculitis in a vessel in the mesenteric fat. Many of the vessels in these sections contain more inflammatory cells than red blood cells (e.g., liver in slide 3B); were these fish anemic?

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. 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).

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.

Peritonitis in the region of the spleen and mesenteric fat is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

/bb/mm



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**Case Report**

**Submission** 2005-04151      **Date** 29-Nov-2005      **Report** 17-Jan-2006

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15447      A 2.4-74

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture and PCR positive samples.

Sample ID: A 2.4 - 74.

HP - 2005-4318.

**Molecular Diagnostics/PCR**

Infectious Hematopoietic Necrosis virus negative by PCR.

Infectious Pancreatic Necrosis virus negative by PCR.

Infectious Salmon Anemia virus negative by PCR.

Piscirickettsia salmonis negative by PCR.

Viral Hemorrhagic Septicemia virus negative by PCR.

\* Results faxed Jan. 17/06.

/bb

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**Case Report**

**Submission** 2005-04153      **Date** 29-Nov-2005      **Report** 14-Dec-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15449      A 2.4 - 76 (1-5)

**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

**History/Symptoms**

Submitted Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture and PCR positive samples.

Sample ID: A 2.4 - 76 (1-5).

Addendum Dec. 2/05 - samples received for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no postfixation dehydration. Large foci of erythrocytes have deposits of acid hematin (e.g., liver in slide 3). Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, abundant
- 1c. Liver: hepatitis, perivascular, lymphocytic, focal, mild
- 2. Heart: epicarditis, focal, lymphoplasmacytic, mild
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

Comment: 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. Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Lymphocytic inflammation around a vessel in the liver is evidence of chronic immune stimulation, e.g., from a bacterial infection. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, none (all organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1c. Liver: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 1d. Liver: parenchymal golden pigment, disseminated, intracellular, mild
- 1e. Liver: hepatitis, perivascular, lymphocytic, focal, mild
- 2. Heart: epicarditis, regionally diffuse, lymphoplasmacytic, mild
- 3a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 3b. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

Comment: 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 peritonitis is consistent with a reaction to foreign material; it sometimes occurs in fish that have been vaccinated. The golden pigment in the liver and spleen most likely is lipofuscin; the liver pigment might also include hemosiderin. 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.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2. Heart: no significant lesions
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: interstitial congestion, diffuse, moderate
- 5. Head kidney: not included on the slide

Comment: 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). Renal congestion and hemorrhage has been associated with VHSV, bacteria, and it may occur as a sampling artifact. Renal congestion and hemorrhage is one of the classic signs of infectious salmon anemia (ISA), but ISA has never been isolated from fish in BC.

Slide 4: autolysis, none (other organs) to mild (liver)

1a. Liver: biliary preductular cell hyperplasia, diffuse, moderate

1b. Liver: cholangitis, lymphocytic, with biliary hyperplasia, focal (300 x 150 µm), mild

1c. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: no significant lesions

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney and Corpuscle of Stannius: no significant lesions

5. Head kidney: not included on the slide

Comment: Lymphocytic cholangitis is an uncommon variant of the more common preductular cell hyperplasia; the affected focus has lymphocytic inflammation that might have resulted from focal accumulation of bacteria.

Slide 5: autolysis, none (other organs) to mild (liver)

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild

1b. Liver: parenchymal golden pigment, disseminated, intracellular, mild

2. Heart: no significant lesions

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

Comment: see other slides

### **Molecular Diagnostics/PCR**

Infectious Hematopoietic Necrosis virus negative by PCR.

Infectious Pancreatic Necrosis virus negative by PCR.

Infectious Salmon Anemia virus negative by PCR.

Piscirickettsia salmonis negative by PCR.

Viral Hemorrhagic Septicemia virus negative by PCR.

\* Results faxed Jan. 19/06.

/bb



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**Case Report**

**Submission** 2005-04154      **Date** 29-Nov-2005      **Report** 07-Dec-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15450      A 3.4 - 90 (1, 5, 6)

**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

**History/Symptoms**

Submitted Provincial Surveillance Program samples for bacteriology - identification of isolates and culture and sensitivity.

Sample ID: A 3.4 - 90 (1, 5, 6).

**Bacteriology**

See attached sheet.

Isolates did not grow on subculture, therefore antibiotic sensitivities were unable to be performed. Isolate A3.4-90 (6) was identified as *Pseudoalteromonas porphyrae* by PCR

\* Results faxed Dec. 7/05.

/bb

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**Case Report**

**Submission** 2005-04162      **Date** 30-Nov-2005      **Report** 02-Dec-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 15454 #5477, 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 histology cassette.

One moribund with hemorrhage of liver. Mortality low at this site. Regular, saltwater entry 2005, Tank ID: 10.

PO# 2562.

### **Histopathology**

1 cassette of preserved tissues was submitted for histopathology in formalin. After processing routinely into paraffin, the organs were split into two cassettes and embedded separately for sectioning.

Slide 1A (Pen 10) - liver, heart, spleen, ovary, skeletal muscle and bone, intestinal ceca and mesenteric fat

Slide 1B (Pen 10) - head kidney, trunk kidney, intestine, gill, and mesenteric fat

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

Quality control: Tissue preservation for most organs is excellent; the tips of intestinal villi in slide 1B have mild autolysis. Organs have no postfixation dehydration. Acid hematin deposits are limited to foci of sinusoidal congestion in the liver.

Measures of physiologic condition:

Hepatocellular glycogen depletion, severe (slide 1A)

Mesenteric adipose tissue depletion: none (slide 1B)

This pattern in the measures of physiologic condition is consistent with a fish that was eating and growing (no depletion of mesenteric fat) but stopped feeding in the past few days (severe hepatocellular glycogen depletion).

### **Diagnosis**

1a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slides 1A)

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1A)

1c. Liver: sinusoidal congestion, acute, multifocal, mild (slide 1A)

2a. Mesenteric fat: peritonitis, multifocal, granulomatous, mild (slide 1B)



**Final Comments**

This fish has microscopic changes consistent with the clinical history. 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. 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).

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. Consider bacteriology and virology and PCR for VHSV, IHNV, and ISAV (if not already done). 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).

Accumulation of acid hematin in the foci of congestion in the liver, and not elsewhere, is evidence that the congested areas were anaerobic, with lactic acid build-up before the fish died.

Peritonitis of mesenteric fat is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

/bb





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**Case Report**

**Submission** 2005-04163      **Date** 30-Nov-2005      **Report** 02-Dec-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 15455 #5478, 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 histology cassette. No growth on Bac-T.

Regular.

PO# 2565.

**Histopathology**

1 cassette of preserved tissues was submitted for histopathology in formalin. After processing routinely into paraffin, the organs were split into two cassettes and embedded separately for sectioning.

Slide 1A (cassette label = "3") - gill, intestinal ceca and mesenteric fat

Slide 1B (cassette label = "3"; special stains = lipofuscin and PAS) - liver, heart, spleen, head kidney, trunk kidney, intestine and mesenteric fat

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

Quality control: Tissue preservation for most organs is excellent; the tips of intestinal villi in slide 1B have mild autolysis. Organs have no postfixation dehydration. Acid hematin deposits are limited to foci of sinusoidal congestion in the liver.

Measures of physiologic condition:

Hepatocellular glycogen depletion, severe (slide 1A)

Mesenteric adipose tissue depletion: none (slide 1B)

This pattern in the measures of physiologic condition is consistent with a fish that was eating and growing (no depletion of mesenteric fat) but stopped feeding in the past few days (severe hepatocellular glycogen depletion).

Trunk kidney (slide 1B) - The mesonephric duct and most of the collecting ducts are lined by hyperplastic and dysplastic epithelial cells. Epithelial cells are irregularly piled two cells deep, and the axis of the nuclei is often oblique and even perpendicular to normal. Some nuclei are slightly larger than normal, and chromatin is vesiculated. Cytoplasm is fairly normal (pale and eosinophilic). Affected tubules rarely contain single necrotic epithelial cells (characterized by condensed hypereosinophilic cytoplasm). The renal interstitium contains scattered cells (about 1 per 40x objective lens field) with cytoplasm distended up to 40 µm by homogeneous glycoprotein (PAS positive); nuclei of these cells are eccentric, oval, and hyperchromatic.



**Diagnosis**

- 1a. Trunk kidney, collecting ducts and mesonephric ducts: renal tubular epithelial hyperplasia, multifocal, with rare single cell necrosis, moderate (slide 1B)
- 1b. Trunk kidney: interstitial cell cytoplasmic distension by homogenous protein deposits, scattered, mild (slide 1B)
- 1c. Trunk kidney: glomerulonephritis, membranous, diffuse, mild (slide 1B)
- 2a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slide 1B)
- 2b. Liver: sinusoidal congestion, with intracytoplasmic eosinophilic to golden hepatocellular inclusions, acute, multifocal, mild (slide 1B)
- 3a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1B)

**Final Comments**

Renal tubular epithelial 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). The pattern of hyperplasia in the renal tubules is unusual, and it is different from any I have seen.

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.

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 infections. Consider bacteriology and virology and PCR for VHSV, IHNV, and ISAV (if not already done). 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 peliosis in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The hepatocellular cytoplasmic inclusions are large, up to twice the size of hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes; they stain negative for lipofuscin. Accumulation of acid hematin in the foci of congestion in the liver, and not elsewhere, is evidence that the congested areas were anaerobic, with lactic acid build-up before the fish died.

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.

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

/bb



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1767 Angus Campbell Road

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

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**Case Report**

**Submission** 2005-04173      **Date** 01-Dec-2005      **Report** 02-Dec-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 15467 #5487, Marine Harvest

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Other

**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

**History/Symptoms**

Submitted 5 histology cassettes.

Increase in mortality. Many moribund.

PO#2583.

**Histopathology**

5 cassettes of preserved tissues were submitted for histopathology in formalin. After processing routinely into paraffin, the gills were removed from each cassette and embedded separately for sectioning. Some of the labels on the cassettes could not be read completely after processing; best estimates are in parentheses below.

Slide 1A (5487-4) - gill

Slide 1B (5487-4) - liver, spleen, heart, head kidney, skeletal muscle with a little bit of trunk kidney, intestinal ceca and mesenteric fat

Slide 2A (ATT?002) - gill

Slide 2B (ATT?002) - liver, spleen (small piece), heart, head kidney, trunk kidney, intestinal ceca and mesenteric fat

Slide 3A (A?03 5487-3) - gill

Slide 3B (A?03 5487-3) - liver, heart, head kidney, trunk kidney, spleen, stomach, intestinal ceca and mesenteric fat

Slide 4A (?1) - gill

Slide 4B (?1) - liver, heart, head kidney, trunk kidney, spleen, intestinal ceca and mesenteric fat

Slide 5A (A?005 5487-5) - gill

Slide 5B (A?005 5487-5) - liver, heart, head kidney, trunk kidney, spleen, 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 for all organs is excellent. Organs have no postfixation dehydration and no acid hematin deposits.

Measures of physiologic condition:

Hepatocellular glycogen depletion, severe (slides 1B, 2B, 3B, 5B), moderate (slide 4B)

Mesenteric adipose tissue depletion: none (slides 1B, 2B, 3B, 4B, 5B)

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 in the past few days (moderate to severe hepatocellular glycogen depletion).





### **Diagnosis**

1. Head kidney: interstitial cell hyperplasia, diffuse, moderate (slide 1B)
2. Spleen and mesenteric fat: peritonitis, granulomatous, multifocal, with intralesional radiating clefts in foci about 50 µm in diameter, mild (slides 2B, 3B), moderate (slides 1B, 4B, 5B)
3. Mesenteric fat and intestinal ceca: and pancreatitis, focal, granulomatous, mild (slides 2B, 3B, 4B, 5B)
- 4a. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 3B, 5B), moderate (slides 2B, 4B)
- 4b. Trunk kidney: renal tubular mineralization, multifocal, mild (slide 3B)
5. Extrahepatic bile ductule: intraluminal mineral, focal mild (slide 5B)

### **Final Comments**

Interstitial cell hyperplasia in the kidney (slide 1B) 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). Most of the hyperplastic cells in slide 1B seem to be forming neutrophils.

Peritonitis and steatitis 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 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.

Renal mineralization is common in cultured fish species; when severe, the condition is termed nephrocalcinosis. The lesion is not considered fatal, although feed conversion may be adversely affected. The cause is unknown, 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.

The single focus of mineral in an extrahepatic bile ductule (slide 5B) probably is of little significance.

/bb



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**Case Report**

**Submission** 2005-04241      **Date** 08-Dec-2005      **Report** 23-Dec-2005

**Report**      **Copies**

**Submitter:** 11899 Grieg Seafoods B.C. Ltd.

**Owner** 11899 Grieg Seafoods B.C. Ltd.

**Farm:**

**Vet Clinic:** 11899 Grieg Seafoods B.C. Ltd.

**Attending** Dr. B. Milligan

**Specimen:** Tissue - Fresh

**Count** 3

**Flock Herd Size:**

**Species:** Atlantic Salmon

**Age**

**Breed:** Mowi

**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 fresh tissue samples for PCR for *Piscirickettsia salmonis*.

Background levels of BKD at both sites, negligible mortality. Suspect *P. salmonis* and would like to confirm.

2-5mm yellow, coalescing nodules in liver, white.

#1 - Barnes Bay.

#2 - Williamson.

**Molecular Diagnostics/PCR**

Samples: Barnes Bay, Williamson, and WP2 - *Piscirickettsia salmonis* negative by PCR.

/mm

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**Case Report**

**Submission** 2005-04258      **Date** 09-Dec-2005      **Report** 17-Jan-2006

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15498      A 2.3 - 82 (1-4)

**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 Provincial Surveillance Program samples for Virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.2 - 82 (1-4).

HP - 2005-4319.

**Molecular Diagnostics/PCR**

Infectious Hematopoietic Necrosis virus negative by PCR.

Infectious Pancreatic Necrosis virus negative by PCR.

Infectious Salmon Anemia virus negative by PCR.

Piscirickettsia salmonis negative by PCR.

Viral Hemorrhagic Septicemia virus negative by PCR.

\* Results faxed Jan. 17/06.

/bb

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**Case Report**

**Submission** 2005-04259      **Date** 09-Dec-2005      **Report** 17-Jan-2006

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15499      A 3.4 - 91 (1-2)

**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 Provincial Surveillance Program samples for Virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.4 - 91 (1-2).

HP - 2005-4322.

**Molecular Diagnostics/PCR**

Infectious Hematopoietic Necrosis virus negative by PCR.

Infectious Pancreatic Necrosis virus negative by PCR.

Infectious Salmon Anemia virus negative by PCR.

Piscirickettsia salmonis negative by PCR.

Viral Hemorrhagic Septicemia virus negative by PCR.

\* Results faxed Jan. 17/06.

/bb

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**Case Report**

**Submission** 2005-04281      **Date** 13-Dec-2005      **Report** 23-Dec-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 15515 #5507, 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 2 samples for virology (tissue culture) and IHN - PCR and VHS - PCR.

Please quote PO# 2679 for billing purposes.

Regular, 2004 saltwater entry.

**Virology**

Two samples inoculated onto tissue culture - both negative.

\* Results faxed on Jan 9/06.

**Molecular Diagnostics/PCR**

IHNV and VHSV negative by PCR.

/mm

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**Case Report**

**Submission** 2005-04282      **Date** 13-Dec-2005      **Report** 23-Dec-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 15516 #5492, 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 bac-t plate for identification.

Fish with clinical signs of *A. salmonicida* (Furunculosis).

PO#2689

**Bacteriology**

Bact plate/slope - *Photobacterium iliopiscarium*.

/mm

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**Case Report**

**Submission** 2005-04283      **Date** 13-Dec-2005      **Report** 15-Dec-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 15517 #5502, 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 bac-t plate for identification.

Previous ERM at site.

Regular.

PO# 2690.

**Bacteriology**

Bact plate - *Yersinia ruckeri*.

/sr

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## Case Report

**Submission** 2005-04318      **Date** 16-Dec-2005      **Report** 23-Dec-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15534      A.2.4-74 (1-3)

**Farm:**

**Vet Clinic:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**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



**History/Symptoms**

Provincial Surveillance Program. Samples for routine histology processing and analysis.

ID: A.2.4-74 (1-3)

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no post fixation dehydration. Large foci of erythrocytes have deposits of acid hematin (e.g., spleen in slide 1). Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (all organs)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1c. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild
- 2. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

Comment: 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. 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.

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).

Slide 2: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 2. Heart: no significant lesions
- 3. Spleen: no significant lesions
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: see slide #1.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1c. Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical amphophilic inclusions, acute, focal (~500 µm in diameter), mild
- 2. Heart:
- 3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: not included on the slide

Comment: Sinusoidal congestion (sometimes called "peliosis") is evidence of sinusoidal damage. In BC Atlantic salmon, peliosis 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 infections.



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). The 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 the congested focus, 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. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

/mb

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**Case Report**

**Submission** 2005-04319      **Date** 16-Dec-2005      **Report** 23-Dec-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15535      A.3.2-82 (1-4)

**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

**History/Symptoms**

Provincial Surveillance Program. Samples for routine histology processing and analysis.

ID: A.3.2-82 (1-4).

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no post fixation dehydration. Large foci of erythrocytes have deposits of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: 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 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.

Slide 2: autolysis, none (other organs) to mild (liver)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: mural thrombosis, focal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

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

Slide 3: autolysis, none (heart) to mild (other organs)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, severe
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

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

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1c. Liver: sinusoidal congestion, with acid hematin granules, acute, multifocal, mild
- 2a. Heart: endocarditis, multifocal, with endothelial cell hypertrophy and scattered foci of macrophages, moderate
- 2b. Heart: endocarditis, focal, granulomatous (a single multinucleate giant cell), mild
3. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: 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.

Sinusoidal congestion (sometimes called "peliosis") 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 infections. 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). 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.

The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Endocardial cells are up to 15 µm thick; some inflammatory foci are primarily hypertrophic endothelial cells, whereas others are primarily macrophages with a few lymphocytes. Inflammation is most prominent at the junction of the inner trabecular and outer compact regions of the heart. The multinucleate giant cell is evidence of foreign material (vaccine?).

/mb



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**Case Report**

**Submission** 2005-04322      **Date** 16-Dec-2005      **Report** 23-Dec-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 15538      A.3.4-91 (1-2)

**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

**History/Symptoms**

Provincial Surveillance Program. Samples for routine histology processing and analysis.

ID: A.3.4-91 (1-2).

**Histopathology**

Quality control/quality assurance: tissues were preserved in 10% neutral buffered formalin for at least 24 hours and then transferred to fresh water for shipment; tissues were processed routinely into paraffin, including initial immersion in formalin. Organs have no postfixation dehydration and no deposits of acid hematin.

Slide 1: autolysis, mild (all organs)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: These organs have no changes to help explain the death of this fish.

Slide 2: autolysis, none (heart) to mild (other organs)

1. Liver: no significant lesions
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney:
5. Head kidney: no significant lesions

Comment: 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 net pen liver disease). Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

/mb



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**Case Report**

**Submission** 2005-04399      **Date** 29-Dec-2005      **Report** 12-Jan-2006

**Report**      **Copies**

**Submitter:** 11899      Grieg Seafoods B.C. Ltd.

**Owner** 11899      Grieg Seafoods B.C. Ltd.

**Farm:**

**Vet Clinic:**

**Attending**      Dr. B. Milligan

**Specimen:** Tissue - Formalized

**Count** 4

**Flock Herd Size:**

**Species:** Atlantic Salmon

**Age**

**Breed:** Mowi

**Sex:**

**Feed:**

**Feed**

**Vaccination**

**Treatmen**

**Diagnosis**

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

**History/Symptoms**

Farm name: Lutes.

As concurrent histology submission. Request PCR - BKD, IHN. (See case 2005-4400).

**Molecular Diagnostics/PCR**

Pools #1, 2, 3, 4, 5 Renibacterium salmoninarum and IHN Virus Negative by PCR.

\* Results faxed on Jan 12/06.

/mm

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**Case Report**

**Submission** 2005-04400      **Date** 29-Dec-2005      **Report** 10-Jan-2006

**Report**      **Copies**

**Submitter:** 11899      Grieg Seafoods B.C. Ltd.

**Owner** 11899      Grieg Seafoods B.C. Ltd.

**Farm:**

**Vet Clinic:**

**Attending**      Dr. B. Milligan

**Specimen:** Tissue - Formalized

**Count** 4

**Flock Herd Size:**

**Species:** Atlantic Salmon

**Age**

**Breed:** Mowi

**Sex:**

**Feed:**

**Feed**

**Vaccination**

**Treatmen**

**Diagnosis**

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



**History/Symptoms**

Farm Name: Lutes

Age: smolt 2006G.

Group size: 1,000,000

Myxobacterial stomatitis 10 - 14 days post-saltwater entry; severity worse in several pens from one particular hatchery; negligible mortality prior to 10 days post-entry and no significant bacteriology or virology on routine fish health checks.

Tx: none - not eating enough at time of sampling (since sampling, on a tribrissen treatment).

PM lesions: oral ulcers (mandible/maxilla/tongue) with yellow filamentous bacteria mats.

Specimens submitted: multi-organ, 10 fish 10112 Dec 22/05, 10 fish 14116 Dec 26/05.

Request histology. Concurrent virology/bacteriology/BKD ELISA's being run.



**Histopathology**

Ten cassettes of preserved tissues were submitted for histopathology in formalin. After processing routinely into paraffin, the gills were removed from each cassette and embedded separately for sectioning. For all slides, slide A has multiple organs (usually heart, liver, head kidney, trunk kidney, spleen, and stomach/intestinal ceca), and slide B has the gills (2-3 arches). Several slides contain sections of 2 pieces of liver. Slide 1-4A contains ovary.

Slide 1-1 - Pen 10/12 Lutes 10/12  
Slide 1-2 - Pen 10/12 Lutes Dec 22  
Slide 1-3 - Pen 10/12 Lutes Dec 22  
Slide 1-4 - Pen 10/12 Lutes Dec 22  
Slide 1-5 - Pen 10/12 Lutes Dec 22  
Slide 2-1 - Pen 14/16 Lutes Dec 26  
Slide 2-2 - Pen 14/16 Lutes Dec 26  
Slide 2-3 - Pen 14/16 Lutes Dec 26  
Slide 2-4 - Pen 14/16 Lutes Dec 26  
Slide 2-5 - Pen 14/16 Lutes Dec 26

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

Quality control: Autolysis for the liver varies from mild (slide 2-5), moderate (slides 1-2, 1-3, 1-5, 2-1, 2-2, 2-3), to severe (slides 1-1, 1-2, 1-3, 1-4, 2-1, 2-4). Large foci of erythrocytes have deposits of acid hematin (e.g., liver in slide 1-2). 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 (all slides)

Mesenteric adipose tissue depletion: none (all slides)

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 (moderate to severe hepatocellular glycogen depletion).



**Diagnosis**

- 1a. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 0.2 - 1 mm in diameter, moderate (slides 1-1, 1-4, 2-1, 2-2, 2-3, 2-4, 2-5), severe (slide 1-2)
- 1b. Spleen: peritonitis, granulomatous, regionally diffuse, mild (slide 1-3)
- 2. Intestinal ceca and liver: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 100 - 500  $\mu$ m in diameter, moderate (slides 1-3, 1-4, 1-5, 2-3, 2-4), severe (slide 2-5)
- 3. Stomach: peritonitis, granulomatous, regionally diffuse, moderate (slide 2-5)
- 4a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 2-1, 2-2, 2-3, 2-5)
- 4b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 2-3, 2-5)
- 4c. Liver: hepatic necrosis, acute, multifocal, moderate (slide 2-5), severe (slide 2-3)
- 4d. Liver: hepatitis, fibrinous, focal, acute, mild (slide 2-5)
- 4e. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 2-5)
- 5a. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 1-1, 1-2, 1-3, 1-4, 1-5, 2-1, 2-2, 2-3, 2-4, 2-5)
- 5b. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, moderate (slide 2-3)
- 6. Gill, lamellar capillaries in tips of filaments: microthrombi, multifocal, mild (slide 1-2)

**Final Comments**

Several changes in these fish are consistent with an acute bacterial or viral infection. External infections with filamentous bacteria have not been associated with lesions in internal organs [Source: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.]. If the lesions are not related to external filamentous bacterial infections, then they might be a result of infection with another bacteria or virus (e.g., viral hemorrhagic septicemia virus).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. These lesions are among the most widespread and severe I have seen in the past year, and they might be contributing to morbidity.

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). Other fish might have had biliary preductular cell hyperplasia, but this change cannot be diagnosed in livers with severe autolysis. 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 (slide 2-3) has no obvious organisms. Fibrinous hepatitis (slide 2-5) might also be related to an acute bacterial or viral infection. 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.

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.

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). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin). Bacteriology and virology might provide additional useful diagnostic information.

Microthrombi in gill lamellar capillaries are consistent with endothelial damage resulting from a bacterial infection.

/sr



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1767 Angus Campbell Road

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

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

## Case Report

**Submission** 2005-04401      **Date** 29-Dec-2005      **Report** 05-Jan-2006

**Report**      **Copies**

**Submitter:** 11899 Grieg Seafoods B.C. Ltd.

**Owner** 11899 Grieg Seafoods B.C. Ltd.

**Farm:**

**Vet Clinic:**

**Attending** Dr. Milligan

**Specimen:** Tissue - Formalized

**Count** 1

**Flock Herd Size:**

**Species:** Atlantic Salmon

**Age**

**Breed:** Mowi

**Sex:**

**Feed:**

**Feed**

**Vaccination**

**Treatmen**

**Diagnosis**

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



### **History/Symptoms**

Farm Name: Williamson Passage.  
Age: 20056. Group size: 450,000  
Negligible mortality. Suspect *Piscirickettsia* at background levels.  
Treatments: none.  
PM lesions: 2-5mm yellow coalescing nodules in liver.  
Specimens: multiorgan - 2 fish 1 histocassette. Request histology.

### **Histopathology**

Two cassettes of preserved tissues were submitted for histopathology in formalin. After processing routinely into paraffin, tissues in cassette WP-1 were split into two cassettes for embedding and sectioning.

Slide 1A (WP-1 Dec 1) - liver, trunk kidney, intestinal ceca, and mesenteric fat

Slide 1B (WP-1 Dec 1) - spleen, liver, trunk kidney, and mesenteric fat

Slide 2 (WP-2 Dec 1) - liver, trunk kidney, spleen

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

Quality control: Autolysis for the liver varies from mild (slide 2, one fish), moderate (slide 2, other fish) to severe (slides 1A, 1B). Large foci of erythrocytes have deposits of acid hematin (e.g., liver in slide 1A). 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 (all sections)

Mesenteric adipose tissue depletion: none (all sections)

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 (moderate to severe hepatocellular glycogen depletion).



**Final Comments**

The fish in slide one probably died of complications related to *Piscirickettsia salmonis* infection, confirming the clinical diagnosis. By comparison, lesions in the fish in slide 2 do not seem sufficient to explain the death of the fish in slide 2. When *P. salmonis* is diagnosed in monitoring studies of pen-reared salmon in British Columbia, it usually affects only 10-25% of the dead fish sampled from a given farm on a given day.

Granulomatous 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 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. 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).

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.

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 fairly common in pen-reared Chinook salmon. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

/sr



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**Case Report**

**Submission** 2005-00512      **Date** 15-Feb-2005      **Report** 22-Feb-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 13521 #5228, 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 5 samples for IHN - PCR and tissue culture (hemorrhage of pyloric ceca on fish #10).

PO# 508371.

Sample submitted by Cilka LaTrace/Dianne Morrison.

**Virology**

5 pooled samples inoculated onto tissue culture - all negative.

\* Results faxed mar. 16/05.

**Molecular Diagnostics/PCR**

Samples 1-2, 3, 4-6, 7-8, 9-10: IHN Virus negative by PCR.

\* Results faxed Feb. 22/05.

/bb

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**Case Report**

**Submission** 2005-00513      **Date** 15-Feb-2005      **Report** 22-Feb-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 13522 #5229, Marine Harvest

**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

**History/Symptoms**

Submitted 6 samples for IHN - PCR and tissue culture.

PO# 508370.

**Virology**

6 pooled samples inoculated onto tissue culture - all negative.

\* Results faxed Mar. 16/05.

**Molecular Diagnostics/PCR**

Samples 1-3, 4-6, 7-9, 10-12, 13-16, 17-20: IHN Virus negative by PCR.

\* Results faxed Feb. 22/05.

/bb



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**Case Report**

**Submission** 2005-00055      **Date** 07-Jan-2005      **Report** 28-Feb-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13267      A 3.1 - 66

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture and PCR positive samples.

Sample ID: A 3.1 - 66 (1-6).

Addendum: Jan. 20/05. Samples submitted for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the sections contain occasional precipitates of acid hematin; this might be a result of transferring tissues to ethanol followed by return to formalin as part of processing the tissue into paraffin. Alternatively, tissues might not have been fixed in neutral buffered formalin.

Slide 1: autolysis, none (other organs) to mild (liver)

1. Liver: vasculitis and hepatic necrosis, acute, multifocal, coalescing, severe, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*)

2a. Heart: epicarditis, regionally diffuse, granulomatous, with karyorrhexis and intracellular basophilic structures consistent with *Piscirickettsia salmonis*, mild

2b. Heart: endocarditis, multifocal to diffuse, lymphohistiocytic, with intracellular basophilic structures consistent with *Piscirickettsia salmonis*, mild

3. Spleen: splenitis, histiocytic, moderate, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*)

4a. Trunk kidney: nephritis, interstitial, histiocytic, moderate, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*)

4b. Trunk kidney: intratubular protein cast, focal, mild

5. Head kidney: not included on the slide

Comment: Intracellular structures in the heart, spleen, liver, and kidney are consistent with the positive PCR results for *Piscirickettsia salmonis*. This fish probably died of complications related to *Piscirickettsia salmonis* infection, particularly in the liver. The protein cast in the kidney is evidence of glomerular or tubular dysfunction.

Slide 2: autolysis, none (other organs) to mild (kidney, liver)

1a. Liver: vasculitis and vascular wall necrosis, multifocal, moderate, with intralesional karyorrhexis and basophilic structures consistent with *Piscirickettsia salmonis* infection

1b. Liver: hepatocellular hydropic degeneration and single cell necrosis, disseminated, acute, moderate

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild

2. Heart: epicarditis, fibrinous, granulomatous, regionally diffuse, severe, with intralesional karyorrhexis and basophilic structures consistent with *Piscirickettsia salmonis*

3a. Spleen: peritonitis, granulomatous, regionally diffuse, moderate, with intralesional karyorrhexis and basophilic structures consistent with *Piscirickettsia salmonis*

3b. Spleen: splenitis, histiocytic, moderate, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*)

4. Trunk kidney: vasculitis, histiocytic, mild, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*)

4. Trunk Kidney: tubular intracytoplasmic protein droplets, multifocal, moderate

5. Head kidney: not included on the slide

Comment: This fish probably died of complications related to *Piscirickettsia salmonis* infection, particularly in the heart. 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 juvenile salmon it might be related to increased protein needed as part an inflammatory response.

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.

Slide 3: autolysis, moderate (other organs) to severe (liver)

1. Liver:

2. Heart: epicarditis and endocarditis, multifocal, granulomatous, with karyorrhexis, mild

3. Spleen: vasculitis, mild, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*)

4. Trunk kidney: no significant lesions



5. Head kidney: interstitial karyorrhexis, diffuse, mild

Comment: Structures consistent with *Piscirickettsia salmonis* are far less common than in sections of fish #s 1 and 2, and associated lesions are less severe. Some of the small karyorrhectic debris in the head kidney might be *Piscirickettsia salmonis*.

Slide 4: autolysis, mild (other organs) to moderate (kidney) to severe (liver)

1. Liver: no significant lesions

2. Heart: endocarditis, multifocal, granulomatous, with karyorrhexis, mild

3. Spleen: parenchymal karyorrhexis, disseminated, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: Some of the small karyorrhectic debris in the heart and spleen might be *Piscirickettsia salmonis*, but this would not be consistent with the PCR results.

Slide 5: autolysis, mild (other organs) to moderate (kidney) to severe (liver)

1. Liver: yellow-brown pigment, intracellular, disseminated, (hemosiderin and lipofuscin?), mild

2. Heart: no significant lesions

3. Spleen: splenitis, histiocytic, moderate, with karyorrhexis and intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*)

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: 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. Despite the negative PCR results for *Piscirickettsia salmonis*, the spleen contains several clusters of bacteria are suspect for *Piscirickettsia salmonis* (location on the stage of my microscope = 36 x 111.3).

Slide 6: autolysis, moderate (other organs) to severe (liver)

1. Liver: hepatocellular single cell necrosis (apoptosis), disseminated, acute, moderate

2. Heart: no significant lesions

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: Hepatocellular single cell necrosis (apoptosis) can occur 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

### **Molecular Diagnostics/PCR**

Samples 1-3: *Piscirickettsia salmonis* positive by PCR.

Samples 4-6: *Piscirickettsia salmonis* negative by PCR.

\* Results faxed Feb. 16/05.

/bb



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**Case Report**

**Submission** 2005-00056      **Date** 07-Jan-2005      **Report** 28-Feb-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13268      A 2.4 - 63

**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 Provincial Surveillance Program samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture and PCR positive samples.

Sample ID: A 2.4 - 63 (1-2).

Addendum: Jan. 20/05. Samples submitted for routine histology processing and analysis.

### **Histopathology**

Quality control/quality assurance: the sections contain abundant precipitates of acid hematin; this might be a result of transferring tissues to ethanol followed by return to formalin as part of processing the tissue into paraffin. Alternatively, tissues might not have been fixed in neutral buffered formalin.

Slide 1: autolysis, mild (other organs) to moderate (kidney)

1. Liver (2 pieces): parenchymal golden pigment, multifocal, intracellular mild
2. Heart: epicarditis, regionally diffuse, lymphocytic, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: The golden pigment in the liver 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. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies. Epicarditis is evidence of chronic immune stimulation, but the cause is otherwise unknown.

Slide 2: autolysis, none (all organs)

- 1a. Liver: parenchymal golden pigment, multifocal, intracellular mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: not included on the slide
3. Spleen: abundant hemorrhage
4. Trunk kidney (2 pieces): no significant lesions
5. Head kidney: no significant lesions

Comment: 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 juvenile salmon it might be related to increased protein needed as part an inflammatory response. Abundant erythrocytes around the spleen are evidence that trauma and a ruptured spleen was the cause of this fish's death; alternatively, abundant erythrocytes might be a post-mortem artefact.

### **Molecular Diagnostics/PCR**

Samples 1-2, 4-6: ISA, IPN, IHN, VHS Virus, Piscirickettsia salmonis negative by PCR.

\* Results faxed Feb. 16/05.

/bb







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**Case Report**

**Submission** 2005-00624      **Date** 23-Feb-2005      **Report** 07-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13586      A 3.2 - 11 (4)

**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 Provincial Surveillance Program samples for bacteriology - identification of isolates and culture and sensitivity.

Sample ID: A 3.2 - 11 (4).

**Bacteriology**

Isolate A 3.2-11 (4) - *Vibrio* *vulnificus* isolated.

\* Results faxed Mar. 7/05.

/bb

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**Case Report**

**Submission** 2005-00626      **Date** 23-Feb-2005      **Report** 18-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13588      A 3.3. - 18 (1-5)

**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

**History/Symptoms**

Submitted Provincial Surveillance Program Samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.3 - 18 (1-5).

Addendum: Mar 14/05 - submitted formalized tissue for histology.

**Histopathology**

Quality control/quality assurance: precipitates of acid hematin are limited to the spleen in slide 1, but slide 3 has acid-hematin in all organs. Tissues might not have been fixed in neutral buffered formalin. Tissues received at the Animal Health Centre were processed directly to alcohol with no formalin intermediate.

Slide 1: autolysis, mild (all organs)

1. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2a. Heart: endocarditis, focal, lymphohistiocytic, mild
- 2b. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney:
5. Head kidney: no significant lesions

Comment: 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). Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation (e.g., low grade bacterial infection), but the cause is otherwise unknown. The cause and significance of myocardial karyomegaly 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). Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, none (other organs) to mild (head kidney, trunk kidney)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, diffuse, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions
6. Mesenteric adipose tissue: vascular congestion, multifocal, mild

Comment: 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 juvenile salmon it might be related to increased protein needed as part an inflammatory response.

Slide 3: autolysis, mild (other organs) to moderate (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

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

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, multifocal, granulomatous, moderate
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
2. Heart: carditis, multifocal, coalescing, granulomatous, severe
3. Spleen: splenitis, multifocal, granulomatous, moderate
4. Trunk kidney: nephritis, interstitial, multifocal, granulomatous, severe
5. Head kidney: nephritis, interstitial, multifocal, granulomatous, severe

Comment: Granulomatous inflammation in multiple organs is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* is the most common organism associated with these lesions.





Slide 5: autolysis, mild (other organs) to severe (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, severe

2. Heart: epicarditis, regionally diffuse, lymphocytic, mild

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: Epicarditis is evidence of chronic immune stimulation, but the cause is otherwise unknown.

**Molecular Diagnostics/PCR**

Samples 1-3, 4-5: IHN, VHS, IPN, ISA, *Piscirickettsia salmonis* negative by PCR.

\* Results faxed Mar. 21/05.

/bb

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**Case Report**

**Submission** 2005-00628      **Date** 23-Feb-2005      **Report** 02-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13590      A 3.1 - 9 (1-2)

**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 Provincial Surveillance Program Samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 3.1 - 9 (1-2).

Addendum: Also submitted samples for routine histology processing and analysis. A3.1 - 9 (1-2).

**Histopathology**

Slide 1: autolysis, none (all organs)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: pericholangitis, lymphocytic, multifocal, mild
- 1c. Liver: hepatocellular single cell necrosis (apoptosis), disseminated, acute, mild
- 1d. Liver: hepatic necrosis, acute, multifocal, mild
- 1e. Liver: yellow-brown pigmented macrophage aggregates and sinusoidal macrophages, disseminated, with intracellular hemosiderin and/or lipofuscin, mild
2. Heart: not included on the slide
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: small numbers of eosinophilic granular cells in interstitial tissue
5. Head kidney: moderate numbers of eosinophilic granular cells in interstitial tissue

Comment: 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. Hepatocellular single cell necrosis (apoptosis) can occur 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). Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., *Piscirickettsia salmonis*). Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis.

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.

Increased numbers of eosinophilic granular cells in the kidney is a fairly common finding in Atlantic salmon. 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*. Increased numbers of eosinophilic granular cells are sometimes associated with chronic parasitic infections, but again, the inciting cause was not included in the sections examined.

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

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.

Slide 2: autolysis, mild (all organs)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: yellow-brown pigmented macrophage aggregates and sinusoidal macrophages, disseminated, with intracellular hemosiderin and/or lipofuscin, mild
- 1c. Liver: pericholangitis, lymphocytic, multifocal, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions



Comment: none

**Molecular Diagnostics/PCR**

Samples 1-2: IHN, VHS, IPN, ISA, *Piscirickettsia salmonis* negative by PCR.

\* Results faxed Mar. 8/05.

/bb

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**Case Report**

**Submission** 2005-00629      **Date** 23-Feb-2005      **Report** 04-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13591      A 3.2-11 (1-3)

**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 Provincial Surveillance Program Samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 3.2 - 11 (1-3).

Addendum: Also submitted samples for routine histology processing and analysis.



**Histopathology**

Slide 1: autolysis, none (all organs)

- 1a. Liver: pericholangitis, lymphocytic, multifocal, moderate
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 2a. Heart: mural cellular thrombus, bifocal (100 to 200 µm in diameter), acute, mild
- 2b. Heart: myocardial karyomegaly, multifocal, mild
- 3. Spleen: parenchymal golden pigment, disseminated, intracellular, mild
- 4a. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
- 4b. Trunk kidney: nephritis, histiocytic, focal (200 µm in diameter), mild
- 5. Head kidney: no significant lesions

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

The cellular thrombi in the heart probably occurred within hours of death, but they are not large enough to have killed the fish. The cause and significance of myocardial karyomegaly 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).

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. The focus of nephritis might be a result of a localized bacterial infection.

The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin 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 have been associated with decreased growth and survival in several studies.

Slide 2: autolysis, none (all organs)

- 1a. Liver: pericholangitis, lymphocytic, multifocal, moderate
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1c. Liver: hepatocellular fatty change (lipidosis), regionally diffuse, mild
- 2. Heart: epicarditis, focal, lymphocytic, mild
- 3. Spleen: not included on the slide
- 4. Trunk kidney: no significant lesions
- 5. Head kidney: no significant lesions

Comment: 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). Epicarditis is evidence of chronic immune stimulation, but the cause is otherwise unknown.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, granulomatous, multifocal, mild
- 1b. Liver: yellow-brown pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart: epicarditis, focal, lymphocytic, mild
- 3. Spleen: parenchymal golden pigment, multifocal, mild
- 4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate
- 5. Head kidney: no significant lesions

Comment: Granulomatous inflammation in the liver is consistent with a chronic bacterial infection, and



*Renibacterium salmoninarum* is the most common organism associated with these lesions; similar lesions have also been associated with chronic *Yersinia ruckeri* infection. Pigment in the liver 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. 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

**Molecular Diagnostics/PCR**

Samples 1-2, 3-4: IHN, VHS, ISA, IPN, *Piscirickettsia salmonis* negative by PCR.

\* Results faxed Mar. 8/05.

/bb

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**Case Report**

**Submission** 2005-00630      **Date** 23-Feb-2005      **Report** 22-Apr-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13592      A 2.3 - 3 (1-2)

**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 Provincial Surveillance Program Samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 2.3 - 3 (1-2).

ADDENDUM: March 24, 2005. Samples submitted for routine histology processing and analysis. Sample ID: A.2.3-3 (1-2).

### **Histopathology**

Quality control/quality assurance: the spleen sections contain small foci 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 evidence of postfixation dehydration artifact.

Slide 1: autolysis, none (all organs)

1. Liver: pericholangitis, lymphocytic, multifocal, mild
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

#### **COMMENT:**

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. The cause and significance of myocardial karyomegaly 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). Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, none (all organs)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

#### **COMMENT:**

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).



**Molecular Diagnostics/PCR**

Samples 1-3, 4-5: IHN, VHS, IPN, ISA, Piscirickettsia salmonis Negative by PCR.

/sr

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**Case Report**

**Submission** 2005-00631      **Date** 23-Feb-2005      **Report** 04-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13593      A 2.4 - 6 (1-2)

**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 Provincial Surveillance Program Samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 2.4 - 6 (1-2).

Addendum: Also submitted samples for routine histology processing and analysis.

**Histopathology**

Slide 1: autolysis, none (all organs)

1a. Liver: yellow-brown pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: Pigment in the liver 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. 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.

The cause and significance of myocardial karyomegaly 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).

Slide 2: autolysis, mild (other organs) to moderate (liver)

1a. Liver: yellow-brown pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, severe

2. Heart: myocardial karyomegaly, multifocal, severe

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

**Molecular Diagnostics/PCR**

Samples 1-2: IHN, VHS, IPN, ISA, Piscirickettsia salmonis negative by PCR.

\* Results faxed Mar. 8/05.

/bb



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**Case Report**

**Submission** 2005-00632      **Date** 23-Feb-2005      **Report** 04-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13594      A 3.2 - 13 (1)

**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 Provincial Surveillance Program Samples for virology - PCR for IHN, ISA, IPN, VHS, and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample ID: A 3.2 - 13 (1).

Addendum: Also submitted samples for routine histology processing and analysis.

**Histopathology**

Slide 1: autolysis, none (other organs) to mild (liver)

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: peritonitis, granulomatous, regionally diffuse, moderate, with occasional fine fibrocellular fronds

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: 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). The cause and significance of myocardial karyomegaly 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). Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

**Molecular Diagnostics/PCR**

Sample 1: IHN, VHS, IPN, ISA, Piscirickettsia salmonis negative by PCR.

\* Results faxed Mar. 8/05.

/bb



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**Case Report**

**Submission** 2005-00763      **Date** 08-Mar-2005      **Report** 10-Mar-2005

**Report**      **Copies**

**Submitter:** 8447      Stolt Sea Farm Inc.  
**Owner** 8447      Stolt Sea Farm Inc.  
**Farm:**  
**Vet Clinic:** 9348      Canadian Animal Hth Mgmt Serv.  
**Attending**      Dr. B. Cox

**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**

Submitted fish tissue for histology and PCR for IHN and VHS.

Increasing mortality.

PO #56472.

**Histopathology**

Seven cassettes of tissues were submitted.

Slide 14-1: heart (2 pieces) and liver (2 pieces)

Slide 14-2: intestinal ceca, mesenteric fat, exocrine pancreas, and skeletal muscle

Slide 14-3: trunk kidney (5 pieces), head kidney, and spleen

Slide 10-1: gill

Slide 10-2: spleen (2 pieces), liver (2 pieces), and heart

Slide 10-3: trunk kidney (4 pieces), head kidney

Slide 10-4: intestinal ceca, mesenteric fat, and exocrine pancreas

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

Quality control: Tissue preservation varies from excellent (most organs), to good (1 liver in slide 10-2), to fair (gill), to poor (intestinal ceca).

Measures of physiologic condition

Hepatocellular glycogen depletion, severe (slides 14-1, 10-2)

Mesenteric adipose tissue depletion, none (slides 14-2, 10-4)

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**

5 pooled samples inoculated onto tissue culture - all negative.

\* Results faxed Apr. 27/05.

**Molecular Diagnostics/PCR**

Samples 1-2, 3-4, 5-7, 13-14: VHS Virus positive by PCR.

Samples 8-12: VHS suspect by PCR.

\* Results faxed Mar. 14/05.





### **Diagnosis**

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, none (one liver in slide 10-2), moderate (slide 14-1, and the other liver in slide 10-2)
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 14-1, 10-2)
- 2a. Heart: myocardial karyomegaly, multifocal, mild (slides 14-1, 10-2)
- 2b. Heart: endocarditis, histiocytic, multifocal, mild (slide 14-1)
- 2c. Heart: endocarditis, lymphocytic, multifocal, with karyorrhexis, mild (slide 10-2)
- 2d. Heart: epicarditis, multifocal, lymphoplasmacytic, mild (slide 14-1)
- 3a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 10-2)
- 3b. Spleen: parenchymal golden pigment, disseminated, intracellular (including multinucleate giant cells), mild (slide 10-2)

### **Final Comments**

The sections have several low grade changes that might have contributed to morbidity, but no clear cause of increased mortality.

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).

The cause and significance of myocardial karyomegaly 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). Histiocytic endocarditis and lymphoplasmacytic epicarditis provide evidence of chronic immune stimulation (e.g., low grade bacterial infection), but the cause is otherwise unknown. Karyorrhexis in the heart in slide 10-2 is an indication of lymphocyte turnover, perhaps part of an active inflammatory response.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin 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 have been associated with decreased growth and survival in several studies (this case was mild).



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**Case Report**

**Submission** 2005-00077      **Date** 11-Jan-2005      **Report** 24-Jan-2005

**Report**      **Copies**

**Submitter:** 11036 Mainstream Canada (M)

**Owner** 11036 Mainstream Canada (M)

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Whole Animal

**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 whole Atlantic salmon for bacteriology and viral cell culture.

Sorka.cerna@mainstreamcanada.com; francisco.miranda.morales@mainstreamcanada.com

**Bacteriology**

No Bacteria isolated from Fish #1 liver and kidney, Fish #2 Liver and Fish #3 Liver and Kidney.

Vibrio sp. NOT Vibrio anguillarum type 1 or 2 or Vibrio ordalii.

\* Results faxed Jan. 24/05.

**Virology**

VHS positive - 3 samples inoculated onto tissue culture.  
All 3 exhibited CPE - identified as VHS by PCR.

\* Results faxed Jan. 24/05.

**Molecular Diagnostics/PCR**

VHS Virus positive by PCR.  
IHN Virus negative by PCR.

\* Results faxed Jan. 24/05.

ADDITIONAL RESULTS (Faxed - Jan. 26/05):

ISA negative by PCR.

/bb



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**Case Report**

**Submission** 2005-00800      **Date** 10-Mar-2005      **Report** 31-Mar-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)

**Owner** 13692 #5244, 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 sample pooled fish (1-3) for virology - tissue culture and PCR for IHNV. No growth on bacteriology.

Please quote PO# 508 393 for billing purposes.

**Virology**

Fish viruses negative by culture.

\* Results faxed Apr. 8/05.

**Molecular Diagnostics/PCR**

IHN negative by PCR.

\* Results faxed Mar. 31/05.

/bb

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## Case Report

**Submission** 2005-00801      **Date** 10-Mar-2005      **Report** 14-Mar-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 13693 #5241, 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 histo sample and one kidney tissue sample for virology - tissue culture and PCR for IHNV.

There is an increase in moribunds in the pen. All are off feed and have severe dermal erosion from rubbing into nets. Internally there are severe vaccine adhesions, reddened liver, and slightly swollen spleen.

Please quote PO# 508392 for billing purposes.

**Histopathology**

One cassette of preserved tissues was submitted (Slide 1, GC 3/6/05). Tissues include the heart (2 pieces) and liver (2 pieces), intestinal ceca, mesenteric fat, exocrine pancreas, skin/skeletal muscle, trunk kidney, head kidney, spleen, gill, and brain. All organs on each slide were examined. Organs not listed below had no significant lesions.

Quality control: Tissue preservation for most organs is good, but preservation of intestinal ceca is poor. The margins of many organs have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cells types stains poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Measures of physiologic condition  
Hepatocellular glycogen depletion, severe  
Mesenteric adipose tissue depletion, mild

This pattern in the measures of physiologic condition is consistent with a fairly healthy growing fish (only mild mesenteric fat depletion) that recently stopped feeding (severe glycogen depletion).

**Virology**

Fish viruses negative by culture.

\* Results faxed Apr. 8/05.

**Molecular Diagnostics/PCR**

IHN negative by PCR.

\* Results faxed Mar. 31/05.



**Diagnosis**

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate
- 1c. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Head kidney: nephritis, interstitial, granulomatous, focal, mild
3. Trunk kidney: edema, interstitial, regionally diffuse, mild
4. Exocrine pancreas: peritonitis, chronic, focal, with fibrocellular fronds, mild
5. Spleen: parenchymal golden pigment, disseminated, intracellular, moderate
6. Skin: dermatitis, ulcerative, diffuse, with superficial filamentous bacteria, severe
7. Skeletal muscle: flocculated sarcoplasm and coagulative necrosis of white myofibres, acute, moderate (~10% of myofibres)
8. Heart: endocarditis, histiocytic, diffuse, moderate

**Final Comments**

The tissues have several low grade changes that might have contributed to morbidity, but no clear cause of increased mortality. Many of the findings here are similar to a recent case that was positive for VHSV, but nothing is particularly specific for VHSV. Also consider bacterial culture, if not already done.

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 juvenile salmon it might be related to increased protein needed as part an inflammatory response. 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).

The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.

Increased space between the tubules in the trunk kidney is interpreted as edema; differential diagnoses include depletion of hematopoietic elements or an artefact. Lack of necrosis is evidence against IHN.

Peritonitis is consistent with a reaction to foreign material and to the adhesions observed grossly; it is common in fish that have been vaccinated (Mutoloki et al. 2004).

The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin 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 have been associated with decreased growth and survival in several studies (this case was mild).

Small ulcers are common in fish, and filamentous bacteria commonly invade skin ulcers. Once filamentous bacteria become established, the ulcers often get larger (as in this case). Enlargement of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection).

Flocculated sarcoplasm is a degenerative change of skeletal muscle that, along with frank necrosis, can result from trauma, bacterial, or parasitic infections. These sections had no evidence of parasitic infections.

Histiocytic endocarditis and lymphoplasmacytic epicarditis provide evidence of chronic immune stimulation (e.g., low grade bacterial or viral infection), but the cause is otherwise unknown

**Literature Cited**

S. Mutoloki, S. Alexandersen, and Ø. Evensen. 2004. Sequential study of antigen persistence and concomitant inflammatory reactions relative to side-effects and growth of Atlantic salmon (*Salmo salar* L.) following intraperitoneal injection with oil-adjuvanted vaccines. *Fish & Shellfish Immunology* 16(5):633-644.

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

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**Case Report**

**Submission** 2005-00084      **Date** 11-Jan-2005      **Report** 25-Jan-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13286      A.3.2-69 (5)

**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**

One culture submitted from specimen A.3.2-69 (5) subculture. Request bacteriology - identification of isolates and C & S.

Provincial surveillance program.

**Bacteriology**

Bact. plate - Gram positive bacillus identified as Microbacterium sp.

Bacteria sensitive to: Erythromycin, Florfenicol, Romet 30, Tri-sulfas, Sulfa-methox-trimeth. and Tetracycline.

\* Results faxed Jan. 25/05.

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**Case Report**

**Submission** 2005-00842      **Date** 14-Mar-2005      **Report** 21-Mar-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13718      A 3.2 - 14 (1-2)

**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 Provincial Program samples for virology - PCR for IHN, ISA, IPN, VHS, and *Piscirickettsia salmonis*. Culture any PCR positive samples.

Sample ID: A 3.2 - 14 (1-2).

Addendum: Also submitted formalized tissue for histology.

**Histopathology**

Quality control/quality assurance: precipitates of acid hematin are limited to the spleen. Tissues received at the Animal Health Centre were processed directly to alcohol with no formalin intermediate.

Slide 1: autolysis, none (other organs) to mild (liver)

- 1a. Liver: single cell necrosis (apoptosis), focal, acute, mild
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: epicarditis and vasculitis, granulomatous, focal (2 mm diameter), with necrosis and basophilic spherical structures (karyorrhectic debris, and *Piscirickettsia salmonis*?), severe
2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, granulomatous, multifocal, with fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: Single cell necrosis (apoptosis) in the liver is evidence of cell turnover. It seems to affect a small focus of lymphocytes and perhaps one or two hepatocytes. Exposure to toxins (endogenous or exogenous) might be related to the focus of apoptosis. 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 juvenile salmon it might be related to increased protein needed as part of an inflammatory response. The large focus of vasculitis and epicarditis is consistent with exposure to an infectious agent; some of the small basophilic structures scattered in the inflammation have features of *Piscirickettsia salmonis*, but this needs to be confirmed by PCR. A differential cause is *Renibacterium salmoninarum*. The cause and significance of myocardial karyomegaly 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). Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, mild (other organs) to severe (liver)

1. Liver: hepatitis, granulomatous, lymphoplasmacytic, multifocal, moderate
- 2a. Heart: epicarditis, diffuse, lymphohistiocytic, severe
- 2b. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages, moderate
3. Spleen: not included on the slide
4. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, with renal tubular necrosis, severe
5. Head kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, severe

Comment: Granulomatous inflammation in the liver and kidney is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* is the most common organism associated with these lesions. Renal tubular necrosis might be secondary to inflammation-induced vascular compromise. The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface throughout most of the ventricle are rarely more than 2 cell layers thick.



**Molecular Diagnostics/PCR**

Samples 1-2: IHN, VHS, IPN, ISA, *Piscirickettsia salmonis* negative by PCR.

\* Results faxed Mar. 31/05.

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**Case Report**

**Submission** 2005-00909      **Date** 17-Mar-2005      **Report** 25-Apr-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13765      A.2.3-4 (1-7)  
A2.3-4

**Farm:**  
**Vet Clinic:**  
**Attending**

**Specimen:** Tissue - Fresh  
**Species:** Atlantic Salmon  
**Breed:**

**Count** 1

**Flock Herd Size:**  
**Age**  
**Sex:**

**Feed:****Feed****Vaccination****Treatmen****Diagnosis**

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

**History/Symptoms**

Provincial Surveillance Program Samples. Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample I.D. A.2.3-4 (1-7).

ADDENDUM: March 24, 2005. Samples submitted for routine histology processing and analysis. Sample ID: A.2.3-4 (1-7).

**Histopathology**

Quality control/quality assurance: the spleen sections contain small foci 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 evidence of postfixation dehydration artefact.

Slide 1: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatic necrosis, acute, multifocal, moderate
- 1b. Liver: pericholangitis, lymphocytic, multifocal, mild
- 1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: epicarditis, focal, lymphocytic, mild
3. Spleen: no significant lesions
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity; in this fish, it is consistent with the positive PCR result for viral hemorrhagic septicaemia virus (VHSV). 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. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Epicarditis is evidence of chronic immune stimulation, but the cause is otherwise unknown.

Slide 2: autolysis, none (all organs)

1. Liver: no significant lesions
2. Heart: epicarditis, focal, histiocytic, neutrophilic, subacute, mild
- 3a. Spleen: ellipsoid hyalinization, diffuse, mild
- 3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: Spleen ellipsoid hyalinization might be part of the fishes' response to infection with VHSV. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 3: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular cytoplasmic vacuoles, regionally diffuse, moderate
- 2a. Heart: myocardial karyomegaly, multifocal, mild
- 2b. Heart: epicarditis, focal, histiocytic, lymphocytic, chronic, mild
3. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: Hepatocellular vacuoles have irregular margins and are filled with variable amounts of foamy eosinophilic material. The origin and significance of the eosinophilic material is unknown; it could be from within the cell (a form of cytotegosome) or from outside the cell (phagocytosed protein). It probably is evidence of altered hepatocellular function. The cause and significance of myocardial karyomegaly 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).

Slide 4: autolysis, none (other organs) to mild (liver)

- 1a. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: endocarditis, focal, lymphohistiocytic, mild
- 3a. Spleen: ellipsoid hyalinization, diffuse, mild
- 3b. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, moderate
4. Trunk kidney: no significant lesions



5. Head kidney: no significant lesions

Comment: 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). Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation (e.g., low grade bacterial infection), but the cause is otherwise unknown.

Slide 5: autolysis, none (other organs) to mild (liver)

1. Liver: hepatic necrosis, acute, multifocal, mild

2. Heart: no significant lesions

3. Spleen: no significant lesions

4. Trunk kidney: no significant lesions

5. Head kidney: no significant lesions

Comment: Hepatic necrosis is consistent with the positive PCR for VHSV.

Slide 6: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

2. Heart: no significant lesions

3. Spleen: peritonitis, granulomatous, chronic, multifocal, with fibrocellular fronds, mild

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: no significant lesions

Comment: 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.

Slide 7: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

2. Heart: myocardial karyomegaly, multifocal, mild

3. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, mild

4. Trunk kidney: no significant lesions

5. Head kidney: not included on the slide

Comment: The cause and significance of myocardial karyomegaly 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).

## **Virology**

2 pooled samples inoculated onto tissue culture - both negative.

\* Results faxed May 4/05.





**Molecular Diagnostics/PCR**

Samples 1-4, 5-7: VHS Virus positive by PCR.

IHN, ISA, IPN, Piscirickettsia salmonis: negative by PCR.

\* Results faxed Apr. 26/05.

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1767 Angus Campbell Road

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

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**Case Report**

**Submission** 2005-00910      **Date** 17-Mar-2005      **Report** 25-Apr-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13766      A.2.3-2 (1-8)

**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**

Provincial Surveillance Program Samples. Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample I.D. A.2.3-2 (1-8).

ADDENDUM: March 24, 2005 - samples submitted for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: the spleen sections contain small foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The edges of some organs have evidence of postfixation dehydration artefact. This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, mild (heart), moderate (spleen, kidney) to severe (liver)

1. Liver: no significant lesions
2. Heart: epicarditis, regionally diffuse, histiocytic, neutrophilic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: Epicarditis is evidence of chronic immune stimulation, but the cause is otherwise unknown. Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated.

Slide 2: autolysis, none (other organs) to mild (liver)

1. Liver: no significant lesions
- 2a. Heart: epicarditis, regionally diffuse, granulomatous, neutrophilic, mild
- 2b. Heart: endocarditis, focal, with endothelial cell hypertrophy and a thin layer of macrophages, mild
3. Spleen: not included on the slide
4. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, moderate
5. Head kidney: not included on the slide

Comment: 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). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as Gentamicin). The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface in the ventricle are rarely more than 2 cell layers thick.

Slide 3: autolysis, none (other organs) to mild (liver)

1. Liver: arteritis, multifocal, acute, neutrophilic, moderate
2. Heart: mural thrombosis, multifocal, mild
3. Spleen: peritonitis, granulomatous, focal, with fibrocellular fronds, moderate
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: Hepatic arteritis and cardiac thrombosis are consistent with VHSV infection. 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.

Slide 4: autolysis, none (all organs)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: not included on the slide
5. Head kidney: no significant lesions

6. Mesenteric fat: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, mild  
Comment: Peritonitis at the margin of the mesenteric fat is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.



Slide 5: autolysis, none (other organs) to mild (liver)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, granulomatous, multifocal, with fibrocellular fronds, severe
4. Trunk kidney: no significant lesions
5. Head kidney: not included on the slide

Comment: see comments on previous slides.

Slide 6: autolysis, none (heart) to mild (kidney and spleen)

1. Liver: not included on the slide
2. Heart: no significant lesions
3. Spleen: no significant lesions
4. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, moderate (consistent with VHSV)
5. Head kidney: not included on the slide

Comment: see comments on previous slides.

Slide 7: autolysis, none (other organs) to mild (liver)

1. Liver: no significant lesions
- 2a. Heart: myocardial necrosis, multifocal, histiocytic, neutrophilic, subacute, moderate
- 2b. Heart: epicarditis, regionally diffuse, histiocytic, neutrophilic, mild
3. Spleen: peritonitis, granulomatous, multifocal, with fibrocellular fronds, moderate
- 4a. Trunk kidney: renal tubular epithelial necrosis, focal, acute, mild (consistent with VHSV)
- 4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: interstitial histiocytosis, bifocal, mild

Comment: Myocardial necrosis and associated inflammation is probably related to VHSV, as is renal tubular necrosis. Interstitial histiocytosis in the head kidney is an unusual lesion that might be related to a VHSV infection.

Slide 8: autolysis, none (other organs) to moderate (kidney)

1. Liver: not included on the slide
2. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild
3. Spleen: peritonitis, granulomatous, multifocal, with fibrocellular fronds, moderate
- 4a. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, mild (consistent with VHSV)
- 4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: Epicarditis is evidence of chronic immune stimulation, but the cause is otherwise unknown.

## **Virology**

2 pooled samples inoculated onto tissue culture - both negative.

\* Results faxed May 4/05.





**Molecular Diagnostics/PCR**

Samples 1-4, 5-8: VHS Virus positive by PCR.  
IHN, ISA, IPN, Piscirickettsia salmonis negative by PCR.

\* Results faxed Apr. 26/05.

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Facsimile: (604) 556-3010  
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**Case Report**

**Submission** 2005-00911      **Date** 17-Mar-2005      **Report** 27-Apr-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13767      A.2.3-1 (1-10)

**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**

Provincial Surveillance Program Samples. Request Virology - PCR for IHN, ISA, IPN, VHS and Piscirickettsia salmonis. Culture any PCR positive samples.

Sample I.D. A.2.3-1 (1-10).

ADDENDUM: March 24, 2005. Samples submitted for routine histology processing and analysis.

**Histopathology**

Quality control/quality assurance: some spleen sections contain foci of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic during fixation. The edges of some organs have evidence of postfixation dehydration artefact. This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, none (all organs)

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, lymphoplasmacytic, granulomatous, regionally diffuse, with fine fibrocellular fronds, moderate
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

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

Slide 2: autolysis, none (other organs) to mild (liver)

- 1a. Liver: peritonitis, lymphoplasmacytic, granulomatous, regionally diffuse, with fine fibrocellular fronds, mild
- 1b. Liver: hepatocellular fatty change (lipidosis), regionally diffuse, moderate
- 1c. Liver: hepatitis, granulomatous, multifocal, with intralesional vacuoles (up to 100 µm in diameter), moderate
2. Heart: no significant lesions
3. Spleen: splenitis, granulomatous, multifocal, with intralesional vacuoles, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: Vacuoles associated with granulomatous splenitis and hepatitis are consistent with a foreign body reaction, most likely related to vaccine injection. A PAS stain on this slide stains material within the granulomas but does not stain the hepatocyte vacuoles.

Slide 3: autolysis, none (other organs) to mild (liver)

1. Liver: hepatitis, perivascular and pericholangial, lymphocytic, multifocal, moderate
- 2a. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages, moderate
- 2b. Heart: epicarditis, regionally diffuse, lymphoplasmacytic, mild
3. Spleen: peritonitis, chronic, diffuse, with fibrocellular fronds, mild
4. Trunk kidney: renal tubular dilation, with epithelial apoptosis and intraluminal hypereosinophilic debris, focal, mild
5. Head kidney: not included on the slide

Comment: Lymphocytic inflammation around vessels in the liver is evidence of chronic immune stimulation, e.g., from a bacterial infection. The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface throughout most of the ventricle, atrium, and bulbus arteriosus are rarely more than 2 cell layers thick. Epicarditis is evidence of chronic immune stimulation, but the cause is otherwise unknown. Lesions related to focal renal tubular dilation are evidence of localized tubular dysfunction, but the cause is unknown.

Slide 4: autolysis, mild (other organs) to moderate (kidney and liver) and severe (intestinal ceca)

1. Liver: no significant lesions



2. Heart (bulbus arteriosus): endocarditis, diffuse, with endothelial cell hypertrophy and a thin layer of macrophages and eosinophilic granular cells, moderate
  3. Spleen: vasculitis and necrosis of vessel walls, multifocal, acute, moderate
  4. Trunk kidney: no significant lesions
  5. Head kidney: no significant lesions
  6. Intestinal ceca: no significant lesions
- Comment: Splenic vasculitis and vascular wall necrosis is consistent with the PCR positive test for VHSV.

Slide 5: autolysis, none (all organs)

1. Liver: hepatocellular fatty change (lipidosis), regionally diffuse, moderate
  2. Heart (bulbus arteriosus): endocarditis, diffuse, with scattered eosinophilic granular cells, mild
  3. Spleen: peritonitis, lymphoplasmacytic, granulomatous, regionally diffuse, with fine fibrocellular fronds, moderate
  4. Trunk kidney: no significant lesions
  5. Head kidney: not included on the slide
  6. Gill: branchial parasites (25 x 150 µm), multifocal, mild
- Comment: Striated muscle in the branchial parasites is consistent with an early stage of copepod parasite.

Slide 6: autolysis, none (other organs) to mild (liver)

1. Liver: no significant lesions
  2. Heart (bulbus arteriosus): endocarditis, diffuse, with scattered eosinophilic granular cells, mild
  3. Spleen: peritonitis, chronic, diffuse, with fibrocellular fronds, moderate
  4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
  5. Head kidney: no significant lesions
- Comment: See comments on previous slides.

Slide 7: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatitis, perivascular and pericholangial, lymphocytic, multifocal, mild
  - 1b. Liver: hepatocellular fatty change (lipidosis), regionally diffuse, moderate
  2. Heart: no significant lesions
  3. Spleen: peritonitis, chronic, diffuse, with fibrocellular fronds, moderate
  4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
  5. Head kidney: not included on the slide
- Comment: See comments on previous slides.

Slide 8: autolysis, mild (other organs) to severe (liver)

1. Liver: no significant lesions
  2. Heart: epicarditis, regionally diffuse, lymphoplasmacytic, mild
  3. Spleen: no significant lesions
  4. Trunk kidney: no significant lesions
  5. Head kidney: no significant lesions
- Comment: See comments on previous slides.

Slide 9: autolysis, mild (other organs) to moderate (liver)

1. Liver: no significant lesions
  2. Heart: epicarditis, regionally diffuse, lymphoplasmacytic, mild
  3. Spleen: no significant lesions
  4. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, moderate
  5. Head kidney: no significant lesions
  6. Mesenteric fat: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, mild
- Comment: 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). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as Gentamicin).

Slide 10: autolysis, none (other organs) to mild (liver)

1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate

1b. Liver: pericholangitis, lymphocytic, multifocal, mild

2. Heart: no significant lesions

3. Spleen: peritonitis, chronic, diffuse, with fibrocellular fronds, moderate

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

5. Head kidney: not included on the slide

Comment: Lymphocytic inflammation around bile ductules (liver) is evidence of chronic immune stimulation.

### **Virology**

2 pooled samples inoculated onto tissue culture - both negative.

\* Results faxed May 11/05.

### **Molecular Diagnostics/PCR**

Samples 1-5, 6-10: VHS Virus positive by PCR.

IHN, ISA, IPN, Piscirickettsia salmonis negative by PCR.

\* Results faxed Apr. 27/05.

/bb





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**Case Report**

**Submission** 2005-00942      **Date** 21-Mar-2005      **Report** 23-Mar-2005

**Report**      **Copies**

**Submitter:** 9439 Marine Harvest Canada (M)  
**Owner** 13787 #5250, 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

### **History/Symptoms**

Log No. 5250. Date: March 20/05. Atlantic salmon. Sex: regular. Saltwater entry: 2005. Netpen/tank ID: 101, 102, 107.

Sample size: 4 tissue samples for tissue culture and PCR. Two histo. samples.

Four tissue samples for IHNV - PCR and tissue culture. Samples are labelled as: 1-3, 4-6, 7, 8-10. Samples are from recent entry Atlantics. Histo taken from fish #1 and #2. Fish rolling over upon saltwater entry; some recovery after entry. No slow simmers. No plankton reports in area. No gross lesions except for #7. Some hemorrhaging of pyloric ceca.

### **Histopathology**

Two cassettes of preserved tissues were submitted wrapped in paper towels soaked in formalin, all sealed in a plastic bag.

Slide 1 (NP-1, 3/16/05) - Heart, liver, stomach, intestine, intestinal ceca, mesenteric fat, exocrine pancreas, skeletal muscle, trunk kidney, head kidney, and gill.

Slide 2 (NP-2, 3/16/05) - Heart, liver, stomach, intestine, intestinal ceca, mesenteric fat, exocrine pancreas, skeletal muscle, trunk kidney, head kidney, spleen, and gill.

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

Quality control: Tissue preservation for most organs is excellent, including intestinal ceca. The margins of the liver, spleen, and kidney have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cells types stains poorly or not at all). Gill and intestine are variably affected. This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Measures of physiologic condition

Hepatocellular glycogen depletion, none (slide 2), severe (slide 1)

Mesenteric adipose tissue depletion, none (slides 1, 2)

This pattern in the measures of physiologic condition is consistent with a healthy growing fish (no mesenteric fat depletion); abundant hepatocellular glycogen in fish #2 is evidence that this fish was eating; severe glycogen depletion in fish #1 is evidence that this fish stopped eating in the last few days.

### **Virology**

4 samples inoculated onto tissue culture - all negative.

\* Results faxed Apr. 15/05.



**Molecular Diagnostics/PCR**

Samples 1-3, 4-6, 7, 8-10: IHN Virus negative by PCR.

**Diagnosis**

1. Liver: pericholangitis, lymphocytic, multifocal, mild
2. Intestine and mesenteric fat: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 2), moderate (slide 1)

**Final Comments**

The tissues have no lesions to explain the clinical signs of rolling over upon saltwater entry. Osmotic imbalance as a result of failure to adapt to saltwater would not produce any specific microscope changes, even though it might kill the fish. Consider analysis of electrolyte levels in plasma as a better indicator of saltwater stress. The fish had two minor lesions.

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.

Peritonitis is consistent with a reaction to foreign material and to the adhesions observed grossly; it is common in fish that have been vaccinated (Mutoloki et al. 2004).

Literature Cited:

S. Mutoloki, S. Alexandersen, and Ø. Evensen. 2004. Sequential study of antigen persistence and concomitant inflammatory reactions relative to side-effects and growth of Atlantic salmon (*Salmo salar* L.) following intraperitoneal injection with oil-adjuvanted vaccines. *Fish & Shellfish Immunology* 16(5):633-644.

/bb



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**Case Report**

**Submission** 2005-00960      **Date** 22-Mar-2005      **Report** 27-Apr-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13798      A.3.3-16

**Farm:**

**Vet Clinic:**

**Attending**

**Specimen:** Tissue - Fresh

**Species:** Atlantic Salmon

**Breed:**

**Count**

**Flock Herd Size:**

**Age**

**Sex:**

**Feed:**

**Feed**

**Vaccination**

**Treatmen**

**Diagnosis**

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

**History/Symptoms**

Provincial Surveillance Program samples. Specimens: A.3.3-16 (1-5). Request virology - PRC for IHN, ISA, IPN, VHS and *Piscirickettsia salmonis* Culture any PCR positive samples.

ADDENDUM: March 24, 2005 - samples submitted for routine histology processing and analysis.



**Histopathology**

Quality control/quality assurance: the spleen sections contain no foci of acid hematin. The margins of a few organs have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cells types stains poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes.

Slide 1: autolysis, mild (other organs) to moderate (liver)

1. Liver: no significant lesions
- 2a. Heart: endocarditis, focal, lymphohistiocytic, mild
- 2b. Heart: epicarditis, regionally diffuse, lymphoplasmacytic, mild
3. Spleen: not included on the slide
4. Trunk kidney: no significant lesions
5. Head kidney: no significant lesions

Comment: Lymphohistiocytic endocarditis and lymphoplasmacytic epicarditis are evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.

Slide 2: autolysis, none (other organs) to mild (liver)

1. Liver: no significant lesions
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: 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 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.

Slide 3: autolysis, none (other organs) to mild (liver)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
- 2a. Heart: endocarditis, multifocal, with endothelial cell hypertrophy and a thin layer of macrophages, mild
- 2b. Heart: epicarditis, regionally diffuse, lymphoplasmacytic, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: no significant lesions
5. Head kidney: nephritis, interstitial, granulomatous, focal, mild

Comment: 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).

The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.

The pattern of inflammation in the heart is consistent with a systemic immune stimulation, probably a bacterial infection. Inflammatory cells lining the endocardial surface in affected foci are rarely more than 2 cell layers thick.

Slide 4: autolysis, none (all organs)

- 1a. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild



2. Heart: myocardial karyomegaly, multifocal, mild
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, minimal
4. Trunk kidney: renal tubular dilation, diffuse, mild
5. Head kidney: no significant lesions

Comment: The cause and significance of myocardial karyomegaly 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). Dilation of renal components is evidence of localized impaired renal function, but the cause is unknown.

Slide 5: autolysis, none (all organs)

- 1a. Liver: hepatocellular fatty change (lipidosis), focal, mild
- 1b. Liver: biliary preductular cell hyperplasia, diffuse, mild
2. Heart: no significant lesions
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild
4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild
5. Head kidney: no significant lesions

Comment: see comments on previous slides.

#### **Molecular Diagnostics/PCR**

Samples 1-3, 4-5: IHN, VHS, ISA, IPN, Piscirickettsia salmonis negative by PCR.

\* Results faxed Apr. 28/05.

/bb



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**Case Report**

**Submission** 2005-00968      **Date** 22-Mar-2005      **Report** 04-Apr-2005

**Report**      **Copies**

**Submitter:** 3409      Ministry of Agriculture & Lands (Dr.  
J.Constantine)

**Owner** 13804      A.2.3-1 (#3 & #10)

**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**

Provincial Surveillance Program. Specimens A.2.3-1 (#3 and #10). For bacteriology - identification of isolates and C & S.

**Bacteriology**

Isolate 10 - *Vibrio* *logei*.

Isolate 3 - *Yersinia* *ruckeri*.

*Yersinia* sensitive to: Florfenicol, Romet 30, Sulfa-methox-trimeth and Tetracycline.

\* Results faxed Apr. 4/05.

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## Case Report

**Submission** 2005-00988      **Date** 23-Mar-2005      **Report** 31-Mar-2005

**Report**      **Copies**

**Submitter:** 12847      Heritage Salmon  
**Owner** 12847      Heritage Salmon  
**Farm:** 13816      Simmonds Pt. (Case 05-12)  
**Vet Clinic:**  
**Attending**      Dr. Peter McKenzie

**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

**History/Symptoms**

Submitter: Tim Talbot. Case #05-12. PO #H15248.

New smolt entries. History of E.R.M. Internal hemorrhage on flesh, pylorus, liver and swim bladder.

Four histo. Cassettes labelled 05-12-1, 05-12-2, 05-12-3, 05-12-4 fixed in formalin - shipped in ethanol.

Please prepare for histo. Exam by Dr. Gary Marty.

**Histopathology**

Four cassettes full of tissues fixed in formalin were submitted in a plastic jar of ethanol.

Slide 1 (05-12-1) - gill, liver, heart, head kidney, skin/skeletal muscle, intestine, and intestinal ceca

Slide 2 (05-12-2) - gill, liver, heart, trunk kidney (3 pieces), skeletal muscle (2 pieces), intestinal ceca, mesenteric fat, and gill

Slide 3 (05-12-3) - gill, liver, heart, head kidney, trunk kidney, skin/skeletal muscle, stomach, and intestinal ceca

Slide 4 (05-12-4) - gill, liver, heart, trunk kidney, skeletal muscle, intestinal ceca, mesenteric fat, and gill

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

Quality control: Tissue preservation for most organs is fair to poor. Tissues have no evidence of postfixation dehydration.

Measures of physiologic condition

Hepatocellular glycogen depletion, severe (slides 1, 2, 3, 4)

Mesenteric adipose tissue depletion, none (slides 2, 3, 4)

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

**Diagnosis**

1. Trunk kidney: renal tubular epithelial necrosis, multifocal, subacute, with fibrin and peritubular fibrosis, moderate (slide 3)

2. Mesenteric fat: peritonitis, lymphoplasmacytic, multifocal, with fine fibrocellular fronds, mild (slide 2)





**Final Comments**

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). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as Gentamicin). Consider bacteriology and virology, if not already done.

The skin/skeletal muscle section (slide 1) contains no epidermis. This could be a result of an ulcer or post mortem separation of the epidermis from the dermis. Small ulcers are common in fish, and filamentous bacteria commonly invade skin ulcers. Once filamentous bacteria become established, the ulcers often get larger (as in this case). Enlargement of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated (Mutoloki et al. 2004).

S. Mutoloki, S. Alexandersen, and Ø. Evensen. 2004. Sequential study of antigen persistence and concomitant inflammatory reactions relative to side-effects and growth of Atlantic salmon (*Salmo salar* L.) following intraperitoneal injection with oil-adjuvanted vaccines. *Fish & Shellfish Immunology* 16(5):633-644.

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## Case Report

**Submission** 2005-00989      **Date** 23-Mar-2005      **Report** 31-Mar-2005

**Report**      **Copies**

**Submitter:** 12847      Heritage Salmon  
**Owner** 12847      Heritage Salmon  
**Farm:** 13814      Wehlis Bay (Case #05-13)  
**Vet Clinic:**  
**Attending**      Dr. Peter McKenzie

**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

### **History/Symptoms**

Submitter: Tim Talbot. Case #05-13. PO #H15248.

Harvest fish. History of BKD and skin lesions. Internal hemorrhage on flesh, pylorics and swim bladder. No bacterial growth on TSA 5% SB.

Two histo. Cassettes labelled 05-13-1, 05-13-2. Fixed in formalin, shipped in ethanol. Please prepare for histo. exam for Dr. Gary Marty.

### **Histopathology**

Two cassettes full of tissues fixed in formalin were submitted in a plastic jar of ethanol. Tissues from each cassette were split and embedded into two blocks, resulting in two slides per cassette.

Slide 1A (05-13-1) - head kidney, spleen, intestine, and mesenteric fat

Slide 1B (05-13-1) - gill, liver, and heart

Slide 2A (05-13-2) - heart, head kidney, spleen, and intestinal epithelium

Slide 2B (05-13-2) - gill, liver, intestine, and mesenteric fat

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

Quality control: Tissue preservation for most organs is good to fair. Tissues have no evidence of postfixation dehydration.

Measures of physiologic condition:

Hepatocellular glycogen depletion, severe (slides 1B, 2B)

Mesenteric adipose tissue depletion, none (slides 1A, 2B)

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

### **Diagnosis**

1. Liver: parenchymal golden pigment, disseminated, intracellular, mild

### **Final Comments**

These organs had no lesions to explain the death of these fish. The golden pigment in the liver most likely is lipofuscin. Accumulation of lipofuscin 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 have been associated with decreased growth and survival in several studies.

/bb



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**Case Report**

**Submission** 2005-00990      **Date** 23-Mar-2005      **Report** 01-Apr-2005

**Report**      **Copies**

**Submitter:** 12847      Heritage Salmon  
**Owner** 12847      Heritage Salmon  
**Farm:** 13817      Maude Island (Case #05-15)  
**Vet Clinic:**  
**Attending**      Dr. Peter McKenzie

**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

### **History/Symptoms**

Submitter: Tim Talbot. Case #05-15. PO #H15248.

Approx. 6 months in salt water. History of BKD at hatchery. Internal swelling of kidney - liver and spleen had small - large granulomas, many of which seemed doughnut shaped and Rickettsia?

Four histo. Cassettes labelled: 05-15-1, 05-15-2, 05-15-3, 05-15-4. Please prepare for histo. exam for Dr. Gary Marty.

### **Histopathology**

Four cassettes full of tissues fixed in formalin were submitted in a plastic jar of ethanol.

Slide 1 (05-15-1) -liver, trunk kidney, and spleen

Slide 2 (05-15-2) - liver, trunk kidney, and spleen

Slide 3 (05-15-3) - liver, trunk kidney, and spleen

Slide 4 (05-15-4) - liver, trunk kidney, and spleen

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

Quality control: Tissue preservation for most organs is fair to poor. Tissues have no evidence of postfixation dehydration.

Measures of physiologic condition

Hepatocellular glycogen depletion, severe (slides 1, 2, 3, 4)

Mesenteric adipose tissue depletion, none (slide 3)

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

### **Diagnosis**

1. Trunk kidney, liver, spleen: granulomatous inflammation, multifocal, moderate to severe (slides 1, 2, 3, 4); Twort's Gram stain on slide 2 contains large numbers of Gram positive bacteria consistent with *Renibacterium salmoninarum*
2. Liver: hepatic necrosis, acute, multifocal, moderate (slide 2); focal, moderate (slide 4)
3. Spleen: peritonitis, lymphoplasmacytic, multifocal, with fine fibrocellular fronds, moderate (slide 1)
4. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, moderate (slide 2)
5. Liver: mural thrombus, 150 µm in diameter, focal, acute, moderate (slide 3)





**Final Comments**

Granulomatous inflammation in multiple organs is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* (Bacterial Kidney Disease) is the most common organism associated with these lesions. The renal tubular epithelial necrosis in slide 2 is probably secondary to altered perfusion resulting from the associated granulomatous inflammation.

Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral or bacterial infections (e.g., *Piscirickettsia salmonis*). Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis.

Thrombosis in the liver (slide 3) is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection. Thrombosis is commonly associated with viral hemorrhagic septicemia virus (VHSV) infection; consider virology, if not already done.

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

/bb



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## Case Report

**Submission** 2005-00991      **Date** 23-Mar-2005      **Report** 31-Mar-2005

**Report**      **Copies**

**Submitter:** 12847      Heritage Salmon  
**Owner** 12847      Heritage Salmon  
**Farm:** 13819      Cliff Bay (Case #05-16)  
**Vet Clinic:**  
**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

### **History/Symptoms**

Submitter: Tim Talbot. Case #05-16. PO #H15248.

Approximately 4 months in salt water. Hatchery has history of BKD. Internal swelling of kidney with granulomas in liver and spleen - some doughnut shaped - Rickettsia?

Submitted 4 histo. Cassettes labelled 05-16-1, 05-16-2, 05-16-3, 05-16-4. Fixed in formalin - shipped in ethanol.

Please prepare for Histo. exam for Dr. Gary Marty.

### **Histopathology**

Four cassettes of tissues fixed in formalin were submitted in a plastic jar of ethanol.

Slide 1 (05-16-1) -liver, trunk kidney, and spleen

Slide 2 (05-16-2) - liver, trunk kidney, and spleen

Slide 3 (05-16-3) - liver, trunk kidney, and spleen

Slide 4 (05-16-4) - liver, trunk kidney, and spleen

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

Quality control: Tissue preservation for most organs is fair to poor. Tissues have no evidence of postfixation dehydration.

Measures of physiologic condition

Hepatocellular glycogen depletion, severe (slides 1, 2, 3, and 4)

This pattern in the measures of physiologic condition is consistent with fish that haven't fed normally for at least a few days (severe glycogen depletion).

### **Diagnosis**

1a. Liver: hepatic necrosis, acute, multifocal, with sinusoidal congestion and fibrin deposition, moderate (slide 1)

1b. Liver: hepatic necrosis, acute, multifocal, mild (slide 2), moderate (3)

1c. Liver: hepatitis, granulomatous, multifocal, severe (slide 4)

1d. Liver: hepatitis, perivascular, lymphocytic, multifocal, mild (slides 2, 3, 4)

2a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, moderate (slide 2), severe (slides 3, 4)

2b. Trunk kidney: renal tubular epithelial necrosis, focal, acute, mild (slide 1), moderate (slides 3, 4), severe (slide 2)

3. Spleen: splenitis, granulomatous, multifocal, with disseminated small foci of fibrin, moderate (slide 3), severe (slide 4)



**Final Comments**

Disseminated granulomatous inflammation and necrosis in all four fish provide sufficient evidence to explain the death of these fish. Hepatic necrosis can be caused by inadequate vascular perfusion or direct cytotoxicity from viral (e.g., Viral hemorrhagic septicemia) or bacterial infections (e.g., *Renibacterium salmoninarum* or *Piscirickettsia salmonis*). Sinusoidal congestion (slide 1 only) has been associated with Infectious Salmon Anemia Virus. Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Consider bacteriology and virology, if not already done.

Lymphocytic inflammation around vessel in the liver (slides 2, 3 and 4) is evidence of chronic immune stimulation, e.g., from a bacterial infection.

The most common organism associated with granulomatous nephritis and splenitis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.

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). Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxicants (e.g., bacterial toxins, or aminoglycoside antibiotics such as Gentamicin). In these fish, it might be a secondary to granulomatous inflammation and *Renibacterium salmoninarum*.

/bb

