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Ministry of Agriculture and Lands

Animal Health Centre

AAVLD - Accredited Laboratory

Ministry of Agriculture and Lands
1767 Angus Campbell Road
Abbotsford BC V3G 2M3
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Final Report AHC Case: 08-99

Last Updated: 01/14/08 3:40 PM
Pathologist: Gary D. Marty
Received Date: 01/09/08
Collected Date: 01/09/08
Client Ref No: PO#2599BM

Veterinarian: **Barry Milligan**
Clinic: **Grieg Seafoods BC Ltd.**
Phone: (250) 286-0838
Fax: (250) 286-1883

Submitter: **Jeanine Sumner**
Phone:
Fax:
Owner: **Grieg Seafoods BC Ltd.**
Phone: (250) 286-0838
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Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 28 cassettes; 15 labeled multi organ tissue cassettes (1 fish/cassette = 15 fish); 13 individual gill samples (not labeled, separate from above group 13 fish in total). Please prepare for histo exam.

Customers complaining of an "off" odor in fish.

Final Diagnosis

1a. Liver: hepatitis, leukocytic, focal, mild (slide 1A)

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 2B, 3A, 4A, 5A, 6A, 8A, 12A, 13A, 14A, 15A)

1c. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slides 2B, 3A, 5A, 7A, 8A, 10A, 12A, 13A, 14A, 15A)

1d. Liver: pericholangitis, lymphocytic, focal, mild (slide 7A)

1e. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 8A)

1f. Liver: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 14A)

2a. Intestinal ceca: mucosal cyst, focal, 500 µm in diameter, with central layered mineral and golden pigment (probably lipofuscin)

2b. Intestinal ceca: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 3B, 12A, 13A), moderate (slide 8A)

3a. Spleen: splenitis, granulomatous, multifocal, coalescing, with small numbers of multinucleate giant cells, severe

3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 9A, 11A)

3c. Spleen: yellow-brown to yellow-green pigmented macrophages, disseminated, mild (slides 14A, 15A)

4. Gill: lamellar telangiectasis, multifocal, mild (slides 4G, 6G)

Final Comment: I can suggest two things that might be related to the "off" odor. First, lysis of gill epithelial cells might be a result of harvesting/shipping in hypotonic water. I use lysis of lamellar epithelial cells to assess the severity of gill autolysis; however, without controlled study I cannot readily differentiate routine postmortem autolysis from postmortem hypotonic lysis. Postmortem hypotonic lysis would increase the rate of breakdown of gill epithelial cells. To test this hypothesis, consider trying to eliminate hypotonic water from harvesting and shipping to see if this slows the rate of gill breakdown. The second thing that might be related to the off odor is the degree of intestinal autolysis (moderate to severe in most fish). Based on my phone conversation with Dr. Milligan, fish are now harvested by being killed and bled at the farm in the afternoon, shipped in chilled water to the processing plant, and then processed (including removal of viscera) the next morning. Under these conditions, intestine has more time to autolyze in the fish than in previously used harvest methods, in which fish were shipped live to the processing plant and the viscera was removed within minutes of death at the processing plant. If any of the autolyzed intestines are associated with bacterial overgrowth, then bacteria or their toxins might leach into other organs before organs are removed at harvest. To test this hypothesis, consider removing the viscera from a sample of harvest fish on site and see if they still develop the off odor. Evidence against this hypothesis is that the intestinal ceca do not contain unusual numbers of bacteria.

Viscera from the 15 fish have several lesions that are fairly common in older Atlantic salmon:

The focus of hepatic parenchymal leukocytes in slide 1A (mostly lymphocytes) is about 400 µm in diameter. Similar foci are fairly common in wild salmonids, but they are rare in pen-reared salmon. These foci probably develop in response to chronic immune stimulation (e.g., focal bacteria, parasites, or other antigens). The section has no obvious organisms.

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

Pigment in the liver probably is lipofuscin, and it might also include hemosiderin. Pigment in the spleen usually is lipofuscin only. Accumulation of lipofuscin in the liver and spleen 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. Mild accumulations of pigment are fairly common in market size Atlantic salmon. Hemosiderin accumulation in the liver might result from increased turnover of red blood cells.

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.

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). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "silvers" that die in marine net pens, in 2007 affecting 12% of the 645 Atlantic salmon but 0% of the 134 Pacific salmon examined as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2007 was sufficient to identify a trend towards greater prevalence in the fall and winter (14-19%) than in the spring and summer (4.1-10%).

The single mucosal cyst in an intestinal cecum on slide7A is an interesting finding. It might be a developmental anomaly, or it might have developed as a result of local trauma. This single cyst probably was of little consequence for fish health.

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

Granulomas in the spleen are evidence of persistent indigestible material. Lack of bacteria (Twort's Gram stain) is consistent with vaccine material as the cause. The primary differential is *Renibacterium salmoninarum*, the cause of bacterial kidney disease; granulomas occasionally have very few organisms.

Telangiectasis in the gill most commonly results from trauma (e.g., handling). It is somewhat surprising that all fish do not have some degree of telangiectasis.

Histopathology

Formalin-fixed tissues from harvest-sized fish were submitted in 28 cassettes for histopathology. After samples were processed into paraffin, cassettes 1A through 6A were split into two cassettes (labeled B), yielding a total of 34 slides.

Slide 1A (Much #1) - spleen, liver, head kidney

Slide 1B - intestinal ceca and mesenteric adipose tissue

Slide 2A (Much #2) - spleen, intestinal ceca, head kidney, liver, mesenteric adipose tissue

Slide 2B - liver, intestinal ceca, mesenteric adipose tissue

Slide 3A (Much #3) - spleen liver, intestinal ceca, mesenteric adipose tissue

Slide 3B - spleen, intestinal ceca, mesenteric adipose tissue

Slide 4A (Much #4) - spleen, liver, intestinal ceca, and thick-walled tubular organ lined by striated muscle on one margin and ciliated epithelium on another (swimbladder?)

Slide 4B - intestinal ceca and mesenteric adipose tissue

Slide 5A (Much #5) - spleen, liver, intestinal ceca

Slide 5B - intestinal ceca, mesenteric adipose tissue

Slide 6A (Much #6) - spleen, liver, intestinal ceca, mesenteric adipose tissue

Slide 6B - liver, intestinal ceca, mesenteric adipose tissue

Slide 7A (Much #7) - liver, intestinal ceca, spleen, mesenteric adipose tissue, and margin of gonad(?)

Slide 8A (Much #8) - spleen liver, intestinal ceca, mesenteric adipose tissue

Slide 9A (Much #9) - spleen liver, intestinal ceca, mesenteric adipose tissue, and margin of gonad(?)

Slide 10A (Much #10) - liver, intestinal ceca, mesenteric adipose tissue

Slide 11A (Much #11) - spleen, liver, stomach, intestinal ceca, mesenteric adipose tissue

Slide 12A (Much #12) - spleen, liver, intestinal ceca, mesenteric adipose tissue

Slide 13A (Much #13) - spleen, liver, intestinal ceca, mesenteric adipose tissue

Slide 14A (Much #14) - spleen, liver, intestinal ceca, mesenteric adipose tissue; a step-section from the same block was stained with Twort's Gram and ZN acid-fast stains.

Slide 15A (Much #15) - stomach, spleen, liver, intestinal ceca, mesenteric adipose tissue

Slides 1G through 13G - gill

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

Quality control:

Liver autolysis: mild (slides 1A, 3A, 4A, 5A, 7A, 8A, 12A), moderate (slides 2B, 6B, 9A, 10A, 11A).

Autolysis of intestinal ceca: moderate (slides 1B, 3A, 11A), severe (slides 2A, 4A, 5A, 6A, 7A, 8A, 9A, 10A, 12A, 13A, 14A, 15A).

Gill autolysis: mild (slide 1G), moderate (slides 4G, 5G, 6G, 8G, 9G, 10G, 11G, 12G, 13G), severe (slides 2G, 3G, 7G)

Many of the cassettes labelled Much #1 through Much #15 were too full; tissues for histopathology need to be thin (<3 mm thick) and small enough so that the tissues in the cassette do not touch each other and so the cassette lid can be closed without squishing the tissues. Large foci of erythrocytes in some fish (e.g., liver in slide 4A and gill in slide 3G) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue). Organs have no postfixation dehydration.

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

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Final Report AHC Case: 08-234

Last Updated: 02/12/08 10:38 AM
Pathologist: Gary D. Marty
Received Date: 01/17/08
Collected Date: 01/17/08
Client Ref No: 6522

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
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Submitter: **Marine Harvest Canada**
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Fax:
Owner: **Marine Harvest Canada**
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Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted two virology samples for PCR for IHN and VHS from 2 fresh dead fish with hemorrhage of pyloric ceca only. Mortality is down. Routine sample to rule out VHS and IHN. Atlantic salmon, regular, 2007 S0, all, saltwater.

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 01/22/08 @ 12:04 PM

Specimen	ID	Test	Result
Tissue	6522-1	PCR - IHNV	Negative
Tissue	6522-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 01/22/08 @ 12:04 PM

Specimen	ID	Test	Result
Tissue	6522-1	PCR - VHSV	Negative
Tissue	6522-2	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 02/12/08 @ 10:38 AM

Specimen	ID	Isolate	Result	Level
Tissue	6522-1		No viruses isolated	
Tissue	6522-2		No viruses isolated	

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Final Report AHC Case: 08-236

Last Updated: 01/22/08 2:18 PM
Pathologist: Gary D. Marty
Received Date: 01/17/08
Collected Date: 01/17/08
Client Ref No: 6521

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
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Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
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Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted histo taken from fish with small discoloured liver with granulomas. Sampled fish was otherwise healthy in appearance. Sample taken during routine ELISA samples.

Atlantic, regular, 2007, S1, Saltwater.

Final Diagnosis

1a. Liver: biliary preductular cell hyperplasia, diffuse and nodular, severe

1b. Liver: hepatitis, granulomatous, multifocal, with an intralesional vacuole 300 µm in diameter, moderate

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

2. Heart: endocarditis, multifocal, lymphohistiocytic, mild

3. Spleen: peritonitis, chronic, regionally diffuse, with fibrocellular fronds, mild

Final Comment: The liver section has a few small foci of granulomatous inflammation; however, lack of bacteria in these foci (Gram stain) rules unlikely *Renibacterium salmoninarum* as the cause. The foci might be a reaction to a vaccine or other persistent immunologic material. 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). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "silvers" that die in marine net pens, affecting 12% of the 645 Atlantic salmon but 0% of the 134 Pacific salmon examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2007 was sufficient to identify a trend towards greater prevalence in the fall and winter (14-19%) than in the spring and summer (4.1-10%). Notably, none of the auditing cases were severe, so the extent of hyperplasia in this fish is rare.

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

Lymphohistiocytic 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, affecting 71% of 1115

Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Histopathology

Formalin-fixed tissues from an Atlantic salmon were received for histopathology. Sections were stained with H&E and Twort's Gram stain.

Slide 1 (6521) - spleen, heart, liver, head kidney, trunk kidney

All organs on the slide were examined. Those not listed elsewhere have no significant lesions.

Quality control: Tissue preservation is excellent for most organs. Large foci of erythrocytes (e.g., splenic vessels) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue). The margins of some organs have evidence 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 (e.g., during shipment).

Gary D. Marty
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Final Report AHC Case: 08-456

Last Updated: 02/08/08 11:10 AM
Pathologist: Gary D. Marty
Received Date: 02/05/08
Collected Date: 02/05/08
Client Ref No: 8-2407

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **Tim Hewison - Microtek**
Phone:
Fax:
Owner: **Microtek International In**
Phone:
Fax:(250) 652-4802

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted three sample sets labeled as: CE-3, CE-4a, CE-4b, CE-6; CL-1, CL-2, CL-3, CL-4, CL-5; M-3, M-10, M-12

All organs were sampled from Atlantic salmon. The samples were preserved in formalin then transferred to tap water for transport. Please refer to case file number 8-2407 on all reports and invoicing. Should you have any questions or require more information, please give me a call at 250 652-4482 ext 201.

Final Diagnosis

Lesions most likely to have caused death of these fish:

1. Mesenteric adipose tissue: capillary congestion, diffuse, severe (slide CL1)

2a. Liver: hepatopathy, diffuse, with hepatocellular lipidosis, basophilic cytoplasm, karyomegaly/megalocytosis, and hydropic degeneration/single cell necrosis, moderate (slide CL2)

2b. Liver: hepatopathy, diffuse, with hepatocellular basophilic cytoplasm, and hydropic degeneration/single cell necrosis, moderate (slide CL2)

3. Spleen: splenitis, fibrinous, multifocal, severe (slide CL5)

4. Multiple organs: granulomatous inflammation and coagulative necrosis, with intralesional short Gram -positive rods consistent with *Renibacterium salmoninarum*, the cause of Bacterial Kidney Disease, severe (slides M3, CE3, CE4A, CE4B, and CE6)

Final Comment: Details for this case are included on an Excel spreadsheet (2008-0456.xls) that is not included with the official final report generated by the Animal Health Centre's VADDS database (the database cannot handle spreadsheets). The "Abbreviation" worksheet includes comments about each lesion. Lesions on the spreadsheet are the same as those included in the histopathology part of the BC Fish Health Auditing and Surveillance Program. Therefore, the spreadsheet includes some lesions that these fish do not have. Specific comments on significant lesions in these fish follow:

Distension of capillaries in the mesenteric adipose tissue is nonspecific evidence of circulating vasodilators; hemorrhage sometimes occurs in severe cases. Mesenteric capillary congestion is most commonly associated with VHSV and bacterial infections; in this case it might be associated with severe peritonitis around the spleen.

Fish CL2 has a combination of liver lesions that could have killed the fish. 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 fish it might be related to increased protein needed as part of an inflammatory response. **Hydropic degeneration** among small numbers of hepatocytes provides evidence that the liver was being exposed to toxins. Potential sources of the inciting toxins include the water, a bacterial infection, or circulating oxygen radicals following a period of hypoxia. In this case, cytoplasm of affected hepatocytes is expanded by fine to large foamy vacuoles. After hydropic degeneration can no longer be reversed, the changes are called **single cell necrosis**. Fish M10 has moderate hepatocellular basophilic cytoplasm, hydropic degeneration, and single cell necrosis.

Fibrinous splenitis is evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin. I have seen this response in salmon that are PCR positive for VHSV. Most of the bacteria in the vessels of fish CL5 probably grew after the fish died; however, intravascular bacteria are not common in Atlantic salmon that die in BC marine netpens (I examine about 600 a year as part of the province's Fish Health Auditing and Surveillance Program), and some bacteria probably grew before the fish died (thereby contributing to morbidity). Bacterial culture is needed for species identification. The bacilli are too large for *Renibacterium salmoninarum*, and the colonies are too small for *Aeromonas salmonicida*.

Five fish probably died of complications related to severe infections with *Renibacterium salmoninarum*, the cause of bacterial kidney disease. This disease can adversely affect nearly all ages of farmed Atlantic salmon in BC.

Lesions in fish CL3, CL4, and M12 are similar to some of the other fish, but they do not seem of sufficient severity to explain their death. Scores for other lesions are included on the spreadsheet.

Histopathology

Formalin-fixed tissues were submitted in 12 cassettes for histopathology.

Slide CL1 (Cl-1) - spleen, liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide CL2 (CL-2) - spleen, liver, trunk kidney, intestinal ceca (and a small piece of stomach), mesenteric adipose tissue

Slide CL3 (CL-3) - brain, spleen, liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide CL4 (CL-4) - brain, spleen, liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide CL5 (CL-5) - brain, spleen, liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide M3 (M-3) - brain, spleen, heart, liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide M10 (M-10) - brain, spleen, liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide M12 (M-12) - brain, spleen, liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide CE3 (CE-3) - brain, spleen, liver, trunk kidney, stomach, intestinal ceca, mesenteric adipose tissue

Slide CE4a (CE-4a) - brain, spleen, liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide CE4b (CE-4b) - brain, spleen, liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide CE6 (CE-6) - liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

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

Quality Control: Details are included on the spreadsheet (2008-0456.xls). Tissue preservation is variable; for example, liver autolysis varies from mild (1 fish) to moderate (5 fish) or severe (5 fish). Large foci of erythrocytes (e.g., spleen in slide M12) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue). Organs have no postfixation dehydration.

Gary D. Marty
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Final Report AHC Case: 08-473

Last Updated: 02/27/08 4:32 PM
Pathologist: Gary D. Marty
Received Date: 02/06/08
Collected Date: 02/06/08
Client Ref No: 6537

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
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Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
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Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh and formalized tissue for histology and PCR for IHNV and VHSV.

Mortality at site is low. One fish with hemorrhage of liver and pale gills. No growth on bacteriology plates. One histo sample collected and one sample for PCR for IHN and VHS.

Atlantic, 2006 SO, vaccinated. Fish died on January 23, 2008

Final Diagnosis

1. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate

2. Mesenteric adipose tissue: peritonitis, chronic, focal, with fibrocellular fronds, mild

Final Comment: The section of liver has no evidence of the hemorrhage observed grossly. None of the lesions are of sufficient severity to explain the death of this fish. Comments on specific lesions follow:

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

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Histopathology

Formalin-fixed tissues were submitted in 1 cassette for histopathology.

Slide 1 (FS 6537 8) - spleen, heart, liver, intestine, head kidney (and margin of trunk kidney), mesenteric adipose tissue

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

Quality control: Liver autolysis: mild; intestinal autolysis: none. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/11/08 @ 1:17 PM

Specimen	ID	Test	Result
Tissue	6537	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/11/08 @ 1:17 PM

Specimen	ID	Test	Result
Tissue	6537	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 02/27/08 @ 4:32 PM

Specimen	ID	Isolate	Result	Level
Tissue	6537		No viruses isolated	

Gary D. Marty
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END OF REPORT

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Final Report AHC Case: 08-474

Last Updated: 02/27/08 4:32 PM
Pathologist: Gary D. Marty
Received Date: 02/06/08
Collected Date: 02/06/08
Client Ref No: 6551

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
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Submitter: **Marine Harvest Canada**
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Fax:
Owner: **Marine Harvest Canada**
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Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh and formalized tissue for histology and PCR for VHS and IHN.

Increase in mortality post transport. Fish off feed with NVL. 9 samples for PCR for IHN and VHS. Full histo from fish #10, #11, #21, #22. No growth on bacteriology - TSA and Blood agar.

Atlantic, 2008, Saltwater, Vaccinated. Date fish died February 1, 2008.

Final Diagnosis

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 10, 21, 22)

1b. Liver: hepatocellular hydropic degeneration, disseminated, acute, mild (slides 10, 21, 22), moderate (slide 11)

1c. Liver: hepatocellular single cell necrosis, disseminated, acute, mild (slide 10)

1d. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 10, 11, 21, 22)

1e. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 10)

2. Spleen and surrounding mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 10, 21)

3. Intestinal mesenteries: capillary congestion, diffuse, mild (slides 10, 11), moderate (slide 21)

4a. Trunk kidney: renal tubular dilation, focal, with intraluminal necrotic cells and interstitial cell atrophy, acute, mild (slide 11)

4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 11)

Final Comment: These fish have a suite of changes that provide evidence of exposure to toxins, but otherwise the changes are nonspecific. The standard list of differentials applies: VHSV, bacteria, or toxins in the water or feed. Comments on specific lesions follow:

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). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "silvers" that die in marine net pens, affecting 12% of the 645 Atlantic salmon but 0% of the 119 Pacific salmon livers examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2007 was sufficient to identify a trend towards greater prevalence in the fall and winter (14-19%) than in the spring and summer (4.1-10%).

Hydropic degeneration among small numbers of hepatocytes provides evidence that the liver was being exposed to toxins. Potential sources of the inciting toxins include the water, a bacterial infection, or circulating oxygen radicals following a period of hypoxia. In this case, cytoplasm of affected hepatocytes is expanded by fine to large foamy vacuoles. After hydropic degeneration can no longer be reversed, the changes are called single cell necrosis.

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

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

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

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Distension of capillaries in the mesenteric adipose tissue is often part of the inflammatory response to many infectious diseases; hemorrhage sometimes occurs in severe cases. In British Columbia, mesenteric congestion and hemorrhage is most commonly associated with VHSV and bacterial infections, but sometimes it seems to be associated with a vaccine reaction.

The combination of renal tubular epithelial cell necrosis, dilation of the tubular lumen, and interstitial cell atrophy is evidence of an acute process. Interstitial cell atrophy can result from sudden increased demand for these cells before the progenitor cells have a chance to replicate in response. The lumen of renal tubules often becomes dilated after patchy epithelial necrosis: remaining epithelial cells spread out to cover the resultant defect in the basil lamina. The change in epithelial height from columnar to squamous results in an increase in the diameter of the lumen, but the diameter of the basil lamina remains unchanged. 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 toxins (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin).

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

Histopathology

Formalin-fixed tissues were submitted in 8 cassettes for histopathology.

Slide 10 and 22 (211108) - spleen, heart, brain, liver, intestine, head kidney, trunk kidney, skin/skeletal muscle, mesenteric adipose tissue

Slide 11 and 21 (211108) - spleen, heart, brain, liver, intestine, trunk kidney, skin/skeletal muscle, mesenteric adipose tissue

Slide 10G, 11G, 21G and 22G (211108) - gill

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

Quality control: Liver autolysis: mild (slides 10, 11, 21, 22). Large foci of erythrocytes (e.g., liver vessels in slide 21) have a few precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue). Organs have no postfixation dehydration.

Measures of physiologic condition:

Hepatocellular glycogen: none (slides 10, 11, 21, 22)

Mesenteric adipose tissue: abundant (all 4 fish)

These measures of physiologic condition are consistent with fairly healthy fish that were not eating normally the last few days before they died.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/11/08 @ 1:17 PM

Specimen	ID	Test	Result
Tissue	6551 1-3	PCR - IHNV	Negative
Tissue	6551 4-6	PCR - IHNV	Negative
Tissue	6551 7-9	PCR - IHNV	Negative
Tissue	6551 12-14	PCR - IHNV	Negative
Tissue	6551 15-17	PCR - IHNV	Negative
Tissue	6551 18-20	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/11/08 @ 1:18 PM

Specimen	ID	Test	Result
Tissue	6551 1-3	PCR - VHSV	Negative
Tissue	6551 4-6	PCR - VHSV	Negative
Tissue	6551 7-9	PCR - VHSV	Negative
Tissue	6551 12-14	PCR - VHSV	Negative
Tissue	6551 15-17	PCR - VHSV	Negative
Tissue	6551 18-20	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 02/27/08 @ 4:32 PM

Specimen	ID	Isolate	Result	Level
Tissue	6551 1-3		No viruses isolated	
Tissue	6551 4-6		No viruses isolated	
Tissue	6551 7-9		No viruses isolated	
Tissue	6551 12-14		No viruses isolated	
Tissue	6551 15-17		No viruses isolated	
Tissue	6551 18-20		No viruses isolated	

Gary D. Marty
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Final Report AHC Case: 08-475

Last Updated: 02/27/08 4:32 PM
Pathologist: Gary D. Marty
Received Date: 02/06/08
Collected Date: 02/06/08
Client Ref No: 6533

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for PCR for IHN and VHSV.

One moribund with exephalmos, swollen vent, petechiation on peritoneum, swim bladder, muscle, and heart. Kidney grey and slightly swollen. One sample taken for PCR for IHN and VHS to rule out viral etiology.

Atlantic, 2006, Saltwater, Vaccinated. Date fish died January 21, 2008.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/11/08 @ 1:18 PM

Specimen	ID	Test	Result
Tissue	6533	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/11/08 @ 1:18 PM

Specimen	ID	Test	Result
Tissue	6533	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 02/27/08 @ 4:32 PM

Specimen	ID	Isolate	Result	Level
Tissue	6533		No viruses isolated	

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

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Final Report AHC Case: 08-476

Last Updated: 02/27/08 4:32 PM
Pathologist: Gary D. Marty
Received Date: 02/06/08
Collected Date: 02/06/08
Client Ref No: 6549

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh and formalized tissue.

Sudden increase in mortality post harvest, suspect over crowding. No visible lesions noted. Histo collected from 4 fish. Fish #3 was moribund, all others fresh dead. No growth on blood agar or TSA. One sample for PCR for IHN and VHS.

Atlantic, 2006, Saltwater, Vaccinated. Date fish died January 31, 2008.

Final Diagnosis

1. Spleen: splenitis, granulomatous, focal, mild (slide 4)

2a. Head kidney: interstitial intracytoplasmic eosinophilic granules, diffuse, mild (slide 4), moderate (slide 3)

2b. Head kidney: interstitial vascular congestion, diffuse, mild (slide 1)

3a. Liver: yellow-brown pigmented macrophage aggregates and sinusoidal macrophages, disseminated, (hemosiderin and lipofuscin?), mild (slides 2, 3), moderate (slide 1)

3b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 3)

4. Heart: myocardial karyomegaly, multifocal, mild (slides 2, 3)

5. Mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 3)

Final Comment: None of these fish have lesions of sufficient severity to explain their death. However, because brain was not included among the tissues sampled, my ability to arrive at a "cause of death" diagnosis is cut in half. As evidence, among the "fresh silvers" that I examined as part of the province's Fish Health Auditing and Surveillance Program, my ability to diagnose "cause of death" increased from 24% in 2006 to 48% in 2007; most of that increase was a result of adding brain to the organs sampled for histopathology. Comments on specific lesions follow:

The most common organism associated with granulomatous splenitis in farmed BC salmon is *Renibacterium salmoninarum*, the cause of

bacterial kidney disease. Differentials include chronic *Yersinia ruckeri* infection and a reaction to a vaccine.

Accumulation of eosinophilic granules in the cytoplasm of cells lining small vessels in the kidney is a distinctive finding in Atlantic salmon. These granules probably accumulate in response to systemic immune stimulation. The granules might be part of eosinophilic granular cells or endothelial cells; granule size seems too large and variable for eosinophilic granular cells. I have seen these granules associated with *Piscirickettsia* salmonis infection and with severe cerebral *Renibacterium salmoninarum* (in at least one case, the affected fish had no granulomatous inflammation in the kidney), but other cases (like this one) have no obvious cause. Fish #s 3 and 4 are good candidates for death due to cerebral *Renibacterium salmoninarum* infection.

Renal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV.

Pigment in the liver probably is lipofuscin, and it might also include hemosiderin. 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. I recently had a case as part of the provincial Fish Health Auditing and Surveillance Program in which increased pigment was associated with algal blooms during the previous summer. 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 might result from increased turnover of red blood cells.

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.1% of the 1142 Atlantic salmon hearts sampled as part of the province's Fish Health Auditing and Surveillance Program during 2006 and 2007). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

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

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

Histopathology

Formalin-fixed tissues from Atlantic salmon were submitted in 6 cassettes for histopathology. The gills were decalcified in Protocol B for about 2 hours before processing routinely into paraffin.

Slide 1 (6549-1) - spleen, heart, liver, head kidney, intestinal ceca, and mesenteric adipose tissue

Slide 2 - spleen, heart, liver, head kidney, intestinal ceca, and mesenteric adipose tissue

Slides 3 and 4 - spleen, heart, liver, head kidney/trunk kidney transition, intestine, and mesenteric adipose tissue

Slide 4Gill and 5 - gill

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

Quality control: Liver autolysis: mild (slide 3), moderate (slide 4), severe (slides 1, 2). Organs have no postfixation dehydration and no acid hematin deposits. Gill decalcification is complete.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/11/08 @ 1:18 PM

Specimen	ID	Test	Result
Tissue	6549	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/11/08 @ 1:18 PM

Specimen	ID	Test	Result

Tissue	6549	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 02/27/08 @ 4:32 PM

Specimen	ID	Isolate	Result	Level
Tissue	6549		No viruses isolated	

Gary D. Marty
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Final Report AHC Case: 08-530

Last Updated: 03/06/08 10:19 AM
Pathologist: Gary D. Marty
Received Date: 02/12/08
Collected Date: 02/12/08
Client Ref No: 6559

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted two samples labeled 6559-1 and 6559-2 for PCR for IHN and VHS. No external lesions on fish. Internally, fish with hemorrhage of liver and p.c. Please run PCR for IHN and VHS.

Molecular Diagnostics

PCR - IHNV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 02/15/08 @ 5:10 PM

Specimen	ID	Test	Result
Tissue	6559-1	PCR - IHNV	Negative
Tissue	6559-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 02/15/08 @ 5:10 PM

Specimen	ID	Test	Result
Tissue	6559-1	PCR - VHSV	Negative
Tissue	6559-2	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 03/06/08 @ 10:19 AM

Specimen	ID	Isolate	Result	Level
Tissue	6559-1		No viruses isolated	
Tissue	6559-2		No viruses isolated	

Gary D. Marty
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Final Report AHC Case: 08-585

Last Updated: 02/19/08 4:17 PM
Pathologist: Gary D. Marty
Received Date: 02/15/08
Collected Date: 02/15/08
Client Ref No: Req.#: 11356

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **Mainstream Canada**
Phone: (250) 725-1255
Fax: (250) 725-1250
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 5 formalized tissues for histo.

A) BareBluff Fish - Pen 107. Fish 1, Atlantic salmon. Approx. 900 g. Gross signs: large white granulomas on/in liver. Cloudy eye. Request histology. Rule out SRS and BKD.

B) BawDen Fish 1- Silv WF, enlarged heart.

C) BawDen Fish 2 - Silv WF. Bloody eye. Congestion pyloric ceca. Patchy gills. Blood in brain.

D) BawDen Fish 3 - Silv WF.

E) BawDen Fish 4 - Silv WF. Enlarged liver and spleen.

BareBluff Fish 1. Pool BawDen Fish 1, 2, 3, 4

Quote Purchase Order 11356. Submitted by Zarah Vansnick.

Final Diagnosis

1a. Liver: hepatic necrosis and fibrosis, chronic- acute, multifocal, coalescing with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), severe (slide A)

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides B1, B3, B4)

1c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides A, B1, B4)

1d. Liver: sinusoidal congestion, acute, multifocal, mild (slide B2)

1e. Liver: pericholangitis, lymphocytic, multifocal, mild (slide B4)

2a. Brain: cerebral and meningeal hemorrhage, focal to multifocal, moderate (slides B1, B2, B3), severe (slide B4)

2b. Brain: capillary (vascular) congestion, diffuse, moderate (slide B3)

3. Head kidney: interstitial congestion, acute, multifocal, mild (slide B2)

4. Eye, lens: phacolysis, central, severe (slide A)

5. Spinal cord/brainstem: meningomyelitis, histiocytic, focal, with intrahistiocytic bacteria (consistent with *Piscirickettsia salmonis*), moderate (slide A)

6a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides B1, B3)

6b. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slide B4)

7. Skeletal muscle, ocular: myonecrosis and regeneration, subacute, multifocal, moderate (slide B4)

Final Comment:

The fish from Bare Bluff probably died of complications related to *Piscirickettsia salmonis* infection. The 4 fish from BawDen seem to have died from traumatic brain injury. Details of specific lesions follow:

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). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "silvers" that die in marine net pens, affecting 12% of the 645 Atlantic salmon livers examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2007 was sufficient to identify a trend towards greater prevalence in the fall and winter (14-19%) than in the spring and summer (4.1-10%).

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

Sinusoidal congestion in the liver and interstitial congestion in the head kidney are nonspecific evidence of endothelial damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum* . Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. Consider bacteriology and PCR for 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.

The most common cause of cerebral and meningeal hemorrhage is trauma. Trauma often results from fish running into something, including other fish, and increased activity that leads to brain trauma might be associated with sea lice infestation or avoidance of predators.

Congestion of brain capillaries is evidence of circulating vasodilators; differentials include viral, bacterial, and parasitic infections. In this case pressure from the hemorrhage might have altered venous blood flow, leading to capillary congestion. [The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section contain erythrocytes.]

Phacolysis refers to the dissolution of the crystalline lens, which usually occurs spontaneously but is occasionally the result of trauma, infectious agents, or malnutrition. Loss of crystalline structure results in the opaque cataract observed grossly. In slide A, the lens seems to be intact, but only a few crystalline fibres remain at the periphery of the lens.

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 (slide B4) 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. It is more common in older fish. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

The section of eye included on slide B2 does not include any hemorrhage to corroborate the "bloody eye" observed grossly.

Skeletal muscle degeneration has been associated with feeding of rancid oils and dietary deficiency of vitamin E and selenium (reference: Fish Pathology, 3rd Edition. 2001. R.J. Roberts). It can also occur in fish that are not eating; muscle tissue is broken down to provide nutrients for critical organ survival. In the ocular muscles, consider also the possibility of trauma associated with exophthalmos. The presence of regenerating fibres is evidence of at least limited ability to repair the damage.

Histopathology

Formalin-fixed tissues were submitted in 5 cassettes for histopathology.

Slide A (BareBluff Fish 1) - brain, eye, liver, periorbital adipose tissue and skeletal muscle

Slide B1 (BawDen Fish 1) - spleen, brain, eye, liver, trunk kidney

Slide B2 (BawDen Fish 2) - spleen, brain, liver, head kidney/trunk kidney transition with Corpuscle of Stannius,

Slide B3 (BawDen Fish 3) - spleen, brain, liver, head kidney, and mesenteric adipose tissue

Slide B4 (BawDen Fish 4) - spleen, brain, retrobulbar adipose tissue and skeletal muscle, retina, liver, trunk kidney

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

Quality control: Liver autolysis: none (slide B1), mild (slides B2, B4), severe (slides A, B3). Large foci of erythrocytes (e.g., liver in slide B2) have a few precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue). Organs have no postfixation dehydration.

Gary D. Marty
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Final Report AHC Case: 08-590

Last Updated: 02/20/08 2:43 PM
Pathologist: Gary D. Marty
Received Date: 02/18/08
Collected Date: 02/18/08
Client Ref No: SS-212

Veterinarian: **Dr. Sonja Saksida**
Clinic: **Sea to Sky Veterinary Ser**
Phone: (250) 287-2363
Fax: (250) 287-2430

Submitter: **Sea to Sky Vet**
Phone:
Fax:
Owner: **SS-212**
Phone:
Fax:

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted formalized tissue for histology.

Acute mortality 10 days post sw entry. Large amount on necrosis around snout (face rot) exposing cartilage. No sign of septicemia. Is there any bacteria associated with face lesions?

Final Diagnosis

1. Head: dermatitis and cellulitis, ulcerative, multifocal, with lytic necrosis and moderate numbers of intralesional filamentous bacteria ("mouthrot"), severe (slides 1A, 2A)

2a. Liver: hepatocellular hydropic degeneration, disseminated, acute, mild (slide 2A), moderate (slide 1A)

2b. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1A, 2A)

2c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1A, 2A)

3. Intestinal ceca: peritonitis, granulomatous, multifocal, with intralesional vacuoles about 150 µm in diameter, moderate (slide 2A), severe (slide 1A)

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 1A), moderate (slide 2A)

5 Intestinal mesenteries: capillary congestion, diffuse, mild (slides 1A, 2A)

Final Comment: Features of the lesions associated with filamentous bacteria in both heads are fairly characteristic of the clinical entity "mouthrot." In slide 1A, bacteria extend to the bone (laterally) and around the teeth.

Hydropic degeneration among small to moderate numbers of hepatocytes provides evidence that the liver was being exposed to toxins. Cytoplasm of affected hepatocytes is expanded by fine to large foamy vacuoles. Potential sources of the inciting toxins include the water, a bacterial infection, or circulating oxygen radicals following a period of hypoxia. For fish #1, the oral bacteria seem the most likely source of the 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). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "silvers" that die in marine net pens, affecting 12% of the 645 Atlantic salmon livers examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2007 was sufficient to identify a trend towards greater prevalence in the fall and winter (14-19%) than in the spring and summer (4.1-10%).

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

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe). Vacuoles probably are a result of vaccine material lost during tissue processing.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

Distension of capillaries in the mesenteric adipose tissue is often part of the inflammatory response to many infectious diseases; hemorrhage sometimes occurs in severe cases. In British Columbia, mesenteric congestion and hemorrhage is most commonly associated with VHSV and bacterial infections, but sometimes it seems to be associated with a vaccine reaction.

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology. For all cassettes, gills were removed from the original (A) cassettes and placed in separate (B) cassettes.

Slide 1A (SS-212) - spleen, brain, liver, swimbladder, trunk kidney, intestinal ceca, mesenteric adipose tissue, and head (skeletal muscle, adipose tissue, cartilage, bone, gill, and teeth)

Slide 2A (SS-212) - spleen, brain, heart, liver, trunk kidney (2 pieces), intestinal ceca, mesenteric adipose tissue, and head (skeletal muscle, adipose tissue, cartilage, bone, cranial nerve, and teeth)

Slides 1B and 2B (SS-212) - gill

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

Quality control: Liver autolysis: mild (slides 1A, 2A); gill autolysis: mild (slides 1B, 2B). Large foci of erythrocytes (e.g., liver in slide 1A) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue). Organs have no postfixation dehydration.

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

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Final Report AHC Case: 08-645

Last Updated: 03/14/08 4:13 PM
Pathologist: Gary D. Marty
Received Date: 02/20/08
Collected Date: 02/20/08
Client Ref No: #6570

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fish tissues for PCR.

2 pools for PCR for IHN and VHS.

Fish shipped down an ice from sea site. NVL internally. Head rot externally.

This is the first virology sample from this group of fish.

Saltwater entry 2008 S1

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/26/08 @ 9:46 AM

Specimen	ID	Test	Result
Tissue	6570-1	PCR - IHNV	Negative
Tissue	6570-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/26/08 @ 9:46 AM

Specimen	ID	Test	Result
Tissue	6570-1	PCR - VHSV	Negative
Tissue	6570-2	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 03/14/08 @ 4:13 PM

Specimen	ID	Isolate	Result	Level
Tissue	6570-1		No viruses isolated	
Tissue	6570-2		No viruses isolated	

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

END OF REPORT

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Final Report AHC Case: 08-682

Last Updated: 02/25/08 1:44 PM
Pathologist: Gary D. Marty
Received Date: 02/21/08
Collected Date: 02/21/08
Client Ref No: 6568

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted formalized tissue for histology.

Increase in mortality in recent entries. Saltwater entry 2008 S1.

Final Diagnosis

1a. Liver: hepatic necrosis, acute, multifocal, moderate (slide A1)

1b. Liver: hepatocellular hydropic degeneration, disseminated, acute, mild (slide A1)

1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides A1, B1, C1)

1d. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides A1, B1, C1)

1e. Liver: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide C1)

2. Trunk kidney: renal tubular mineralization, multifocal, mild (slide C1), moderate (slide B1)

3. Mesenteric adipose tissue: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides B1, C1)

4a. Gill: chloride cell hyperplasia, and hypertrophy, regionally diffuse, mild (slides A2, B2)

4b. Gill: lamellar clubbing, multifocal, mild (slides A2, B2)

4c. Gill, filament blood vessel: mural thrombosis, focal, acute, mild (slide B2)

Final Comment: These fish have a number of lesions consistent with the history of increased mortality. The most significant is hepatic necrosis. Hepatocellular necrosis can be caused by inadequate vascular perfusion (e.g., as occurs with harmful algal blooms or hypoxia) or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Renibacterium salmoninarum* , or *Piscirickettsia salmonis*); the cause is not determined in most cases. 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. Hepatic necrosis is somewhat common in salmon that die in marine net

pens, affecting 11% of the 645 Atlantic salmon and 6% of the 119 Pacific salmon examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program.

Hydropic degeneration among small numbers of hepatocytes provides evidence that the liver was being exposed to toxins. Potential sources of the inciting toxins include the water, a bacterial infection, or circulating oxygen radicals following a period of hypoxia. In this case, cytoplasm of affected hepatocytes is expanded by fine to large foamy vacuoles. After hydropic degeneration can no longer be reversed, the changes are called single cell necrosis.

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

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

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 pathogenesis is not fully understood, but renal mineralization has been experimentally reproduced through high carbon dioxide levels, magnesium deficiency, selenium toxicity, and a diet low in minerals (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Clinically, renal mineralization is most commonly associated with high carbon dioxide levels.

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

Chloride cell hyperplasia has been associated with high carbon dioxide levels (Fivelstad et al. 2003) and with chronic exposure to acid waters with elevated dissolved aluminum (reference: Fish Pathology, 3rd Edition. 2001. R.J. Roberts; p. 68).

Focal thickening of the peripheral lamella (clubbing) might be a result of damage to the marginal channel, the most peripheral vessel in the lamella (and the only lamellar channel with an endothelial lining). Gill clubbing has been associated with parasitic infections (e.g., amoeba), but the affected gills in this case have no obvious organisms.

Thrombosis in the gill is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection; the section has no obvious organisms.

Literature cited:

Fivelstad, S., R. Waagbo, S.F. Zeitz, A.C.D. Hosfeld, A.B. Olsen, and S. Stefansson. 2003. A major water quality problem in smolt farms: combined effects of carbon dioxide, reduced pH and aluminium on Atlantic salmon (Salmo salar L.) smolts: physiology and growth. Aquaculture 215: 339-357.

Histopathology

Formalin-fixed tissues were submitted in 6 cassettes for histopathology.

Slide A1 (A, 2/1808) - spleen, heart, brain, liver, head kidney, trunk kidney, intestinal ceca, skin/skeletal muscle, mesenteric adipose tissue

Slide B1 (B) - spleen, heart, brain, liver, intestine, head kidney, trunk kidney, intestinal ceca, skin/skeletal muscle, mesenteric adipose tissue

Slide C1 (C) - spleen, heart, brain, liver, intestine, trunk kidney (2 pieces), intestinal ceca, skin/skeletal muscle, mesenteric adipose tissue

Slides A2 (A, 2/1808), B2 (B) and C2 (C) - gill (2 pieces)

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

Quality control: Liver autolysis: none (slides A1, B1, C1). Organs have no postfixation dehydration and no acid hematin deposits.

Case: 08-682

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

END OF REPORT

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Final Report AHC Case: 08-683

Last Updated: 03/14/08 4:13 PM
Pathologist: Gary D. Marty
Received Date: 02/21/08
Collected Date: 02/21/08
Client Ref No: 6568

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 4 samples for PCR for IHN and VHS.

Recent increase in mortality upon saltwater entry. Fish with head rot - mechanical damage? Samples kept frozen prior to shipment.

Histo submitted 24 hours ago. Saltwater entry 2008, S1

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/26/08 @ 11:23 AM

Specimen	ID	Test	Result
Tissue	Pen14 1-3	PCR - IHNV	Negative
Tissue	Pen14 4-6	PCR - IHNV	Negative
Tissue	Pen14 7-9	PCR - IHNV	Negative
Tissue	Pen14 10-12	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/26/08 @ 11:23 AM

Specimen	ID	Test	Result
Tissue	Pen14 1-3	PCR - VHSV	Negative
Tissue	Pen14 4-6	PCR - VHSV	Negative
Tissue	Pen14 7-9	PCR - VHSV	Negative
Tissue	Pen14 10-12	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 03/14/08 @ 4:13 PM

Specimen	ID	Isolate	Result	Level
Tissue	Pen14 1-3		No viruses isolated	
Tissue	Pen14 4-6		No viruses isolated	
Tissue	Pen14 7-9		No viruses isolated	
Tissue	Pen14 10-12		No viruses isolated	

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

END OF REPORT

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Final Report AHC Case: 08-690

Last Updated: 03/14/08 4:13 PM
Pathologist: Gary D. Marty
Received Date: 02/21/08
Collected Date: 02/21/08
Client Ref No: 11364

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **Mainstream Canada**
Phone:
Fax:
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 8 fresh tissue samples for PCR for VHS and IHN. Pool samples.

Biotech Services, contact Zarah Vansnick (250) 725-1255 and (250) 725-1250 fax.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/26/08 @ 11:23 AM

Specimen	ID	Test	Result
Tissue	A-(1-4)	PCR - IHNV	Negative
Tissue	B-(5-8)	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/26/08 @ 11:23 AM

Specimen	ID	Test	Result
Tissue	A-(1-4)	PCR - VHSV	Negative
Tissue	B-(5-8)	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 03/14/08 @ 4:13 PM

Specimen	ID	Isolate	Result	Level
Tissue	A-(1-4)		No viruses isolated	
Tissue	B-(5-8)		No viruses isolated	

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

END OF REPORT

Case: 08-690

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Final Report AHC Case: 08-697

Last Updated: 02/26/08 4:08 PM
Pathologist: Gary D. Marty
Received Date: 02/22/08
Collected Date: 02/22/08
Client Ref No: PO 11366

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **Zaran Vansnick**
Phone:
Fax:
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 6 bags of cassettes for histology. PO #11366.

BawDen Feb. 19/08. Gross signs: high mortality. Torn fins. Hemorrhage on bellies. Blood clots in pericardial cavity and belly. Torn operculums, wounds.

Samples labelled Fish 1,2,3, 4 and Fish 5,6.

Feb. 19/08:

Fish 1, Pen 112. Exophthalmic. With feed.

Fish 2, Pen 112. Hem. belly. Blood clots, internal

Fish 3, Pen 107

Fish 4, Pen 111. Enlarged liver, congested ceca. Blood clots

Fish 5, Pen 107

Fish 6, Pen 111, Silv WF. Exophthalmic. Torn hem. fins. Hem. belly and vent. Pale enlarged liver, enlarged spleen. Ascites. Scale loss.

Fish 7, Pen 111, Silv WF. Hem. fins and belly. Broken maxilla. Torn fins. Blood in eye. Scale loss. Ascites.

Feb. 20/08

Fish 1, Pen 107, Silv. WF. Ascites. Some blood in eye. hem. vent and belly. Pale liver, some hem. pyloric.

Fish 2, Pen 107. Silv WF. Hem. fins and belly. Ascites. Some hem. pyloric ceca.

Fish 3, Pen 107. Silv. WF. Hem. vent, pop eye.

Fish 4, Pen 107. Silv. WF. Hem. and torn fins. Enlarged liver and spleen. Slight sw. kidney.

Final Diagnosis

1. Head kidney: interstitial intracytoplasmic eosinophilic granules, diffuse, moderate numbers (slide 5A, 6A), abundant (slides 1, 2A, 4A)

2. Trunk kidney: interstitial intracytoplasmic eosinophilic granules, diffuse, small numbers (slide 5A), moderate numbers (slides 1, 2A, 4A)

3a. Liver: hepatic necrosis, acute, multifocal, moderate (slide 5A)

3b. Liver: hepatocellular cytoplasmic vacuolation (lipidosis?), diffuse, mild (slides 2A, 4A), moderate (slides 1, 5A, 6A)

3c. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 1, 4A, 5A, 6A)

3d. Liver: peritonitis, chronic, focal, with fibrocellular fronds, moderate (slide 3A)

3e. Liver: hepatocellular single cell necrosis, disseminated, acute, mild (slide 3A)

4a. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slides 1, 3A, 5A, 6A)

4b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 2A, 3A)

4c. Spleen: parenchymal necrosis, focal, acute, mild (slide 6A)

5a. Heart: myocardial karyomegaly, multifocal, mild (slide 3A)

5b. Heart: endocarditis, focal, lymphohistiocytic, mild (slide 5A)

6a. Brain: encephalitis, multifocal, lymphohistiocytic, with neuropil and intraneuronal microsporidia ("*Microsporidium cerebralis*"), moderate (slide 3A)

6b. Brain: capillary (vascular) congestion, diffuse, mild (slides 3A, 4A, 6A)

Final Comment: In addition to the cerebral microsporidian in fish #3, these fish have several lesions that could be a result of a bacterial or viral infection. I recommend bacterial culture and PCR for VHSV (if not already done). Comments on specific lesions follow:

Accumulation of eosinophilic granules in the cytoplasm of cells lining small vessels in the kidney is a distinctive finding in Atlantic salmon. These granules probably accumulate in response to systemic immune stimulation. The granules might be part of eosinophilic granular cells or endothelial cells; granule size seems too large and variable for eosinophilic granular cells. I have seen these granules associated with *Piscirickettsia* salmonis infection and with severe cerebral *Renibacterium salmoninarum* (in at least one case, the affected fish had no granulomatous inflammation in the kidney), but other cases (like this one) have no known cause. Renal eosinophilic granules have also been described in Atlantic salmon naturally infected with chronic pancreas disease in Norway (Salmonid alphavirus subtype 3, SAV3; McLoughlin and Graham 2007), but SAV3 has not been identified in BC salmon.

Hepatocellular necrosis can be caused by inadequate vascular perfusion (e.g., as occurs with harmful algal blooms or hypoxia) or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Renibacterium salmoninarum* , or *Piscirickettsia salmonis*); the cause is not determined in most cases. This case has no obvious organisms, but consider PCR to rule out VHSV. Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Hepatic necrosis is somewhat common in salmon that die in marine net pens, affecting 11% of the 645 Atlantic salmon and 6% of the 119 Pacific salmon examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program.

Vacuoles in hepatocytes of many of the fish are usually round and well-demarcated. In about 10% of the cells, the vacuoles seem to have coalesced into larger vacuoles, up to 10 µm in diameter. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. The primary differentials for these vacuoles are lipid or hydropic degeneration. Hydropic degeneration is evidence that the liver was being exposed to toxins. Potential sources of the inciting toxins include the water, a bacterial infection, or circulating oxygen radicals following a period of hypoxia.

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). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "silvers" that die in marine net pens, affecting 12% of the 645 Atlantic salmon livers examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2007 was sufficient to identify a trend towards greater prevalence in the fall and

winter (14-19%) than in the spring and summer (4.1-10%).

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

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. It is also consistent with the history of hemorrhage. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Splenic and hepatic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Parenchymal necrosis is an uncommon lesion in the spleen. It might be a result of a bacterial or viral infection. Consider bacteriology and PCR for VHSV.

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.1% of the 1142 Atlantic salmon hearts sampled as part of the province's Fish Health Auditing and Surveillance Program during 2006 and 2007). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. 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.

Inflammation associated with microsporidians in the brain of fish #3 probably contributed to its death. Parasite spores occur in clusters, but they do not seem to form distinct xenomas (as occur with another microsporidian parasite, *Loma salmonae*). The brain microsporidian species in this fish has not been officially described, but it was given the temporary name of *Microsporidian cerebralis* when described as part of a case report by Brocklebank et al. (1995).

Congestion of brain capillaries is evidence of circulating vasodilators; differentials include viral, bacterial, and parasitic infections. In smolts, brain congestion is commonly associated with filamentous bacteria in the mouth (mouthrot). [The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section contain erythrocytes.]

Literature Cited:

Brocklebank, J.R., Speare, D.J., and M.L. Kent. 1995. Microsporidian encephalitis of farmed Atlantic salmon (Salmo salar) in British-Columbia. Canadian Veterinary Journal 36 (10): 631- 633.

McLoughlin, M.F. and D.A. Graham. 2007. Alphavirus infections in salmonids ¿ a review. Journal of Fish Diseases. 30(9):511-531.

Histopathology

Formalin-fixed tissues were submitted in 6 cassettes for histopathology. For cassettes 2, 3, 4, 5 and 6, gills were removed from the original (A) cassettes and placed in separate (B) cassettes. Paraffin block 4A was subjected to additional surface decalcification with 8% formic acid before sectioning. All cassettes were labelled with the data "Feb 20/08," but the submission sheet labeled "Feb 20/08" listed only 4 fish. By comparison, the submission sheet labeled "Feb 19/08" listed 7 fish, but none of the cassettes were labeled "Feb 19/08."

Slide 1 and 2A (Baw Den Feb 20/08) - spleen, heart, brain, liver, intestine, head kidney, trunk kidney, and mesenteric adipose tissue

Slide 3A and 6A (Baw Den Feb 20/08) - spleen, heart, brain, liver, intestine, head kidney, and trunk kidney

Slide 4A (Baw Den Feb 20/08) - spleen, heart, brain, cranial cartilage and semicircular canal, liver, head kidney, trunk kidney (2 pieces), intestinal ceca, mesenteric adipose tissue

Slide 5A (Baw Den Feb 20/08) - spleen, heart, brain, liver, intestine, head kidney, trunk kidney (2 pieces)

Slides 2B, 3B, 4B, 5B and 6B (Baw Den Feb 20/08) - gill

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

Quality control: Liver autolysis: none (slides 1, 3A), mild (slides 5A, 6A), moderate (slides 2A, 4A). Large foci of erythrocytes (e.g., spleen in slide 5A) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue). Organs have no postfixation dehydration.

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

END OF REPORT

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Final Report AHC Case: 08-711

Last Updated: 02/25/08 3:33 PM
Pathologist: Gary D. Marty
Received Date: 02/22/08
Collected Date: 02/22/08
Client Ref No: #8-2410

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **Microtek International Inc.**
Phone:
Fax:
Owner: **Microtek International In**
Phone:
Fax:(250) 652-4802

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Please find enclosed samples for histology testing. The samples have been fixed in a 10% formalin buffered solution then transferred to water for transportation. Please refer thecase file number (8-2410) on all results and invoices.

Case file #8-2410 - 4 atlantic smolts that exhibited erosion around the lower jaw. Multiorgansamples from each fish including a portion of the lower jaw from fish #1.

Submitted by Tim Hewison, Fish Health Services Manager. Phone (250) 652-4482 ext 201. or by email thewisonatmicrotek-intl.com.

Final Diagnosis

1a. Liver: sinusoidal congestion, acute, multifocal, mild (slides 1A, 3A)

1b. Liver: hepatitis, granulomatous, bifocal, with intralesional vacuoles (~60 µm in diameter; consistent with vaccine material), mild (slide 2A)

1c. Liver: hepatitis, perivascular, neutrophilic to granulomatous, multifocal, mild (slide 3A)

2a. Spleen: splenitis, granulomatous, multifocal, with intralesional vacuoles (~60 µm in diameter; consistent with vaccine material, severe (slide 3A)

2b. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds and intralesional vacuoles 50 - 100 µm in diameter, severe (slide 1A)

3a. Trunk kidney: interstitial edema, diffuse, moderate (slide 2A)

3b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 2A), moderate (slides 1A, 3A)

4a. Brain: meningeal and neuropil hemorrhage, multifocal, moderate (slide 2A)

4b. Brain: capillary (vascular) congestion, diffuse, mild (slide 3A), moderate (slide 2A)

Final Comment: These fish have several lesions that might have contributed to mortality or provide a clue to the cause of mortality. Comments

on specific lesions follow:

Sinusoidal congestion in the liver is nonspecific evidence of endothelial damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Consider bacteriology and PCR for VHSV and IHNV.

Vaccine material is uncommon in the parenchyma of several organs. Vaccine material might have migrated to the liver and spleen via vessels. Inflammation in the liver and spleen in slide 3A includes a multinucleate giant cell, and these cells are common with foreign body reactions.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Interstitial cells in the kidney (slide 2A) are separated by clear space characteristic of edema. Edema is evidence of increased vascular leakage that can result from exposure to vasodilators. Common sources of vasodilators include bacterial toxins and inflammatory mediators (as might be released in a viral infection). I have associated *Yersinia ruckeri* infection with interstitial renal edema, but this case has no obvious organisms. Consider bacteriology and PCR for VHSV and IHNV (if not already done).

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

The most common cause of meningeal hemorrhage is trauma, but the small foci of hemorrhage in the neuropil of this fish (slide 2A) might be a result of vascular damage (e.g., from bacterial or viral infection). Trauma often results from fish running into something, including into other fish, and increased activity that leads to brain trauma might be associated with sea lice infestation or avoidance of predators.

Congestion of brain capillaries is evidence of circulating vasodilators; differentials include viral, bacterial, and parasitic infections. In smolts, brain congestion is commonly associated with filamentous bacteria in the mouth (mouthrot). [The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section contain erythrocytes.]

The piece of the jaw on slide 1A has foci of skin that lack epidermis, but none of these foci contain filamentous bacteria (the primary cause of oral lesions in salmon smolts). Sometimes the epidermis separates from the dermis as a postmortem artefact. Therefore, the gross appearance of jaw lesions cannot be confirmed based on the section examined.

Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology. For cassettes 1, 2 and 3, gills were removed from the original (A) cassettes and placed in separate (B) cassettes.

Slide 1A (Fish 1, 8-2410) - spleen, heart, brain, liver, trunk kidney, and lower jaw (skin, skeletal muscle, bone, and cartilage)

Slide 2A (Fish 2, 8-2410) - heart, brain, liver, trunk kidney

Slide 3A (Fish 3, 8-2410) - spleen, brain, liver, trunk kidney

Slide 4 (Fish 4, 8-2410) - spleen, heart, brain, liver, head kidney, trunk kidney

Slides 1B (Fish 1, 8-2410), 2B (Fish 2, 8-2410) and 3B (Fish 3, 8-2410) - gill

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

Quality control: Liver autolysis: mild (slides 1A, 3A, 4), severe (slide 2A). Organs have no postfixation dehydration and no acid hematin deposits.

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

END OF REPORT

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1767 Angus Campbell Road
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Toll-Free : 1-800-661-9903

Final Report AHC Case: 08-713

Last Updated: 02/26/08 9:16 AM
Pathologist: Gary D. Marty
Received Date: 02/22/08
Collected Date: 02/22/08
Client Ref No: #8-2413

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **Microtek International Inc.**
Phone:
Fax:
Owner: **Microtek International In**
Phone:
Fax:(250) 652-4802

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Please find enclosed samples for histology testing. The samples have been fixed in a 10% formalin buffered solution then transferred to water for transportation. Please refer the case file number (8-2413) on all results and invoices.

Case file #8-2413 - 5 atlantic smolts. Multi-organ samples for a general health check.

Submitted by Tim Hewison, Fish Health Services Manager. Phone (250)652-4482 ext 201 or by email thewisonatmicrotek- intl.com

Final Diagnosis

1. Trunk kidney: nephritis, interstitial, granulomatous, with intralesional vacuoles up to 200 µm in diameter, moderate (slide 5A)

2a. Liver: sinusoidal congestion, acute, multifocal, mild (slides 2A, 3A, 5A)

2b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 1A)

3. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 1A, 2A, 4A, 5A)

Final Comment: The measures of physiologic condition provide evidence that two of the fish (#s 4 and 5) were eating fairly normally before they died. Basophilic hepatocellular cytoplasm in fish #1 is the only clue that any of these fish were sick before they died. Comments on specific lesions follow:

Granulomatous inflammation with intralesional vacuoles in farmed Atlantic salmon is most commonly associated with vaccine material.

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

Sinusoidal congestion in the liver is nonspecific evidence of endothelial damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. If this change is associated with increased mortality, consider bacteriology and PCR for VHSV and IHNV.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology. For all cassettes, gills were removed from the original (A) cassettes and placed in separate (B) cassettes.

Slides 1A (Fish 1, 8-2413), 2A (Fish 2, 8-2413), 3A (Fish 3, 8-2413), Slide 4A (Fish 4, 8-2413) - spleen, liver, head kidney, trunk kidney

Slide 5A (Fish 5, 8-2413) - spleen, trunk kidney, liver

Slides 1B (Fish 1, 8-2413), 2B (Fish 2, 8-2413), 3B (Fish 3, 8-2413), Slide 4B (Fish 4, 8-2413), Slide 5B (Fish 5, 8- 2413) - gill

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

Quality control: Liver autolysis: mild (slides 1A, 2A, 3A, 4A, 5A). Organs have no postfixation dehydration and no acid hematin deposits.

Measure of physiologic condition: Hepatocellular glycogen: none (slides 1A, 3A), small amounts (slide 2A), moderate amounts (slides 4A, 5A).

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

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Final Report AHC Case: 08-766

Last Updated: 02/28/08 2:22 PM
Pathologist: Gary D. Marty
Received Date: 02/26/08
Collected Date: 02/26/08
Client Ref No: 8-2416

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **Microtek**
Phone: (250) 652-4482
Fax: (250) 652-4802
Owner: **Microtek International In**
Phone:
Fax:(250) 652-4802

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 3 cassettes for histology testing. The samples were fixed in formalin in the field and transferred to tap water for transport. Please refer to case file 8-2416 on all result reports and invoices. There are 3 multi-organ samples labeled Raza #4, Raza #4, and Raza #5 taken from 3 individual atlantic smolts. If you require any further information, please contact me at 250-652-4482 ext 201.

Additional information from phone call with Tim Hewison (28 Feb 2008, 13:35): *Aeromonas salmonicida* is growing from tissues from the same group of fish.

Final Diagnosis

1a. Liver: bacterial rods, multifocal, consistent with *Aeromonas salmonicida*, mild (slide 1), moderate (slides 2, 3)

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

1c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild to moderate (slides 1, 2)

2a. Trunk kidney: bacterial rods, multifocal, moderate (slides 1, 2)

2b.Trunk kidney: interstitial vascular congestion, diffuse, mild (slide 1)

3a. Spleen: peritonitis, granulomatous, focal, moderate (slide 2); regionally diffuse, with intralesional vacuoles about 50 µm in diameter, mild (slide 1)

3b. Spleen: splenitis, granulomatous, focal, with a central vacuole 500 µm in diameter, moderate (slide 1)

3c. Spleen: parenchymal fibrin, multifocal, acute, with intralesional bacterial rods, moderate (slide 3)

3d. Spleen: bacterial rods, multifocal, mild (slide 3)

4a. Mesenteric adipose tissue: capillary congestion, multifocal, moderate (slide 3)

4b. Mesenteric adipose tissue: steatitis, granulomatous, focal, with an intralesional vacuole 350 µm in diameter, mild (slide 2) and 3 mm in

diameter, moderate (slide 3)

5a. Brain: capillary (vascular) congestion, diffuse, mild (slide 2), moderate (slide 1)

5b. Brain: bacterial rods, multifocal, intravascular, mild (slide 2)

Final Comment: These fish probably died from a bacterial septicemia. Size of the bacteria is consistent with *Aeromonas salmonicida*, and one section of kidney in slide 3 has characteristic large colonies. However, because of small colony size in other organs, without culture results I would also consider *Yersinia ruckeri* as a differential. Comments on specific lesions follow:

Slide 1 has foci of plump bacterial rods that form small colonies between hepatocytes and in the renal interstitium. In slide 2, similar bacteria are in the liver, kidney, and brain. In slide 3, similar bacteria are in the trunk kidney, liver, and spleen. Bacteria are rare in Atlantic salmon livers and kidney, even after significant autolysis. Therefore, these bacteria are probably significant. Bacterial culture or PCR is needed for species confirmation.

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). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "silvers" that die in marine net pens, affecting 12% of the 645 Atlantic salmon livers examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in2007 was sufficient to identify a trend towards greater prevalence in the fall and winter (14-19%) than in the spring and summer (4.1-10%).

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

Renal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. In this case, the interstitial bacteria are the most likely source for the vasodilators.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe). Vacuoles probably are a result of vaccine material lost during tissue processing. Vacuoles within the spleen (slide 1) and mesenteric adipose tissue (slides 2 and 3) probably have a similar pathogenesis. The 3-mm -diameter vacuole in slide 3 is the largest I have ever seen.

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

Distension of capillaries in the mesenteric adipose tissue is often part of the inflammatory response to many infectious diseases; hemorrhage sometimes occurs in severe cases. In British Columbia, mesenteric congestion and hemorrhage is most commonly associated with VHSV and bacterial infections, and sometimes it seems to be associated with a vaccine reaction.

Congestion of brain capillaries is evidence of circulating vasodilators; differentials include viral, bacterial, and parasitic infections. In smolts, brain congestion is commonly associated with filamentous bacteria in the mouth (mouthrot). In this case, it probably is related to *Aeromonas salmonicida* infection.

Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology.

Slide 1 (Raza #4) - spleen (2 pieces), brain (2 pieces), liver (3 pieces), stomach, trunk kidney (3 pieces), intestinal ceca, mesenteric adipose tissue

Slide 2 (Raza #4) - spleen, brain (2 pieces), liver (2 pieces), trunk kidney (3 pieces), intestinal ceca, mesenteric adipose tissue

Slide 3 (Raza #5) - spleen (3 pieces), brain, liver (3 pieces), stomach, trunk kidney (3 pieces), intestinal ceca, mesenteric adipose tissue

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

Quality control: Liver autolysis: moderate (slide 1), severe (slides 2, 3). Organs have no postfixation dehydration and no acid hematin

deposits.

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

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Final Report AHC Case: 08-900

Last Updated: 03/11/08 2:02 PM
Pathologist: Gary D. Marty
Received Date: 03/06/08
Collected Date: 03/06/08
Client Ref No: 8-2418

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **Microtek International**
Phone:
Fax:
Owner: **Microtek International In**
Phone:
Fax:(250) 652-4802

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 14 cassettes for histology.

The cassettes contain multi-organ samples taken from atlantic salmon. The samples were preserved in 10% buffered formalin solution then transferred to tap water for shipping. Please refer case file #8-3418 on all results sheets and invoices. The samples are identified as #1-14.

Final Diagnosis

Lesions most likely to have caused death of these fish:

1. Liver and Kidney: granulomatous hepatitis and nephritis, necrosis, and hepatic thrombosis, multifocal, acute, severe (slide 13A)

2. Kidney and spleen: splenitis and nephritis, granulomatous, with renal coagulative necrosis, multifocal, and small to moderate numbers of intralesional short Gram -positive rods consistent with *Renibacterium salmoninarum* (the cause of bacterial kidney disease), severe (slide 14)

Final Comment: Details for this case are included on an Excel spreadsheet (2008-0900.xls) that is not included with the official final report generated by the Animal Health Centre's VADDS database (the database cannot handle spreadsheets). The "Abbreviation" worksheet includes comments about each lesion. Lesions on the spreadsheet are the same as those included in the histopathology part of the BC Fish Health Auditing and Surveillance Program. Therefore, the spreadsheet includes many lesions that these fish do not have. Specific comments on significant lesions in these fish follow:

Fish #13 probably died of complications related to coagulative necrosis in the kidney and large thrombi in the liver. Thrombosis in the liver is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection. Sections contain no obvious organisms on H&E or Gram stain.

Fish #14 probably died of complications related to severe infections with *Renibacterium salmoninarum*, the cause of bacterial kidney disease. This disease can adversely affect nearly all ages of farmed Atlantic salmon in BC.

Lesions in the other 12 fish are not of sufficient severity to explain their death. Scores for other lesions are included on the spreadsheet.

Value of brain histopathology for determining cause of death - I recommend sampling of brain (including brainstem) in cases like this one where the cause of death is unknown. At the beginning of 2007, brain was added to the list of organs to sample for histopathology as part of the Provincial government's Fish Health Auditing and Surveillance Program. Of the 168 Atlantic salmon examined during the first quarter of

2007, liver, kidney, and heart had lesions sufficient to explain the cause of death in 27% of the cases. The addition of brain histopathology allowed me to determine the cause of death of another 20% of the fish. Spleen, intestine, and mesenteric adipose tissue did not provide any unique information for determining the cause of death. Gill and mouth, selected only from fish with lesions in these structures, added a cause of death to another 4% of the fish.

Histopathology

Formalin-fixed tissues were submitted in 14 cassettes for histopathology. After processing routinely into paraffin, the gills were removed the original cassette (A) and placed into a second cassette (B). All organs were examined. A Twort's Gram stain was done on sections from the same paraffin block as slides 13A and 14. Organs not listed elsewhere have no significant lesions.

Quality Control: Details are included on the spreadsheet (2008-0900.xls). Tissue preservation is poor for nearly all organs, significantly decreasing the sensitivity and specificity of histopathology. Problems with autolysis can be decreased by harvesting the organs and preserving them at the farm site (nearly all of my aquaculture clients do this); if tissues cannot be harvested and preserved on site, then each fish needs to be wrapped individually and packed in an insulted container completely surrounded by snow or shaved ice (a single block of gel ice on the side of a bag full of fish does not provide sufficient cooling). Gills were not decalcified before sectioning; however, these gills have little bone, and sectioning quality is adequate. Organs have no acid hematin deposits and no postfixation dehydration.

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

END OF REPORT

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Final Report AHC Case: 08-984

Last Updated: 03/17/08 3:25 PM
Pathologist: Gary D. Marty
Received Date: 03/12/08
Collected Date: 03/12/08
Client Ref No: PO#2599BM

Veterinarian: **Barry Milligan**
Clinic: **Grieg Seafoods BC Ltd.**
Phone: (250) 286-0838
Fax: (250) 286-1883

Submitter: **Grieg Seafood**
Phone:
Fax:
Owner: **Grieg Seafoods BC Ltd.**
Phone: (250) 286-0838
Fax:(250) 286-1883

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 5 fish total, individual fish in separate cassettes for histology.

PO# 2599BM

Final Diagnosis

1a. Brain (brainstem): encephalitis, diffuse, lymphohistiocytic, with neuropil and intraneuronal microsporidia ("*Microsporidium cerebralis*"), moderate (slides 1A, 2A, 5A), severe (slides 3A, 4A)

1b. Brain: capillary (vascular) congestion, diffuse, mild (slide 4A), moderate (slide 1A)

2. Skin: dermatitis, ulcerative, focal, with moderate numbers of superficial filamentous bacteria invading full thickness of the dermis, moderate (slide 1A)

3a. Trunk kidney: interstitial congestion and hemorrhage, diffuse, mild (slide 4A), moderate (slide 1A)

3b. Trunk kidney: tubular dilation, focal (250 µm in diameter), moderate (slide 5A)

4a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1A, 5A)

4b. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slides 2A, 4A, 5A)

5a. Mesenteric adipose tissue: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 2A, 4A)

6a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 2A, 5A)

6b. Liver: hepatitis, perivascular, focal, with eosinophilic granular cells, mild (slide 2A)

6c. Liver: sinusoidal congestion, acute, multifocal, mild (slide 5A), moderate (slide 4A)

Final Comment: All five fish probably died of complications related to cerebral microsporidiosis. Parasite spores often occur in clusters, but they do not seem to form distinct xenomas (as occur with another microsporidian parasite, *Loma salmonae*). The brain microsporidian species in these fish has not been officially described, but it was given the temporary name of *Microsporidian cerebralis* when described as part of a case report by Brocklebank et al. (1995). Because this organism localizes in the brainstem, brainstem needs to be included with any brain sample (in this case, all 5 cassettes contained good brain samples). These fish also have several other lesions:

Congestion of brain capillaries, including the meninges, is nonspecific evidence of circulating vasodilators or a mass-occupying intracranial lesion; hemorrhage sometimes occurs in severe cases. Capillary congestion can be associated with bacterial infections (e.g. mouthrot in smolts), but it also results when venous return is blocked (e.g, with thrombi and massive intracranial hemorrhage or inflammation). Congestion of brain capillaries is not common with VHSV. [The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section contain erythrocytes.]

Small ulcers are common in fish (but not as common in 3 Kg fish), and filamentous bacteria commonly invade skin ulcers. Once filamentous bacteria become established, the ulcers often get larger. Enlargement of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection). Identification of the bacteria requires culture or PCR. In saltwater, *Tenacibaculum maritimum* is likely.

Renal and hepatic congestion is commonly associated with circulating toxins (e.g., from inflammatory cells or bacterial or viral infections). Renal congestion and hemorrhage in BC has been associated with VHSV and bacteria. Renal congestion is one of the classic signs of infectious salmon anemia (ISA), but ISA has never been isolated from fish in BC. Consider bacteriology and virology (if not already done).

Renal tubular dilation is evidence that flow of urine is abnormal. The most probable cause is some type of flow blockage.

Peritonitis involving the spleen and mesenteric adipose tissue is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

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.

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

The focus of eosinophilic granular cells in the liver of slide 2A is unusual (I have never seen a similar focus of EGCs in the liver of Atlantic salmon). However, the single focus probably did not contribute to the death of this fish.

Literature Cited: Brocklebank, J.R., Speare, D.J., and M.L. Kent. 1995. Microsporidian encephalitis of farmed Atlantic salmon (Salmo salar) in British-Columbia. Canadian Veterinary Journal 36 (10): 631-633.

Histopathology

Formalin-fixed tissues from 5 Atlantic salmon were received in cassettes for histopathology. The gills were decalcified over the weekend in 10% EDTA before processing routinely into paraffin. For all cassettes, gills were removed from the original (A) cassettes and placed in separate (B) cassettes.

Slide 1A - spleen, brain, liver, trunk kidney, skeletal muscle (with dermis but no epidermis), intestinal ceca, and mesenteric adipose tissue

Slides 2A, 3A, 4A and 5A - spleen, brain, liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slides 1B, 2B, 3B, 4B and 5B - gill

All organs on each slide were examined. Those not listed elsewhere have no significant lesions.

Quality control: Liver autolysis: none (slides 2A, 3A), mild (slide 1A), moderate (slide 4A). Organs have no acid hematin deposits and no postfixation dehydration.

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

END OF REPORT

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Final Report AHC Case: 08-1071

Last Updated: 04/14/08 1:36 PM
Pathologist: Gary D. Marty
Received Date: 03/18/08
Collected Date: 03/18/08
Client Ref No: #6598

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Diane Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 2 samples for PCR for IHN and VHS. Also submitted 1 TSA plate for ID from fish #2.

Tissues are from broodstock with septicemia.

Species: Atlantic salmon. Saltwater entry: 2006 brood.. Fish died Mar. 12/08.

Ref. #6598.

Submitted by C. LaTrace for Dr. Diane Morrison.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Erin Zabek on 03/28/08 @ 3:49 PM

Specimen	ID	Isolate	Result	Level
Isolate		Photobacterium sp.	Positive	

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 03/20/08 @ 4:33 PM

Specimen	ID	Test	Result
Tissue	6598-1	PCR - IHNV	Negative
Tissue	6598-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 03/20/08 @ 4:34 PM

Specimen	ID	Test	Result

Tissue	6598-1	PCR - VHSV	Negative
Tissue	6598-2	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Liisa Nielsen Verified by: Dr. J. Robinson on 04/14/08 @ 1:36 PM

Specimen	ID	Isolate	Result	Level
Tissue	6598-1		No viruses isolated	
Tissue	6598-2		No viruses isolated	

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

END OF REPORT

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Final Report AHC Case: 08-1250

Last Updated: 04/28/08 3:31 PM
Pathologist: Gary D. Marty
Received Date: 04/02/08
Collected Date: 04/02/08
Client Ref No: 6614

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh and formalized tissue for histology and viral culture.

One cassette from one fresh mort and one sample for PCR for IHN and VHS. Some hemorrhaging internally, anemic. No bacterial growth, resulted.

Final Diagnosis

1. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, severe

2a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild

2b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild

3. Spleen and intestinal ceca: peritonitis, granulomatous, multifocal, with intralesional vacuoles about 50 µm in diameter, moderate

4. Mesenteric adipose tissue: capillary congestion, multifocal, moderate

Final Comment: This fish probably died of complications related to renal tubular epithelial necrosis. Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Renal tubular epithelial necrosis was fairly common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 8.4%; n = 643) and Pacific salmon (prevalence = 4.2%; n = 120); the cause was not determined in many cases. Differentials include viral hemorrhagic septicemia virus (VHSV) and exposure to toxins (e.g., bacterial toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin).

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It is normal in mature females producing protein for deposition in their eggs. In other fish it might be related to increased protein needed as part of an inflammatory response. Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program

(44% were mild, 22% were moderate, and 5.0% were severe). Vacuoles probably are a result of vaccine material lost during tissue processing.

Distension of capillaries in the mesenteric adipose tissue is often part of the inflammatory response to many infectious diseases; hemorrhage sometimes occurs in severe cases. In British Columbia, mesenteric congestion and hemorrhage is most commonly associated with VHSV and bacterial infections, and sometimes it seems to be associated with a vaccine reaction.

Histopathology

Formalin-fixed tissues were submitted in 1 cassette for histopathology. Slide 1 (6641-1) contains heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, and mesenteric adipose tissue. All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Liver autolysis: none. Organs have no postfixation dehydration and no acid hematin deposits.

Measures of physiologic condition:

Hepatocellular glycogen: none

Mesenteric adipose tissue: abundant

These measures of physiologic condition are consistent with a fairly healthy fish that has not eaten normally for the past couple days.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/07/08 @ 2:55 PM

Specimen	ID	Test	Result
Tissue	6614-1	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/07/08 @ 2:55 PM

Specimen	ID	Test	Result
Tissue	6614-1	PCR - VHSV	Negative

PCR - Yersinia ruckeri Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 04/24/08 @ 11:37 AM

Specimen	ID	Test	Result
Tissue	Organs	PCR - Yersinia ruckeri	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 04/28/08 @ 3:31 PM

Specimen	ID	Isolate	Result	Level
Tissue	6614-1		No viruses isolated	

Gary D. Marty

Case: 08-1250

END OF REPORT

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Final Report AHC Case: 08-1251

Last Updated: 04/28/08 3:31 PM
Pathologist: Gary D. Marty
Received Date: 04/02/08
Collected Date: 04/02/08
Client Ref No: 6618

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 3 samples for PCR for IHN and VHS from 3 fresh mortalities.

Fish #1 with internal hemorrhage, #2, #3 NVL. No disease concerns, but fish to be checked prior to moving them.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/07/08 @ 2:55 PM

Specimen	ID	Test	Result
Tissue	6618-1	PCR - IHNV	Negative
Tissue	6618-2	PCR - IHNV	Negative
Tissue	6618-3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/07/08 @ 2:55 PM

Specimen	ID	Test	Result
Tissue	6618-1	PCR - VHSV	Negative
Tissue	6618-2	PCR - VHSV	Negative
Tissue	6618-3	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 04/28/08 @ 3:31 PM

Specimen	ID	Isolate	Result	Level
Tissue	6618-1		No viruses isolated	
Tissue	6618-2		No viruses isolated	

Tissue	6618-3		No viruses isolated

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

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Final Report AHC Case: 08-1252

Last Updated: 04/28/08 3:31 PM
Pathologist: Gary D. Marty
Received Date: 04/02/08
Collected Date: 04/02/08
Client Ref No: 6607

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for PCR for IHN and VHS.

Overall mortality is low at site, with some morbidity in some pens. Fresh morts with no signs of disease, virology taken to rule out IHN and VHS.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/07/08 @ 2:56 PM

Specimen	ID	Test	Result
Tissue	6607-1	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/07/08 @ 2:56 PM

Specimen	ID	Test	Result
Tissue	6607-1	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 04/28/08 @ 3:31 PM

Specimen	ID	Isolate	Result	Level
Tissue	6607-1		No viruses isolated	

Gary D. Marty
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Final Report AHC Case: 08-1267

Last Updated: 04/08/08 1:18 PM
Pathologist: Gary D. Marty
Received Date: 04/03/08
Collected Date: 04/03/08
Client Ref No: PO#2599B

Veterinarian: **Barry Milligan**
Clinic: **Grieg Seafoods BC Ltd.**
Phone: (250) 286-0838
Fax: (250) 286-1883

Submitter: **Grieg Seafood**
Phone:
Fax:
Owner: **Grieg Seafoods BC Ltd.**
Phone: (250) 286-0838
Fax:(250) 286-1883

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted formalized tissue for histology.

Fish have been treated for BKD. Harvest fish from processing plant with external lesion/ulcer. Three separate tissue samples were taken from the same fish. Causing down grades at the plant (approx 5%).

PO# 2599B

Final Diagnosis

1. Skin: ulcerative, granulomatous, fibrinous, regionally diffuse, severe

2. Skeletal muscle: myonecrosis and myositis, lymphocytic, focal, mild

Final Comment: The lesions noted clinically are severely inflamed ulcers. The pieces of skin contain no scales, but fibrin and neutrophils fill the space where scales used to be. The inflammation might have started in foci of lost scales. Foci of granulomatous inflammation are focally more prominent at the edge of the fibrinous inflammation, on the surface of the ulcer, and on the superficial margin of the subdermal adipose tissue (panniculus). Bacteria might have been involved in the pathogenesis of the lesion, but no organisms stain with Twort's Gram stain (i.e., no evidence of *Renibacterium salmoninarum*). The focus of myositis is in one of the pieces of tissue that includes only skeletal muscle and adipose tissue; I suspect that the inflammation is secondary to overlying ulcerative dermatitis.

Histopathology

Formalin-fixed tissues were submitted in a cassette for histopathology; sections were stained with H&E and Twort's Gram stain. The resultant slide contains two cross sections of skin, with underlying skeletal muscle and adipose tissue, and two sections that include only adipose tissue and skeletal muscle (these might be pieces of skin that were too wide to embed on edge; note that skin pieces need to be less than 3 mm thick to be embedded in transverse section). All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Preservation is good. Organs have no postfixation dehydration and no acid hematin deposits.

Gary D. Marty
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Final Report AHC Case: 08-1446

Last Updated: 05/07/08 3:20 PM
Pathologist: Gary D. Marty
Received Date: 04/15/08
Collected Date: 04/15/08
Client Ref No: 6623

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted tissue, one histo and one virology collected from one fish with hemorrhage of p.c, liver, and belly wall.

Very low mortality at site, mostly pred hits. ID: 6623

Final Diagnosis

1a. Liver: vasculitis, fibrinous, acute, multifocal, moderate, with mild sinusoidal congestion with spherical golden to amphophilic inclusions

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

2. Spleen: vasculitis, fibrinous, acute, multifocal, mild, with small numbers of spherical golden to amphophilic inclusions and moderate numbers of Gram-negative bacterial rods

3. Intestine: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate

Final Comment:

Neither hepatic nor splenic vasculitis are common in farmed Atlantic salmon in British Columbia. The primary differentials are a bacterial infection (e.g., *Yersinia ruckeri*) and VHSV. The spleen contains small foci of Gram - negative bacteria. Lesions in the liver have no bacteria on the Twort's Gram stain, and the tissues are negative for VHSV. The inclusions are probably degenerating erythrocytes.

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

Intestinal peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe). Vacuoles probably are a result of vaccine material lost during tissue processing.

Histopathology

Formalin -fixed tissues were submitted in 1cassette for histopathology. Sections were stained with H&E and Twort's Gram stain.

Slide 1 (6623, RAY 4/3/08) - brain, heart, spleen, liver, trunk kidney, swimbladder, stomach, intestine, intestinal ceca, mesenteric adipose tissue

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

Quality control: Liver autolysis: none. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 04/18/08 @ 9:41 AM

Specimen	ID	Test	Result
Tissue	#6623	PCR - IHNV	Negative

PCR - VHSV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 04/18/08 @ 9:40 AM

Specimen	ID	Test	Result
Tissue	#6623	PCR - VHSV	Negative

PCR - Yersinia ruckeri Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 04/24/08 @ 11:38 AM

Specimen	ID	Test	Result
Tissue	organs	PCR - Yersinia ruckeri	Negative

Virology

Tissue Culture Resulted by: Cheryl Cecconi Verified by: Dr. J. Robinson on 05/07/08 @ 3:20 PM

Specimen	ID	Isolate	Result	Level
Tissue			No viruses isolated	

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

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Final Report AHC Case: 08-1447

Last Updated: 04/17/08 5:16 PM
Pathologist: Gary D. Marty
Received Date: 04/15/08
Collected Date: 04/15/08
Client Ref No: 6636

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted tissue for histology.

Moribund fish with side lesions. Mortality is decreasing at sea site, but many fish with lesions remain. No other signs of disease. Temp 7 degrees C.

Final Diagnosis

1a. Skin and underlying skeletal muscle: dermatitis, ulcerative, focal, severe, with coagulative necrosis of skeletal muscle, and moderate numbers of superficial filamentous bacteria (slide 1)

1b. Skin: dermatitis, ulcerative, focal, with small numbers of superficial filamentous bacteria, mild (slide 2), moderate (slide 3)

2a. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate (slides 2, 3), severe (slide 1)

2b. Intestinal ceca: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 2)

3a. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, mild (slide 2)

3b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 1)

4a. Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, mild (slide 1)

4b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 2), moderate (slides 1, 3)

4c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 1, 2, 3)

5. Heart: endocarditis, multifocal, with endothelial cell hypertrophy, moderate (slide 3)

Final Comment: The skin lesions noted at gross exam are ulcers infiltrated by filamentous bacteria. 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). Identification of the bacteria requires culture or PCR, but in salt water *Tenacibaculum maritimum* is most likely.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe). Vacuoles probably are a result of vaccine material lost during tissue processing.

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

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

Sinusoidal congestion in the liver is evidence of endothelial damage. I have seen it associated with viral hemorrhagic septicemia virus and *Listonella anguillarum* infection. Consider bacteriology and virology and PCR for VHSV, IHNV (if not already done). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).

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

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

The pattern of inflammation in the heart in slide 3 is consistent with a systemic immune stimulation, probably a bacterial infection. Hypertrophic endothelial cells are basophilic and up to 10 µm thick.

Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology.

Slides 1 (1-6636), 2 (2-6636) and 3 (3-6636) ¿ heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, skin/skeletal muscle, mesenteric adipose tissue

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

Quality control: Liver autolysis: none (slides 1,2), mild (slide 3). Organs have no postfixation dehydration and no acid hematin deposits.

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

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Final Report AHC Case: 08-1463

Last Updated: 05/07/08 3:20 PM
Pathologist: Gary D. Marty
Received Date: 04/15/08
Collected Date: 04/15/08
Client Ref No: 6616

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted tissue for PCR for IHN and VHS.

Routine sample.

Molecular Diagnostics

PCR - IHNV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 04/18/08 @ 9:40 AM

Specimen	ID	Test	Result
Tissue	organs #6616	PCR - IHNV	Negative

PCR - VHSV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 04/18/08 @ 9:41 AM

Specimen	ID	Test	Result
Tissue	organs #6616	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Cheryl Cecconi Verified by: Dr. J. Robinson on 05/07/08 @ 3:20 PM

Specimen	ID	Isolate	Result	Level
Tissue			No viruses isolated	

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

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Final Report AHC Case: 08-1696

Last Updated: 05/06/08 10:57 AM
Pathologist: Gary D. Marty
Received Date: 05/02/08
Collected Date: 05/02/08
Client Ref No:

Veterinarian: **Barry Milligan**
Clinic: **Grieg Seafoods BC Ltd.**
Phone: (250) 286-0838
Fax: (250) 286-1883

Submitter: **Grieg Seafood BC**
Phone:
Fax:
Owner: **Cliff Cove**
Phone:
Fax:

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fish tissues & skin/skeletal muscle for Histo.

Fish have been treated for BKD. Tissue samples are from harvest fish. External ulcers/lesions present causing down grades @ the plant.

Saltwater. 7kg. Vaccinated. PO# 2599BM

Final Diagnosis

1. Skin and underlying adipose tissue and skeletal muscle: dermatitis, ulcerative, with deep granulation tissue and superficial fibrin, focal, severe (slide 1 and 2)

Final Comment: Skin lesions in these fish are ulcers filled with granulation tissue and covered by fibrin. Histopathology of chronic ulcers often is unrewarding when it comes to identifying the original cause; this case is no exception. Small ulcers are common in fish (e.g., small wounds or perforations from sea lice attachements), and filamentous bacteria commonly invade skin ulcers. Once filamentous bacteria become established, the ulcers often get larger. This case has no obvious filamentous bacteria, so their role in the development of the lesion is unknown. Enlargement of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection).

Histopathology

Formalin-fixed tissues from an Atlantic salmon were received for histopathology. Although none of the sections examined contain epidermis, I infer from the history that the sections include subcutaneous adipose tissue and skeletal muscle.

Slide 1 - skeletal muscle and adipose tissue (2 pieces)

Slide 2 - skeletal muscle and adipose tissue (3 pieces)

All organs on each slide were examined. Those not listed elsewhere have no significant lesions.

Quality control: Tissue preservation is good for all tissues. Organs have no acid hematin deposits and no postfixation dehydration.

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

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Final Report AHC Case: 08-1752

Last Updated: 05/08/08 4:53 PM
Pathologist: Gary D. Marty
Received Date: 05/06/08
Collected Date: 05/06/08
Client Ref No: 6642

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted two cassettes from two fish taken during culling and sea lice counts for histology.

No visible lesions noted. 215g and 95g.

Final Diagnosis

1a. Liver: hepatitis, granulomatous, multifocal, with multinucleate giant cells surrounding globular yellow-green material that is consistent with vaccine material, mild (slide 2), moderate (slide 1)

1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1, 2)

1c. Liver: pericholangitis, lymphocytic, focal, mild (slide 2)

2a. Heart: epicarditis and endocarditis, granulomatous, multifocal, with multinucleate giant cells surrounding globular yellow-green material that is consistent with vaccine material, moderate (slide 1)

2b. Heart: epicarditis, regionally diffuse, and endocarditis, focal, lymphohistiocytic, mild (slide 2)

3a. Head kidney: nephritis, interstitial, granulomatous, multifocal, with multinucleate giant cells surrounding globular yellow-green material that is consistent with vaccine material, moderate (slide 1)

4a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, with multinucleate giant cells surrounding globular yellow-green material that is consistent with vaccine material, mild (slides 1, 2)

4b. Trunk kidney: renal tubular intraluminal protein casts, multifocal, mild (slide 1)

4c. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 1, 2)

5. Spleen: peritonitis and splenitis, granulomatous, multifocal, with multinucleate giant cells surrounding globular yellow-green material that is consistent with vaccine material, moderate (slides 1, 2)

Final Comment:

Putative vaccine material occurs 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 included in nearly all foci of granulomatous inflammation, a concurrent infection with *Renibacterium salmoninarum* seems unlikely.

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

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 and endocarditis in slide 2 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 might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

Renal tubular protein casts result from glomerular or tubular dysfunction; excess protein leaks through glomeruli, or tubules are unable to reabsorb protein.

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology.

Slide 1 (6642-2) and Slide 2 (6642-1) - heart, spleen, liver, head kidney, trunk kidney

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

Quality control: Liver autolysis: none (both slides). Organs have no postfixation dehydration and no acid hematin deposits.

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

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Final Report AHC Case: 08-1753

Last Updated: 05/28/08 4:44 PM
Pathologist: Gary D. Marty
Received Date: 05/06/08
Collected Date: 05/06/08
Client Ref No: 6645

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted one sample for PCR for IHN and VHS. No gross lesions noted.

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 05/08/08 @ 4:00 PM

Specimen	ID	Test	Result
Tissue	#6645-1	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 05/08/08 @ 4:01 PM

Specimen	ID	Test	Result
Tissue	#6645-1	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 05/28/08 @ 4:44 PM

Specimen	ID	Isolate	Result	Level
Tissue	#6645-1		No viruses isolated	

Gary D. Marty
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Final Report AHC Case: 08-1757

Last Updated: 05/28/08 4:45 PM
Pathologist: Gary D. Marty
Received Date: 05/06/08
Collected Date: 05/06/08
Client Ref No: 6654

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for PCR for IHN and VHS. Fish with signs of hemorrhage of muscle. Mostly BKD at site.

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 05/08/08 @ 4:01 PM

Specimen	ID	Test	Result
Tissue	#6654-Pen16	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 05/08/08 @ 4:01 PM

Specimen	ID	Test	Result
Tissue	#6654-Pen16	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 05/28/08 @ 4:45 PM

Specimen	ID	Isolate	Result	Level
Tissue	#6654-Pen16		No viruses isolated	

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

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Final Report AHC Case: 08-1897

Last Updated: 07/23/08 10:50 AM
Pathologist: Gary D. Marty
Received Date: 05/15/08
Collected Date: 05/15/08
Client Ref No: 6676

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for viral culture.

Hemorrhage in swim bladder, liver, spleen, peritoneum lining, and p. ceaca.

ID: 6676.

Molecular Diagnostics

PCR-Fish Iridovirus Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/30/08 @ 11:41 AM

Specimen	ID	Test	Result
Tissue	6676-1	PCR-Fish Iridovirus	Negative
Fluid	EPC	PCR-Fish Iridovirus	Negative

PCR-Salmon Alphavirus Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 07/23/08 @ 10:50 AM

Specimen	ID	Test	Result
Tissue	6676-1	PCR-Salmon Alphavirus	Negative
Fluid	EPC	PCR-Salmon Alphavirus	Negative

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/30/08 @ 1:58 PM

Specimen	ID	Test	Result
Tissue	6676-1	PCR - IHNV	Negative
Fluid	EPC	PCR - IHNV	Negative

PCR - IPNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/29/08 @ 2:50 PM

Specimen	ID	Test	Result
Tissue	6676-1	PCR - IPNV	Negative

Fluid	EPC	PCR - IPNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/29/08 @ 2:50 PM

Specimen	ID	Test	Result
Tissue	6676-1	PCR - ISA	Negative
Fluid	EPC	PCR - ISA	Negative

PCR-Piscirickettsia salmo Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/30/08 @ 11:41 AM

Specimen	ID	Test	Result
Tissue	6676-1	PCR - Piscirickettsia salmonis	Negative
Fluid	EPC	PCR - Piscirickettsia salmonis	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/30/08 @ 11:41 AM

Specimen	ID	Test	Result
Tissue	6676-1	PCR - VHSV	Negative
Fluid	EPC	PCR - VHSV	Negative

PCR - Herpesvirus salmoni Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/30/08 @ 11:41 AM

Specimen	ID	Test	Result
Tissue	6676-1	PCR - Herpesvirus salmonis	Negative
Fluid	EPC	PCR - Herpesvirus salmonis	Negative

Virology

Electron Microscopy Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 07/04/08 @ 5:01 PM

Specimen	ID	Isolate	Result	Level
Fluid	EPC		Negative	
**: Alphavirus negative by EM				

Gary D. Marty
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Final Report AHC Case: 08-2092

Last Updated: 07/03/08 8:44 AM
Pathologist: Gary D. Marty
Received Date: 05/29/08
Collected Date: 05/29/08
Client Ref No: 6692

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

6 samples for PCR for IHN and VHS. Disease screen of fish prior to movement to another site. BKD present at site. Morts and random fish represented in the sample. One histo of fish with hepatic granuloma. Question vaccine?

ID: 6692

Final Diagnosis

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

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

1c. Liver: yellow-brown to yellow-green pigmented macrophage aggregates (hemosiderin and lipofuscin?), multifocal, mild

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

2a. Trunk kidney: renal tubular casts of yellow-green pigment, multifocal, mild

2b. Trunk kidney: interstitial cell hyperplasia, diffuse, mild

2c. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

3. Heart: epicarditis, multifocal, lymphohistiocytic, mild

4a. Spleen and surrounding fatty mesenteries: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, moderate

5b. Spleen: parenchymal golden pigment, scattered, intracellular, mild

Final Comment: This fish has several changes consistent with chronic stress and a low-grade bacterial infection. The granuloma noted grossly is more consistent with a vaccine reaction than with *Renibacterium salmoninarum* infection. Comments on specific lesions follow:

Hepatic peritonitis is consistent with a reaction to foreign material; it sometimes occurs in fish that have been vaccinated. Similar lesions on the margin of the spleen are more common.

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

Pigment in the liver, spleen, and kidney tubules probably includes lipofuscin; in the liver, it 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 hepatic lipofuscin have been associated with decreased growth and survival in several studies. Hemosiderin accumulation in the liver might result from increased turnover of red blood cells.

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

Interstitial cell hyperplasia in the kidney results from increased demand for erythrocytes or white blood cells somewhere in the body (renal interstitial cells are the blood- forming or hematopoietic cells in the kidney).

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

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

Histopathology

Formalin -fixed tissues were submitted in a cassette for histopathology.

Slide 1 (6692) - spleen, heart, liver, head kidney, trunk kidney

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

Quality control: Liver autolysis: mild. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/02/08 @ 11:51 AM

Specimen	ID	Test	Result
Tissue	6692-1	PCR - IHNV	Negative
Tissue	6692-2	PCR - IHNV	Negative
Tissue	6692-3	PCR - IHNV	Negative
Tissue	6692-4	PCR - IHNV	Negative
Tissue	6692-5	PCR - IHNV	Negative
Tissue	6692-6	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/02/08 @ 11:51 AM

Specimen	ID	Test	Result
Tissue	6692-1	PCR - VHSV	Negative
Tissue	6692-2	PCR - VHSV	Negative
Tissue	6692-3	PCR - VHSV	Negative
Tissue	6692-4	PCR - VHSV	Negative

Tissue	6692-5	PCR - VHSV	Negative
Tissue	6692-6	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Cheryl Cecconi on 07/03/08 @ 8:44 AM

Specimen	ID	Isolate	Result	Level
Tissue	6692-1		No viruses isolated	
Tissue	6692-2		No viruses isolated	
Tissue	6692-3		No viruses isolated	
Tissue	6692-4		No viruses isolated	
Tissue	6692-5		No viruses isolated	
Tissue	6692-6		No viruses isolated	

Gary D. Marty
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Final Report AHC Case: 08-2094

Last Updated: 07/03/08 8:45 AM
Pathologist: Gary D. Marty
Received Date: 05/29/08
Collected Date: 05/29/08
Client Ref No: 6685

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Histo collected from one fish with hem p.c. Pooled virology sample taken from fish with similar lesions. Please run PCR for IHN and VHS.

6685.

Final Diagnosis

1. Liver: sinusoidal congestion, multifocal, mild

2. Intestinal ceca and mesenteric adipose tissue: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate

3. Brain: capillary (vascular) congestion, diffuse, mild

Final Comment: This fish has lesions that are fairly common in pen-reared Atlantic salmon in British Columbia; however, none of the lesions are of sufficient severity to explain this fish's death. The "hem in p.c." noted grossly is a result of congestion of the villus lamina propria: an unusual pattern in my experience (congestion of the intestinal ceca usually involves capillaries in the mesenteric adipose tissue). Accumulation erythrocytes in intestinal villi might be evidence of enteritis, but the tissues are too autolyzed to speculate further about the contribution of the intestinal cecal lesions to the death of this fish.

Sinusoidal congestion in the liver is nonspecific evidence of endothelial damage. In BC Atlantic salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*; PCR rules out VHSV in this case.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Vacuoles probably are a result of vaccine material lost during tissue processing.

Congestion of brain capillaries, including the meninges, is nonspecific evidence of circulating vasodilators or a mass-occupying intracranial lesion. Capillary congestion can be associated with bacterial infections (e.g., mouthrot in smolts), but it also results when venous return is blocked (e.g, with thrombi and massive intracranial hemorrhage or inflammation). However, the section has no thrombi or intracranial hemorrhage. Congestion of brain capillaries is not common with VHSV. [The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section contain erythrocytes.]

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology.

Slide 1 (6685-2) - heart, brain, liver, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

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

Quality control: Liver autolysis: mild; intestinal ceca autolysis: moderate. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/02/08 @ 11:51 AM

Specimen	ID	Test	Result
Tissue	6685	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/02/08 @ 11:51 AM

Specimen	ID	Test	Result
Tissue	6685	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Cheryl Cecconi on 07/03/08 @ 8:45 AM

Specimen	ID	Isolate	Result	Level
Tissue	6685		No viruses isolated	

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Final Report AHC Case: 08-2165

Last Updated: 06/27/08 9:12 AM
Pathologist: Gary D. Marty
Received Date: 06/04/08
Collected Date: 06/04/08
Client Ref No: 6683

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

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

Fish with signs of hemorrhage on the liver. High mortality on site mostly d/t BKD; but is coming down.

Molecular Diagnostics

PCR-Renibacterium salmoni Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/13/08 @ 2:11 PM

Specimen	ID	Test	Result
Tissue	#668	PCR-Renibacterium salmoninaru	Positive

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/06/08 @ 2:12 PM

Specimen	ID	Test	Result
Tissue	#668	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/06/08 @ 2:13 PM

Specimen	ID	Test	Result
Tissue	#668	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Cheryl Cecconi Verified by: Melissa Trapp on 06/27/08 @ 9:12 AM

Specimen	ID	Isolate	Result	Level
Tissue	#668		No viruses isolated	

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

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Final Report AHC Case: 08-2166

Last Updated: 06/27/08 9:11 AM
Pathologist: Gary D. Marty
Received Date: 06/04/08
Collected Date: 06/04/08
Client Ref No: 6702

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh and formalized tissue.

Low mortality at site, however, sampled fish was on feed. Gross lesions were hemorrhage of swim bladder, p.c. and intestine. Please run PCR for IHN and VHS.

ID: 6702.

Final Diagnosis

1. Liver: sinusoidal congestion, multifocal, mild

2. Head kidney: capillary congestion, diffuse, mild

3. Brain: capillary congestion, diffuse, mild

Final Comment: Congestion in the liver and head kidney is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria; PCR results rule out VHSV or IHNV. Capillary congestion in the brain might have the same pathogenesis as in the liver and head kidney, or it might be a result of percussion euthanasia.

Histopathology

Formalin-fixed tissues were submitted in a cassette for histopathology and processed onto 1 slide labeled "GC Pen 7 5/28/08" (heart, liver, spleen, brain, head kidney, trunk kidney, intestine, intestinal ceca, and mesenteric adipose tissue). All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Liver autolysis: moderate; intestinal autolysis: severe. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/06/08 @ 2:13 PM

Specimen	ID	Test	Result
Tissue	#6702	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/06/08 @ 2:13 PM

Specimen	ID	Test	Result
Tissue	#6702	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Cheryl Cecconi Verified by: Melissa Trapp on 06/27/08 @ 9:11 AM

Specimen	ID	Isolate	Result	Level
Tissue	#6702		No viruses isolated	

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

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Final Report AHC Case: 08-2248

Last Updated: 06/12/08 2:30 PM
Pathologist: Gary D. Marty
Received Date: 06/10/08
Collected Date: 06/10/08
Client Ref No: File #2471

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **Microtek International**
Phone:
Fax:
Owner: **Microtek International In**
Phone:
Fax:(250) 652-4802

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted four fixed tissue samples for histology testing.

Case file #2471.

Final Diagnosis

1a. Liver: hepatocellular karyomegaly, diffuse, mild (slide 4)

1b. Liver: hepatocellular single cell necrosis, diffuse, moderate (slide 4)

1c. Liver: golden pigmented sinusoidal macrophages (probably lipofuscin and hemosiderin), diffuse, moderate (slides 4)

1d. Liver: hepatitis, perivascular, lymphoplasmacytic, multifocal, mild (slide 4)

1e. Liver: sinusoidal congestion, multifocal, moderate (slide 4)

1f. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 3)

2a. Trunk kidney: renal tubular epithelial necrosis, focal, acute, mild (slides 2B, 4)

2b. Trunk kidney: renal tubular casts of mineral, protein, and yellow-brown pigment, with tubular dilation, multifocal, mild (slide 4)

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 4)

Final Comment:

Many features of the liver in slide 4 are consistent with netpen liver disease. Netpen liver disease can be reproduced in the laboratory with water exposure to the algal toxin microcystin- LR, but the source of the toxin in the field is unknown. Other toxins in the water or feed (e.g., aflatoxins) are likely differentials. Changes in the affected liver include single cell necrosis, hepatocellular karyomegaly, sinusoidal lipofuscin accumulation, and variable amounts of lymphoplasmacytic inflammation. Widespread parenchymal collapse is evidence that the process has been going on for a long time (months?). Lack of lipid stores in the mesenteric adipose tissue of this fish is further evidence of chronic

impairment. Hepatic megalocytosis can result from exposure to several types of toxins, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. Hydropic degeneration is a precursor to single cell necrosis. 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; it is a common feature of netpen liver disease. Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies.

Sinusoidal congestion in the liver is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. Consider bacteriology and virology, if not already done.

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 necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Renal tubular epithelial necrosis was fairly common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 8.4%; n = 643) and Pacific salmon (prevalence = 4.2%; n = 120); the cause was not determined in many cases. Differentials include viral hemorrhagic septicemia virus (VHSV) and exposure to toxins (e.g., bacterial toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin). Consider bacteriology and virology, if not already done.

Renal mineralization is common in cultured fish species. The lesion is not considered fatal, although feed conversion may be adversely affected. The pathogenesis is not fully understood, but renal mineralization has been experimentally reproduced through high carbon dioxide levels, magnesium deficiency, selenium toxicity, and a diet low in minerals (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Clinically, renal mineralization is most commonly associated with high carbon dioxide levels.

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

The head kidney in slide 2B has a greater concentration of melanocytes than any other Atlantic salmon strains I have seen in British Columbia.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology (slides from these cassettes are labeled 1 - 4). Before processing into paraffin, gills were removed from their original cassettes and placed in separate cassettes.

Slides 1A, 2A, 2C and 2D (2471) - gill

Slide 1B (2471) - liver, trunk kidney

Slides 2B and 4 (2471) - liver, head kidney, trunk kidney

Slide 3 (2471) - liver, spleen, head kidney, trunk kidney

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

Quality control: Liver autolysis: moderate (slide 4), severe (slides 1B, 2B, 3); gill autolysis: severe (slides 1A, 2A, 2C, 2D). Liver autolysis in slides 1B and 2B is too severe to diagnose most lesions. Organs have no postfixation dehydration and no acid hematin deposits.

Gary D. Marty

Case: 08-2248

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Final Report AHC Case: 08-2280

Last Updated: 07/04/08 5:01 PM
Pathologist: Gary D. Marty
Received Date: 06/12/08
Collected Date: 06/12/08
Client Ref No: 6719

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **D. Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted two histo and one virology collected to rule out disease etiology. Please run PCR for IHN and VHS.

Histo collected by site Manager. Externally, fish showing signs of net rub. Site staff have corrected the issue, but not billowing.

Case 6719

Final Diagnosis

1. Heart: mural microthrombi, multifocal, mild (slide 1), moderate (slide 2B)

2. Gill, tips of filaments: microthrombi, mild, focal (slide 1B), multifocal (slide 2)

3a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1)

3b. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slides 1, 2B)

4a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1, 2B)

4b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 2B)

Final Comment: Microthrombi in the heart and gill are probably the most significant lesions in these fish. Thrombosis is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection, but the sections contain no obvious organisms. PCR results rule out common salmonid viruses. Consider bacteriology, if not already done.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

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; it is more common in older fish. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in

several studies.

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

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

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology. Gills were removed from the original cassettes and placed in separate cassettes prior to processing into paraffin

Slides 1 (6719-7) and 2B (6719-8) - spleen, heart, liver, trunk kidney

Slides 1B and 2 - gill

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

Quality control: Liver autolysis: mild (slides 1, 2B); gill autolysis, none (slides 1B, 2). Large foci of erythrocytes (e.g., gill in slide 2) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue, or in tissues immersed in an acid decalcifier). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/15/08 @ 2:43 PM

Specimen	ID	Test	Result
Tissue	6719	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/15/08 @ 2:43 PM

Specimen	ID	Test	Result
Tissue	6719	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 07/04/08 @ 5:01 PM

Specimen	ID	Isolate	Result	Level
Tissue	6719		No viruses isolated	

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

END OF REPORT

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Animal Health Centre

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Ministry of Agriculture and Lands
1767 Angus Campbell Road
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Toll-Free : 1-800-661-9903

Final Report AHC Case: 08-2281

Last Updated: 06/13/08 2:01 PM
Pathologist: Gary D. Marty
Received Date: 06/12/08
Collected Date: 06/12/08
Client Ref No: 6713

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **D. Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted two whole hearts in formalin. Atrial abnormalities. Please let Dr. Marty look at whole samples prior to making slides.

Mortality is low at this site.

Case 6713.

Final Diagnosis

1. Heart: Atrial dysplasia, diffuse, with endothelial cell hypertrophy and myocardiocytes with lack of cross striations, severe (slides A1, A2, B1, B2)

2. Heart, atrium: mural thrombosis, multifocal, coalescing, with karyorrhexis and reorganization, mild (slide B2), moderate (slide B1), severe (slides A1, A2)

Final Comment: The primary lesion in these hearts is atrial dysplasia. Myocardiocytes in the atrium lack prominent cross striations that are common in the normal myocardiocytes of the ventricle. The atrium, therefore, probably had little contractile function, instead serving only as a sac for pooling blood before it entered the ventricle.

The white foci noted grossly in the atria of heart A are thrombi. The thrombi in heart B are about 1 mm in diameter (not noted grossly). Atrial thrombi are probably secondary to turbulence and pooling associated with atrial dysplasia. Several different patterns of thrombi, from mostly fibrinous to mostly cellular, with minimal to moderate reorganization, are evidence that the thrombi formed and reformed several times before the heart was sampled. The thrombi contain many karyorrhectic cells, but no obvious microorganisms.

Although this lesion could be called cardiomyopathy, the features are not the same as cardiomyopathy in farmed salmon described in farmed Atlantic salmon in Norway (Ferguson et al. 1990) and elsewhere. The lesions described by Ferguson et al. (1990) involved both the ventricle and atrium, whereas lesions in this case are limited to the atrium. Thrombosis and endothelial cell hypertrophy are common features of cardiomyopathy in Norwegian fish and in this case.

Literature cited:

Ferguson, H.W., T. Poppe, and D.J. Speare. 1990. Cardiomyopathy in farmed Norwegian salmon. Dis. Aquat. Org. 8:225- 231.

Histopathology

Two formalin-fixed intact hearts from Atlantic salmon (*Salmo salar*) were received for histopathology wrapped in moist paper towel in a zip-seal bag. The atria were dilated and thin-walled; the volume of the atria was greater than the ventricle and bulbus arteriosus combined. Each heart was transected mid- sagittally and each half was put in a separate cassette (A1 and A2 for one heart; B1 and B2 for the other heart). The apex of heart A had firm white regions on each side: on one side it was about 3 × 2 × 10 mm (slide A1); on the other side it was about 3 mm in diameter (slide A2).

Quality control: Tissue preservation is excellent, with no acid hematin deposits and no postfixation dehydration.

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

END OF REPORT

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Final Report AHC Case: 08-2282

Last Updated: 07/04/08 5:01 PM
Pathologist: Gary D. Marty
Received Date: 06/12/08
Collected Date: 06/12/08
Client Ref No: 6708

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **D. Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

10 pooled (3 fish/pool) samples for PCR for IHN and VHS. Six histo taken from broodstock with suspect livers. (NPLDz)?

No other visible lesions.

Case 6708

Final Diagnosis

1a. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 4), with biliary hyperplasia, moderate (slide 1)

1b. Liver: granular green-yellow pigmented macrophage aggregates (hemosiderin and lipofuscin confirmed in slide 2) and pericholangial macrophages, disseminated, mild (slides 1, 3, 4), moderate (slides 2, 5, 6)

1c. Liver: hepatocellular fatty change (lipidosis), multifocal, mild (slide 6), diffuse, moderate (slides 2, 3)

1d. Liver: fibrous capsule granuloma, focal (80 µm in diameter), mild (slide 2)

1e. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate (slides 5, 6), abundant (slide 4)

1f. Liver: sinusoidal congestion, multifocal, mild (slide 5)

2. Mesenteric adipose tissue: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, mild (slides 2, 3), moderate (slide 1)

3a. Heart: endocarditis, focal, lymphohistiocytic, mild (slide 1)

3b. Heart: myocardial karyomegaly, multifocal, mild (slides 2, 4, 6)

Final Comment: These sections have several lesions that are common in older Atlantic salmon in British Columbia. Lesions are not consistent with net pen liver disease. Comments on specific lesions follow:

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 association of biliary hyperplasia with the inflammation is not common; it might be a result of exposure to toxins.

Pigment in the liver includes lipofuscin and hemosiderin (both confirmed in slide 2). 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; it is common in older fish. In slide 1, most of the pigment is adjacent to the inflamed biliary system. 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 might result from increased turnover of red blood cells.

Fibrous capsule granulomas are fairly common in fish that are captured from the wild and/or reared outdoors. The granuloma in the liver might represent a focus where parasites died long ago; all that is left is the indigestible remnants. This focus probably is of little significance for fish health. The negative ZN acid-fast stain rules out the primary differential: mycobacteriosis.

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

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These types of vacuoles were rare before 2007 and probably are a result of the significant increase in the proportion of plant-based components in commercial feeds that occurred in 2007. The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown.

Sinusoidal congestion in the liver is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. PCR results rule out VHSV and IHNV. Consider bacteriology, if not already done.

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

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

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.1% of the 1142 Atlantic salmon hearts sampled as part of the province's Fish Health Auditing and Surveillance Program during 2006 and 2007). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Histopathology

Formalin-fixed tissues were submitted in 6 cassettes for histopathology.

Slide 1 (6708-1) - spleen, heart, liver, trunk kidney, mesenteric adipose tissue

Slides 2 (6708-2), 3 (6708-3), 4 (6708-4), 5 (6708-5) and 6 (6708-6) - spleen, heart, liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

All organs were examined. Organs not listed elsewhere have no significant lesions. Special stains applied to sections from block 2 include Perl's iron, Schmorl's lipofuscin, ZN acid-fast (on a de-stained original H&E section), and a new H&E.

Quality control: Liver autolysis: none (slides 2, 3, 4), mild (slides 1, 5, 6). Large foci of erythrocytes (e.g., spleen in slides 1, 2) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/15/08 @ 2:43 PM

Specimen	ID	Test	Result
Tissue	6708-1 1-3	PCR - IHNV	Negative

Tissue	6708-2 4-6	PCR - IHNV	Negative
Tissue	6708-3 7-9	PCR - IHNV	Negative
Tissue	6708-4 10-12	PCR - IHNV	Negative
Tissue	6708-5 13-15	PCR - IHNV	Negative
Tissue	6708-6 16-18	PCR - IHNV	Negative
Tissue	6708-7 19-21	PCR - IHNV	Negative
Tissue	6708-8 22-24	PCR - IHNV	Negative
Tissue	6708-9 25-27	PCR - IHNV	Negative
Tissue	6708-10 28-30	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/15/08 @ 2:43 PM

Specimen	ID	Test	Result
Tissue	6708-1 1-3	PCR - VHSV	Negative
Tissue	6708-2 4-6	PCR - VHSV	Negative
Tissue	6708-3 7-9	PCR - VHSV	Negative
Tissue	6708-4 10-12	PCR - VHSV	Negative
Tissue	6708-5 13-15	PCR - VHSV	Negative
Tissue	6708-6 16-18	PCR - VHSV	Negative
Tissue	6708-7 19-21	PCR - VHSV	Negative
Tissue	6708-8 22-24	PCR - VHSV	Negative
Tissue	6708-9 25-27	PCR - VHSV	Negative
Tissue	6708-10 28-30	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 07/04/08 @ 5:01 PM

Specimen	ID	Isolate	Result	Level
Tissue	6708-1 1-3		No viruses isolated	
Tissue	6708-2 4-6		No viruses isolated	
Tissue	6708-3 7-9		No viruses isolated	
Tissue	6708-4 10-12		No viruses isolated	
Tissue	6708-5 13-15		No viruses isolated	
Tissue	6708-6 16-18		No viruses isolated	
Tissue	6708-7 19-21		No viruses isolated	
Tissue	6708-8 22-24		No viruses isolated	
Tissue	6708-9 25-27		No viruses isolated	
Tissue	6708-10 28-30		No viruses isolated	

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

END OF REPORT

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Final Report AHC Case: 08-2408

Last Updated: 07/16/08 4:06 PM
Pathologist: Gary D. Marty
Received Date: 06/20/08
Collected Date: 06/20/08
Client Ref No: 6726

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh and formalized tissue for viral culture and histology.

Slight increase in mortality as well as change in classification; more silvers with no visible lesions. Histo taken from two of these fish and samples pooled into one virology sample.

ID: 6726

Final Diagnosis

1a. Liver: hepatocellular cytoplasmic vacuoles, diffuse, abundant (slides 1, 2)

1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 1)

1c. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages (hemosiderin and lipofuscin?), disseminated, mild (slide 1)

2. Spleen: parenchymal golden pigment (lipofuscin?), scattered, intracellular, mild (slides 1, 2)

3. Brain: capillary (vascular) congestion, diffuse, mild (slide 1)

4. Perirenal connective tissue: cellulitis, granulomatous, diffuse, with multifocal vacuoles consistent with foreign (vaccine) material, severe (slide 1)

5. Heart: myocardial karyomegaly, multifocal, mild (slides 1, 2)

6. Gill: lamellar telangiectasis, multifocal, with thrombosis and neutrophils, mild (slide 1A)

Final Comment: These fish have several lesions that are common in fish that die in British Columbia net pens. However, none of the lesions seem of sufficient severity to explain their death. Comments on specific lesions follow:

Hepatocellular cytoplasmic vacuoles vary from round (probably lipid) to angular (possible glycogen). These types of vacuoles were rare before

2007 and probably are a result of the significant increase in the proportion of plant-based components in commercial feeds that occurred since 2006. The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown.

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

Pigment in the liver probably is lipofuscin, and it might also include hemosiderin. 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; it is more common in older fish. 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 might result from increased turnover of red blood cells.

The golden pigment in the spleen most likely is lipofuscin. Pathogenesis and significance is the same as for lipofuscin in the liver.

Congestion of brain capillaries, including the meninges, is nonspecific evidence of circulating vasodilators or a mass-occupying intracranial lesion. Capillary congestion can be associated with bacterial infections (e.g., mouthrot in smolts), but it also results when venous return is blocked (e.g, with thrombi and massive intracranial hemorrhage or inflammation). Congestion of brain capillaries is not common with VHSV. This case has no obvious cause.

Granulomatous inflammation with vacuoles is common in fish that have been vaccinated. However, the location and extent of the inflammation in slide 1 is unusual. This might be a result of vaccine material being injected into an uncommon location (perirenal).

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.1% of the 1142 Atlantic salmon hearts sampled as part of the province's Fish Health Auditing and Surveillance Program during 2006 and 2007). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Telangiectasis in the gill most commonly results from trauma (e.g., handling). Thrombosis and neutrophils provide evidence that telangiectasis preceded fish death by at least a few hours.

Histopathology

Formalin -fixed tissues from 2 fish were submitted in 4 cassettes for histopathology. The gills were decalcified in Protocol B (hydrochloric acid solution) for 4 hours before being rinsed with water and processed routinely into paraffin.

Slide 1 (6726-1) - brain, heart, spleen, liver, trunk kidney (2 pieces), intestinal ceca, mesenteric adipose tissue

Slide 2 (6726-2) - heart, spleen, liver, intestine, trunk kidney, mesenteric adipose tissue

Slides 1A (6726-1A) and 2A (6726-2A) - gill

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

Quality control: Liver autolysis: mild (slides 1, 2); gill autolysis, mild (slide 2A), moderate (slide 1A). Gill decalcification is complete. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/24/08 @ 11:47 AM

Specimen	ID	Test	Result
Tissue	6726	PCR - IHNV	Negative

PCR - IPNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/04/08 @ 1:22 PM

Specimen	ID	Test	Result
Tissue	6726	PCR - IPNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/04/08 @ 1:21 PM

Specimen	ID	Test	Result
Tissue	6726	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/24/08 @ 11:47 AM

Specimen	ID	Test	Result
Tissue	6726	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 07/16/08 @ 4:06 PM

Specimen	ID	Isolate	Result	Level
Tissue	6726		No viruses isolated	

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

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Final Report AHC Case: 08-2436

Last Updated: 06/26/08 3:49 PM
Pathologist: Gary D. Marty
Received Date: 06/24/08
Collected Date: 06/24/08
Client Ref No: 6663

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue. Please run PCR for *Renibacterium salmoninarum* on 3 enclosed kidney tissue samples (diluted with PBS).

ID: 6663.

Molecular Diagnostics

PCR-Renibacterium salmoni Resulted by: A Scouras Verified by: Dr. J. Robinson on 06/26/08 @ 3:49 PM

Specimen	ID	Test	Result
Kidney	6663-A	PCR-Renibacterium salmoninaru	Negative
Kidney	6663-B	PCR-Renibacterium salmoninaru	Negative
Kidney	6663-C	PCR-Renibacterium salmoninaru	Negative

Gary D. Marty
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Final Report AHC Case: 08-2437

Last Updated: 07/17/08 4:39 PM
Pathologist: Gary D. Marty
Received Date: 06/24/08
Collected Date: 06/24/08
Client Ref No: 6733

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted one pooled sample of 4 fish for PCR for IHN and VHS.

Some mouthrot visible at site, but 4 fish found with hemorrhage of the p.c, so virology sample taken for rule out.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/26/08 @ 3:50 PM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/26/08 @ 3:50 PM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 07/17/08 @ 4:39 PM

Specimen	ID	Isolate	Result	Level
Tissue			No viruses isolated	

Gary D. Marty
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Final Report AHC Case: 08-2521

Last Updated: 07/24/08 2:25 PM
Pathologist: Gary D. Marty
Received Date: 07/02/08
Collected Date: 07/02/08
Client Ref No: 6746

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest-Brad Boyce**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted head kidney and spleen for PCR IHN and VHS.

4 fresh morts samples (2 fish pools). No signs of clinical disease. Saltwater entry: 2007 S1. Vaccinated, yes.

Client: 6746

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/04/08 @ 12:28 PM

Specimen	ID	Test	Result
Tissue	6746-1	PCR - IHNV	Negative
Tissue	6746-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/04/08 @ 12:29 PM

Specimen	ID	Test	Result
Tissue	6746-1	PCR - VHSV	Negative
Tissue	6746-2	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 07/24/08 @ 2:25 PM

Specimen	ID	Isolate	Result	Level
Tissue	6746-1		No viruses isolated	
Tissue	6746-2		No viruses isolated	

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

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Final Report AHC Case: 08-2522

Last Updated: 07/24/08 2:25 PM
Pathologist: Gary D. Marty
Received Date: 07/02/08
Collected Date: 07/02/08
Client Ref No: 6750

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted three fresh tissues for PCR for IHN and VHS.

Samples taken from pens 7, 8 and 9. Low do's at site. Fish with hem of heart, +/- kidney, +/- fat and p.c. Saltwater entry 2006, S0. Vaccinated. DODL: June 27, 08.

Client ID# 6750.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/04/08 @ 12:28 PM

Specimen	ID	Test	Result
Tissue	6750-Pen7	PCR - IHNV	Negative
Tissue	6750-Pen8	PCR - IHNV	Negative
Tissue	6750-Pen9	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/04/08 @ 12:28 PM

Specimen	ID	Test	Result
Tissue	6750-Pen7	PCR - VHSV	Negative
Tissue	6750-Pen8	PCR - VHSV	Negative
Tissue	6750-Pen9	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 07/24/08 @ 2:25 PM

Specimen	ID	Isolate	Result	Level

Tissue	6750-Pen7	No viruses isolated
Tissue	6750-Pen8	No viruses isolated
Tissue	6750-Pen9	No viruses isolated

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

END OF REPORT

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Final Report AHC Case: 08-2614

Last Updated: 08/01/08 2:41 PM
Pathologist: Gary D. Marty
Received Date: 07/09/08
Collected Date: 07/09/08
Client Ref No: 6752

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 30 samples for PCR for IHN and VHS and tissue culture, 3 groups of fish from 3 pens (#1, 2, and 5). Routine check prior to fish move.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/11/08 @ 10:20 AM

Specimen	ID	Test	Result
Tissue	1	PCR - IHNV	Negative
Tissue	2	PCR - IHNV	Negative
Tissue	3	PCR - IHNV	Negative
Tissue	4	PCR - IHNV	Negative
Tissue	5	PCR - IHNV	Negative
Tissue	6	PCR - IHNV	Negative
Tissue	7	PCR - IHNV	Negative
Tissue	8	PCR - IHNV	Negative
Tissue	9	PCR - IHNV	Negative
Tissue	10	PCR - IHNV	Negative
Tissue	11	PCR - IHNV	Negative
Tissue	12	PCR - IHNV	Negative
Tissue	13	PCR - IHNV	Negative
Tissue	14	PCR - IHNV	Negative
Tissue	15	PCR - IHNV	Negative
Tissue	16	PCR - IHNV	Negative
Tissue	17	PCR - IHNV	Negative
Tissue	18	PCR - IHNV	Negative
Tissue	19	PCR - IHNV	Negative
Tissue	20	PCR - IHNV	Negative

Tissue	21	PCR - IHNV	Negative
Tissue	22	PCR - IHNV	Negative
Tissue	23	PCR - IHNV	Negative
Tissue	24	PCR - IHNV	Negative
Tissue	25	PCR - IHNV	Negative
Tissue	26	PCR - IHNV	Negative
Tissue	27	PCR - IHNV	Negative
Tissue	28	PCR - IHNV	Negative
Tissue	29	PCR - IHNV	Negative
Tissue	30	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/11/08 @ 10:20 AM

Specimen	ID	Test	Result
Tissue	1	PCR - VHSV	Negative
Tissue	2	PCR - VHSV	Negative
Tissue	3	PCR - VHSV	Negative
Tissue	4	PCR - VHSV	Negative
Tissue	5	PCR - VHSV	Negative
Tissue	6	PCR - VHSV	Negative
Tissue	7	PCR - VHSV	Negative
Tissue	8	PCR - VHSV	Negative
Tissue	9	PCR - VHSV	Negative
Tissue	10	PCR - VHSV	Negative
Tissue	11	PCR - VHSV	Negative
Tissue	12	PCR - VHSV	Negative
Tissue	13	PCR - VHSV	Negative
Tissue	14	PCR - VHSV	Negative
Tissue	15	PCR - VHSV	Negative
Tissue	16	PCR - VHSV	Negative
Tissue	17	PCR - VHSV	Negative
Tissue	18	PCR - VHSV	Negative
Tissue	19	PCR - VHSV	Negative
Tissue	20	PCR - VHSV	Negative
Tissue	21	PCR - VHSV	Negative
Tissue	22	PCR - VHSV	Negative
Tissue	23	PCR - VHSV	Negative
Tissue	24	PCR - VHSV	Negative
Tissue	25	PCR - VHSV	Negative
Tissue	26	PCR - VHSV	Negative
Tissue	27	PCR - VHSV	Negative
Tissue	28	PCR - VHSV	Negative
Tissue	29	PCR - VHSV	Negative
Tissue	30	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 08/01/08 @ 2:41 PM

Specimen	ID	Isolate	Result	Level
Tissue	1		No viruses isolated	
Tissue	2		No viruses isolated	
Tissue	3		No viruses isolated	
Tissue	4		No viruses isolated	
Tissue	5		No viruses isolated	
Tissue	6		No viruses isolated	
Tissue	7		No viruses isolated	
Tissue	8		No viruses isolated	
Tissue	9		No viruses isolated	
Tissue	10		No viruses isolated	
Tissue	11		No viruses isolated	
Tissue	12		No viruses isolated	
Tissue	13		No viruses isolated	
Tissue	14		No viruses isolated	
Tissue	15		No viruses isolated	
Tissue	16		No viruses isolated	
Tissue	17		No viruses isolated	
Tissue	18		No viruses isolated	
Tissue	19		No viruses isolated	
Tissue	20		No viruses isolated	
Tissue	21		No viruses isolated	
Tissue	22		No viruses isolated	
Tissue	23		No viruses isolated	
Tissue	24		No viruses isolated	
Tissue	25		No viruses isolated	
Tissue	26		No viruses isolated	
Tissue	27		No viruses isolated	
Tissue	28		No viruses isolated	
Tissue	29		No viruses isolated	
Tissue	30		No viruses isolated	

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

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Final Report AHC Case: 08-2720

Last Updated: 08/06/08 9:46 AM
Pathologist: Stephen Raverty, DVM
Received Date: 07/15/08
Collected Date: 07/15/08
Client Ref No: 6759

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh and formalized tissue for histology, viral culture, PCR for IHN and VHS.

Large numbers of poor performers, appears to be NPLD.

Final Diagnosis

MORPHOLOGIC DIAGNOSES:

Fish 1 and 2:

1). Liver: Hepatocellular degeneration and necrosis, marked, multifocally extensive with lobular collapse, lipofuscinosis, ceroidosis, biliary ductular hyperplasia and compensative karyocytomegally

2). Pancreas: Peritonitis, mild, multifocal, granulomatous with intralesional clear vacuoles (presumptive vaccine induced)

3). Heart: Pericarditis, mild, segmental, granulomatous (possible vaccine induced)

There are no significant lesions within the gills, pyloric cecae, adipose tissue or spleen.

COMMENTS:

The changes within the liver are consistent with net pen liver disease and would have been sufficiently severe to have contributed significantly to impaired hepatobiliary function and the loss of these animals. The pericarditis and peritonitis are suggestive of vaccine induced inflammation, although the possibility of an opportunistic or secondary bacterial infection cannot be discounted. PCR was negative for VHS and IHN.

Histopathology

Refer to Morphologic Diagnoses

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/17/08 @ 12:06 PM

Specimen	ID	Test	Result
Tissue	6759-1	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/17/08 @ 12:06 PM

Specimen	ID	Test	Result
Tissue	6759-1	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 08/06/08 @ 9:46 AM

Specimen	ID	Isolate	Result	Level
Tissue	6759-1		No viruses isolated	

Stephen Raverty, DVM
Stephen.Raverty@gov.bc.ca

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Final Report AHC Case: 08-2721

Last Updated: 07/21/08 4:59 PM
Pathologist: Stephen Raverty, DVM
Received Date: 07/15/08
Collected Date: 07/15/08
Client Ref No: 6761

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted two formalized tissue samples for histology.

Suspected NPLD.

Log 6761

Final Diagnosis

MORPHOLOGIC DIAGNOSES:

1). Heart: Myocarditis, moderate, multifocal, granulomatous and necrotizing with scattered endocardial hyperplasia

2). Liver: Individualization and degeneration, hepatocellular, mild to moderate, multifocal, random, acute with scattered biliary ductular hyperplasia and periductular fibrosis

There are no significant lesions within the pyloric caecae, adipose tissue, kidney or spleen.

COMMENTS:

There is no conclusive microscopic indication of net pen liver disease; the changes within the heart are suggestive of BKD and follow up IFA, culture or PCR of representative clinical case material is recommended. The hepatocellular changes are suggestive of acute sepsis, although the possibility of early stage NPLD in 1 of the 2 sections cannot be entirely discounted. Follow up histology of the liver from representative fish may provide additional insights into the pathogenesis and etiology of this condition.

Histopathology

Refer to Morphologic Diagnoses

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Final Report AHC Case: 08-2722

Last Updated: 08/06/08 9:45 AM
Pathologist: Gary D. Marty
Received Date: 07/15/08
Collected Date: 07/15/08
Client Ref No: 6760

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for viral culture, PCR for IHN and VHS.

No clinical disease suspected in the fish sampled. Four morts sampled for routine virus screen.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/17/08 @ 12:06 PM

Specimen	ID	Test	Result
Tissue	6760-1	PCR - IHNV	Negative
Tissue	6760-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/17/08 @ 12:06 PM

Specimen	ID	Test	Result
Tissue	6760-1	PCR - VHSV	Negative
Tissue	6760-2	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 08/06/08 @ 9:45 AM

Specimen	ID	Isolate	Result	Level
Tissue	6760-1		No viruses isolated	
Tissue	6760-2		No viruses isolated	

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

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Final Report AHC Case: 08-2723

Last Updated: 08/06/08 9:45 AM
Pathologist: Stephen Raverty, DVM
Received Date: 07/15/08
Collected Date: 07/15/08
Client Ref No: 6762

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh and formalized tissue for histology, viral culture, PCR for IHN and VHS.

Fish starved for 26 days due to convolutes bloom. On feed for 2 days now. Morts elevated. Three sampled fish had petechial hemorrhage in pc/fat, liver, peritoneum. Fish #1 was a fresh mort and 2, 3, were moribunds.

Final Diagnosis

MORPHOLOGIC DIAGNOSES:

Fish 1:

Brain: Encephalitis, mild, multifocal, granulomatous

Heart: Myocarditis, perivascular and intramural, mild, focal, granulomatous

There are no significant lesions within the pyloric caecae, adipose tissue, kidney or spleen.

Fish 2 and 3:

Liver, slide 3: Cholangiohepatitis, mild, multifocal, subacute with biliary ductular hyperplasia

There are no significant lesions within the brain, heart, posterior kidney, anterior kidney, pyloric caecae, adipose tissue or spleen.

COMMENTS:

There are no consistent lesions within the examined tissues which may account for the increased morbidity and mortality within this stock of fish; due to the chronicity of the encephalitis and lack of discernible pathogens, the etiology of this condition is unknown. However, BKD is a prime consideration. The myocarditis is minimal and suggestive of a generalized antigenemia. In fish 3, there is a mild cholangiohepatitis suggestive of an ascending infection from the bowel. The lack of other overt microscopic findings tends to discount NPLD. Should fish continue to exhibit clinical signs, follow up resubmission of case material for evaluation is recommended. PCR for VHS and IHN was negative.

Histopathology

Refer to Morphologic Diagnoses

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/17/08 @ 12:06 PM

Specimen	ID	Test	Result
Tissue	6762-1	PCR - IHNV	Negative
Tissue	6762-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/17/08 @ 12:06 PM

Specimen	ID	Test	Result
Tissue	6762-1	PCR - VHSV	Negative
Tissue	6762-2	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 08/06/08 @ 9:45 AM

Specimen	ID	Isolate	Result	Level
Tissue	6762-1		No viruses isolated	
Tissue	6762-2		No viruses isolated	

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Final Report AHC Case: 08-2735

Last Updated: 08/01/08 11:33 AM
Pathologist: Stephen Raverty, DVM
Received Date: 07/16/08
Collected Date: 07/16/08
Client Ref No:

Veterinarian: **Barry Milligan**
Clinic: **Grieg Seafoods BC Ltd.**
Phone: (250) 286-0838
Fax: (250) 286-1883

Submitter: **Grieg Seafood**
Phone:
Fax:
Owner: **Grieg Seafoods BC Ltd.**
Phone: (250) 286-0838
Fax:(250) 286-1883

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted formalized tissue for histology.

8 fish sampled, 3 fish/cassette, 1 cassette with 2 fish. Increased mortality. Suspect BKD or Rickettsia.

Final Diagnosis

MORPHOLOGIC DIAGNOSES:

1). Liver, multiple sections: Hepatitis, marked, variably extensive, necrohemorrhagic, acute

2). Spleen, multiple sections: Splenitis, moderate to marked, diffuse, acute with numerous intralesional cocci and diplococci

3). Kidney, select sections: Necrosis, hematopoietic, moderate, diffuse with scattered hemosiderin laden macrophages and dispersed melanin

4). Brain, select sections: Meningitis, mild, focal, nonsuppurative

There are no significant lesions within the adipose tissue, pyloric cecae, or pancreas.

COMMENTS:

The hepatitis would have been sufficiently severe to account for the increased morbidity within the stock. Additional recuts and special stains were negative for Rickettsia and there were sparse numbers of Gram positive coccobacilli suggestive of BKD. Should mortalities persist, follow up PCR for confirmation is recommended. The hematopoietic necrosis, meningitis, and splenitis are consistent with antigenemia.

Histopathology

Refer to Morphologic Diagnoses

Stephen Raverty, DVM
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Final Report AHC Case: 08-2773

Last Updated: 07/24/08 11:06 AM
Pathologist: Gary D. Marty
Received Date: 07/18/08
Collected Date: 07/18/08
Client Ref No:

Veterinarian:
Clinic: **Mainstream Canada-T**
Phone: (250) 725-1255
Fax: (250) 725-1250

Submitter: **Zarah Vansnick**
Phone:
Fax:
Owner: **Romit Point**
Phone:
Fax:

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted histo. cassettes and one culture plate for bacteriology (Note: Bacteriology plates were highly contaminated).

Fish numbers 1 - 4 had the following: no food in their digestive tract, small greenish livers, and friable spleens. Fish number 5 had no food in its digestive tract, a small liver, and an enlarged spleen.

Final Diagnosis

1a. Liver: hepatocellular karyomegaly, diffuse, mild (slide 1), moderate (slides 2, 4)

1b. Liver: hepatocellular single cell necrosis, diffuse, mild (slide 2)

1c. Liver: lipofuscin-pigmented sinusoidal macrophages, diffuse, moderate (slides 1, 2, 4)

1e. Liver: hepatitis, lymphoplasmacytic, multifocal, mild (slides 2, 4)

1f. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 5)

2a. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, mild (slide 5), moderate (slide 4), severe (slide 2)

2b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1)

3. Heart: epicarditis, multifocal, lymphoplasmacytic, mild (slide 2)

Final Comment: Many features of slides 1 - 4 are consistent with netpen liver disease. Netpen liver disease can be reproduced in the laboratory with water exposure to the algal toxin microcystin-LR, but the source of the toxin in the field is unknown. Differentials include other toxins in the water or feed (e.g., aflatoxins). Changes in the affected liver include single cell necrosis, hepatocellular karyomegaly, sinusoidal lipofuscin accumulation, and variable amounts of lymphoplasmacytic inflammation. Lack of lipid stores in the mesenteric adipose tissue of affected fish is further evidence of chronic impairment. Hepatic megalocytosis can result from exposure to several types of toxins, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. 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; it is a common feature of netpen liver disease. Conditions that lead to

moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies.

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

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe). Vacuoles probably are a result of vaccine material lost during tissue processing.

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

Because microscopic lesions are not consistent with death due to bacterial septicemia, bacteriology results are probably a result of plate contamination.

Histopathology

Formalin-fixed tissues were submitted in 5 cassettes for histopathology. Slide numbers correspond to fish numbers on the cassettes.

Slides 1, 2, 3, 4 - liver, heart, head kidney, spleen, intestinal ceca and exocrine pancreas

Slide 5 - liver, spleen

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

Quality control: Liver autolysis: mild (slide 5), moderate (slides 2, 4), severe (slide 1), or too autolyzed for analysis (slide 3). Organs have no postfixation dehydration and no acid hematin accumulation.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 07/22/08 @ 11:21 AM

Specimen	ID	Isolate	Result	Level
Isolate	3	Aeromonas sp.	Positive	
Isolate	4	Aeromonas sp.	Positive	
**: Please note: Plates were highly contaminated with Proteus sp. and other contaminant species.				

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 07/22/08 @ 11:21 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Aeromonas sp.	3	s	s	s	r	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline							

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

These results relate only to the animals or items tested.

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Final Report AHC Case: 08-2774

Last Updated: 07/24/08 2:33 PM
Pathologist: Gary D. Marty
Received Date: 07/18/08
Collected Date: 07/18/08
Client Ref No:

Veterinarian:
Clinic: **Mainstream Canada-T**
Phone: (250) 725-1255
Fax: (250) 725-1250

Submitter: **Zarah Vansnick**
Phone:
Fax:
Owner: **West Side**
Phone:
Fax:

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 7 histo. cassettes and one culture plate for bact. West Side, July 9/08.

Fish 1 - Silv. No feed. Deformed fish, ascites, melanin

Fish 2 - Silv. W/feed, pale flesh. Gr3 melanin ??? kidney. Some hemorrhage swim bladder. Congestion digestive tract.

Fish 3 - Silv. w feed. Small??? Melanin.

Fish 4 - Silv. No feed. Palish gills. Melanin. Congestion digestive tract.

Fish 5 - Bloody ?? Pale liver ???

Fish 6 - Small Silv. w/feed, 600 g.

Fish 7 - Large Silv. w feed.

Final Diagnosis

1a. Liver: hepatic necrosis, acute, multifocal, moderate (slide 5)

1b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slide 5), moderate (slide 1)

1c. Liver: hepatocellular single cell necrosis (apoptosis), disseminated, acute, mild (slide 1)

1d. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 7)

2a. Spleen: leukocytic karyorrhexis, disseminated, moderate (slide 2)

2b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1, 3, 4, 5)

3. Intestinal ceca: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 6)

4. Head kidney: interstitial vascular congestion, diffuse, moderate (slide 6)

Final Comment: These fish have several lesions that are common in fish that die in marine net pens in British Columbia. Among the bacteria isolated from these fish, *Psychrobacter* spp. are not considered to be fish pathogens. *Vibrio tubiashii* is a halophilic, gram -negative bacterium that is common in estuary and seawater environments. It is considered a significant pathogen of oysters (Fouz et al. 1990), but it has not been described as a significant pathogen in fish; however, few vibrios have been critically evaluated for their pathogenicity in fish. Comments on specific lesions follow:

Hepatocellular necrosis can be caused by inadequate vascular perfusion (e.g., as occurs with harmful algal blooms or hypoxia) or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Renibacterium salmoninarum* , or *Piscirickettsia salmonis*); the cause is not determined in most cases. This case has no obvious organisms. If the problem continues and is not associated with low dissolved oxygen or a harmful algal bloom, consider submitting fresh tissues for VHSV PCR. Lack of proliferative lesions in the biliary system of fish #5 is evidence against a chronic toxic cause for the hepatic necrosis. Hepatic necrosis is somewhat common in salmon that die in marine net pens, affecting 11% of the 645 Atlantic salmon and 6% of the 119 Pacific salmon examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These types of vacuoles were rare before 2007 and might be related to the significant increase in the proportion of plant-based components in commercial feeds that has occurred since 2006. The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown.

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

Renal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria (e.g., *Vibrio tubiashii*), and infection with VHSV.

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). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "silvers" that die in marine net pens, affecting 12% of the 645 Atlantic salmon livers examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2007 was sufficient to identify a trend towards greater prevalence in the fall and winter (14-19%) than in the spring and summer (4.1-10%). Biliary preductular cell hyperplasia is rare in farmed Pacific salmon, affecting only 2.4% of the 253 Pacific salmon examined in 2006 and 2007 as part of the Province's Fish Health Auditing and Surveillance Program.

The presence of degenerating nuclei (karyorrhexis) in the spleen is evidence of increased cell turnover, possibly as part on an active inflammatory response.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Literature cited:

Fouz, B.; Conchas, R.F.; Bolinches, J.; Romalde, J.L.; Barja, J.L.; Toranzo, A.E. 1990. Relationship among pathogenic *Vibrio anguillarum* and *Vibrio tubiashii* with environmental vibrios. pp. 77-89 in, Third International Colloquium on Pathology in Marine Aquaculture, Gloucester Point, VA (USA), 2-6 Oct 1988. Academic Press, San Diego, CA (USA).

Histopathology

Formalin -fixed tissues were submitted in 7 cassettes for histopathology. Slide numbers correspond to fish numbers on the cassettes.

Slides 1 - 7: liver, heart, head kidney, spleen, intestinal ceca and exocrine pancreas

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

Quality control: Liver autolysis: mild (slides 5, 7), moderate (slides 1), or severe (slides 2, 3, 4, 6). Organs have no postfixation dehydration and no acid hematin accumulation.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 07/22/08 @ 11:23 AM

Specimen	ID	Isolate	Result	Level
Isolate	3	Psychrobacter sp.	Positive	
Isolate	6	Vibrio tubiashii	Positive	

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 07/22/08 @ 11:23 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Psychrobacter sp.	3	s	s	r	r	r	r
Vibrio tubiashii	6	s	s	s	s	r	s

**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline

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

END OF REPORT

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Final Report AHC Case: 08-2775

Last Updated: 07/24/08 4:18 PM
Pathologist: Gary D. Marty
Received Date: 07/18/08
Collected Date: 07/18/08
Client Ref No:

Veterinarian:
Clinic: **Mainstream Canada-T**
Phone: (250) 725-1255
Fax: (250) 725-1250

Submitter: **Zarah Vansnick**
Phone:
Fax:
Owner: **Fortune Channel**
Phone:
Fax:

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted histo. cassettes and Two culture plates for bacteriology.

Please note: Bacti plates were highly contaminated.

Final Diagnosis

1a. Liver: hepatocellular karyomegaly, diffuse, mild (slides 1, 3, 5, 6, 7), moderate (slides 2)

1b. Liver: hepatocellular single cell necrosis, diffuse, mild (slide 1, 6)

1c. Liver: lipofuscin-pigmented sinusoidal macrophages, diffuse, moderate (slides 2, 5), abundant (slides 1, 3, 6, 7)

1d. Liver: pericholangitis and hepatitis, lymphoplasmacytic, multifocal, mild (slides 2, 5, 6), moderate (slides 1, 3, 7)

2a. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, mild (slide 5), moderate (slides 1, 3)

2b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 2), moderate (slide 6)

3. Intestine/stomach: peritonitis, chronic, focal, with fibrocellular fronds, moderate (slide 2), severe (slide 4)

Final Comment: Many features of these slides are consistent with netpen liver disease. Netpen liver disease can be reproduced in the laboratory with water exposure to the algal toxin microcystin-LR, but the source of the toxin in the field is unknown. Differentials include other toxins in the water or feed (e.g., aflatoxins). Changes in the affected liver include single cell necrosis, hepatocellular karyomegaly, sinusoidal lipofuscin accumulation, variable amounts of lymphoplasmacytic inflammation, and general parenchymal collapse (e.g., in some livers the volume of lipofuscin is about equal to the volume of hepatocytes). Hepatic megalocytosis can result from exposure to several types of toxins, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. 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; it is a common feature of netpen liver disease. Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe). Vacuoles probably are a result of vaccine material lost during tissue processing.

Although netpen liver disease is the primary disease in these fish, the ultimate cause of death might have been a *Vibrio ordalii* septicemia. ***Vibrio ordalii*** is a halophilic gram negative rod that is a major cause of mortality in both wild and farmed fishes in the Pacific. It is closely related to *Listonella anguillarum*. Unlike many other vibrios, which are common in marine sediments, *Vibrio ordalii* is more common in the digestive tract of marine mammals. Septicemia is common, and infection tends to target the muscle and skin. [source: Fish Pathology, 3rd Edition. 2001. R.J. Roberts]

Histopathology

Formalin-fixed tissues were submitted in 7 cassettes for histopathology. Slide numbers correspond to fish numbers on the cassettes.

Slides 1, 3, 5, 6, and 7: liver and spleen

Slide 2 - liver, tubular organ (stomach or intestine), and spleen

Slide 4 - liver, granulomatous inflammation (margin of spleen or intestine?)

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

Quality control: Liver autolysis: mild (slides 1, 3, 5, 6, 7), moderate (slide 2), or too autolyzed for analysis (slide 4). Organs have no postfixation dehydration and no acid hematin accumulation.

Bacteriology

Aerobic Culture - Prod Resulted by: Verified by: Sean Byrne on 07/24/08 @ 3:51 PM

Specimen	ID	Isolate	Result	Level
Isolate	1	Proteus sp.		
Isolate	2	Proteus sp.		
Isolate	3	Proteus sp.		
Isolate	4	Proteus sp.		
**: Isolate 1-4 overgrown with Proteus sp., unable to isolate pure organism. Please resubmit if possible.				
Isolate	5	Vibrio sp.	Positive	
Isolate	6	Vibrio sp.	Positive	
Isolate	7	Vibrio sp.	Positive	
Isolate	8	Vibrio sp.	Positive	
**: Isolates 5,6,7,8 are all the same isolate. Identified as Vibrio ordalii by DNA sequencing				

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 07/24/08 @ 3:51 PM

Organism	ID	e	ffc	sor	s3	sxt	ot
Vibrio sp.	5	s	s	s	r	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline							

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

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Final Report AHC Case: 08-2816

Last Updated: 08/13/08 1:23 PM
Pathologist: Gary D. Marty
Received Date: 07/22/08
Collected Date: 07/22/08
Client Ref No: 6775

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted four fish salmon tissues for histology and PCR for IHN and VHS.

Mouth rot present at site. 3 fish sampled with hemorrhaging in the muscle around the head kidney and along side the kidney; hemorrhaging of the swim bladder and lower intestine. All (4) fish sampled for PCR for IHN and VHS. Histo taken from fish #1 (fresh dead) some autolysis may be present. No growth on bacteriology.

Saltwater entry: 2008 S1. Vaccinated. Euthanized: no. DOD: July 17, 08. Ref# 6775

Final Diagnosis

1a. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, mild (probable cause: VHSV)

1b. Trunk kidney: interstitial hemorrhage, diffuse, with hematopoietic cell necrosis, moderate (probable cause: VHSV)

1c. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild

2. Liver: basophilic hepatocellular cytoplasm, diffuse, mild

3. Skeletal muscle: myonecrosis, peracute, multifocal, with hemorrhage, moderate

4. Spleen and intestinal ceca: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate

Final Comment: This fish has several lesions consistent with the PCR+ results for VHSV. VHSV is rarely isolated in July, but it can occur in stressed fish.

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Renal tubular epithelial necrosis was fairly common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 8.4%; n = 643) and Pacific salmon (prevalence = 4.2%; n = 120); the cause was not determined in many cases. This case is probably a result of infection with viral hemorrhagic septicemia virus (VHSV). Differentials include exposure to toxins (e.g., bacterial toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin).

Renal interstitial hemorrhage and necrosis is an uncommon manifestation of infection with viral hemorrhagic septicemia virus (VHSV).

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

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 this fish it might be related to increased protein needed as part of an inflammatory response.

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

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe). Vacuoles probably are a result of vaccine material lost during tissue processing.

Histopathology

Formalin -fixed tissues were submitted in 1 cassette for histopathology.

Slide 1 (6775) - brain, heart, spleen, liver, trunk kidney, skin/skeletal muscle, intestinal ceca, and mesenteric adipose tissue

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

Quality control: Liver autolysis: severe; kidney autolysis, moderate. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 07/24/08 @ 9:48 AM

Specimen	ID	Test	Result
Tissue	6775-1	PCR - IHNV	Negative
Tissue	6775-2	PCR - IHNV	Negative
Tissue	6775-3	PCR - IHNV	Negative
Tissue	6775-4	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 07/24/08 @ 9:48 AM

Specimen	ID	Test	Result
Tissue	6775-1	PCR - VHSV	Positive
Tissue	6775-2	PCR - VHSV	Positive
Tissue	6775-3	PCR - VHSV	Positive
Tissue	6775-4	PCR - VHSV	Positive

Virology

Tissue Culture Resulted by: Cheryl Cecconi Verified by: Melissa Trapp on 08/13/08 @ 1:23 PM

Specimen	ID	Isolate	Result	Level
Tissue	6775-1	Viral Hemorrhag Septicem Virus	Positive	
Tissue	6775-2	Viral Hemorrhag Septicem Virus	Positive	
Tissue	6775-3	Viral Hemorrhag Septicem Virus	Positive	

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

END OF REPORT

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Final Report AHC Case: 08-2914

Last Updated: 08/20/08 2:55 PM
Pathologist: Gary D. Marty
Received Date: 07/28/08
Collected Date: 07/28/08
Client Ref No: 6791

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 10 samples for PCR for IHN and VHS. Each sample is a pool of 3 fish.

Routine site visit and sample after transfer. No gross lesions noted.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/30/08 @ 10:37 AM

Specimen	ID	Test	Result
Tissue	6791-1	PCR - IHNV	Negative
Tissue	6791-2	PCR - IHNV	Negative
Tissue	6791-3	PCR - IHNV	Negative
Tissue	6791-4	PCR - IHNV	Negative
Tissue	6791-5	PCR - IHNV	Negative
Tissue	6791-6	PCR - IHNV	Negative
Tissue	6791-7	PCR - IHNV	Negative
Tissue	6791-8	PCR - IHNV	Negative
Tissue	6791-9	PCR - IHNV	Negative
Tissue	6791-10	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/30/08 @ 10:37 AM

Specimen	ID	Test	Result
Tissue	6791-1	PCR - VHSV	Negative
Tissue	6791-2	PCR - VHSV	Negative
Tissue	6791-3	PCR - VHSV	Negative

Tissue	6791-4	PCR - VHSV	Negative
Tissue	6791-5	PCR - VHSV	Negative
Tissue	6791-6	PCR - VHSV	Negative
Tissue	6791-7	PCR - VHSV	Negative
Tissue	6791-8	PCR - VHSV	Negative
Tissue	6791-9	PCR - VHSV	Negative
Tissue	6791-10	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 08/20/08 @ 2:55 PM

Specimen	ID	Isolate	Result	Level
Tissue	6791-1		No viruses isolated	
Tissue	6791-2		No viruses isolated	
Tissue	6791-3		No viruses isolated	
Tissue	6791-4		No viruses isolated	
Tissue	6791-5		No viruses isolated	
Tissue	6791-6		No viruses isolated	
Tissue	6791-7		No viruses isolated	
Tissue	6791-8		No viruses isolated	
Tissue	6791-9		No viruses isolated	
Tissue	6791-10		No viruses isolated	

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

END OF REPORT

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Final Report AHC Case: 08-2947

Last Updated: 08/21/08 4:11 PM
Pathologist: Gary D. Marty
Received Date: 07/30/08
Collected Date: 07/30/08
Client Ref No: 6784

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for IHN/VHS PCR testing.

Three fresh morts with hemorrhage in liver. 07/S1. Vaccinated. Case ID: 6784

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 08/01/08 @ 10:45 AM

Specimen	ID	Test	Result
Tissue	6784-1	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 08/01/08 @ 10:45 AM

Specimen	ID	Test	Result
Tissue	6784-1	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 08/21/08 @ 4:11 PM

Specimen	ID	Isolate	Result	Level
Tissue	6784-1		No viruses isolated	

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

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Final Report AHC Case: 08-3070

Last Updated: 08/28/08 11:56 AM
Pathologist: Gary D. Marty
Received Date: 08/07/08
Collected Date: 08/07/08
Client Ref No: 6803

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted two salmon tissues for PCR IHN and VHS.

Samples labeled as 4-A and 4-B. From same site as our case #6775. Fish with typical VHS lesions. Part of routine health check.

Euthanized: N. TMS. DOD: July 31, 08. Saltwater entry: 2008 S1.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: A Scouras on 08/11/08 @ 10:23 AM

Specimen	ID	Test	Result
Tissue	6803 Pen 4-A	PCR - IHNV	Negative
Tissue	6803 Pen 4-B	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: A Scouras on 08/11/08 @ 10:23 AM

Specimen	ID	Test	Result
Tissue	6803 Pen 4-A	PCR - VHSV	Positive
Tissue	6803 Pen 4-B	PCR - VHSV	Positive

Virology

Tissue Culture Resulted by: Cheryl Cecconi Verified by: Melissa Trapp on 08/28/08 @ 11:56 AM

Specimen	ID	Isolate	Result	Level
Tissue	6803 Pen 4-A		No viruses isolated	
Tissue	6803 Pen 4-B		No viruses isolated	

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

END OF REPORT

Case: 08-3070

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Final Report AHC Case: 08-3071

Last Updated: 08/28/08 11:56 AM
Pathologist: Gary D. Marty
Received Date: 08/07/08
Collected Date: 08/07/08
Client Ref No: 6804

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted ten fresh tissues for PCR for IHN and VHS.

10 samples labeled #11 through #20. Samples taken during cull grade. NVL. 3 fish per virology pool. ie 30 fish sampled in total.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: A Scouras on 08/11/08 @ 10:23 AM

Specimen	ID	Test	Result
Tissue	6804-11	PCR - IHNV	Negative
Tissue	12	PCR - IHNV	Negative
Tissue	13	PCR - IHNV	Negative
Tissue	14	PCR - IHNV	Negative
Tissue	15	PCR - IHNV	Negative
Tissue	16	PCR - IHNV	Negative
Tissue	17	PCR - IHNV	Negative
Tissue	18	PCR - IHNV	Negative
Tissue	19	PCR - IHNV	Negative
Tissue	20	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: A Scouras on 08/11/08 @ 10:24 AM

Specimen	ID	Test	Result
Tissue	6804-11	PCR - VHSV	Negative
Tissue	12	PCR - VHSV	Negative
Tissue	13	PCR - VHSV	Negative
Tissue	14	PCR - VHSV	Negative

Tissue	15	PCR - VHSV	Negative
Tissue	16	PCR - VHSV	Negative
Tissue	17	PCR - VHSV	Negative
Tissue	18	PCR - VHSV	Negative
Tissue	19	PCR - VHSV	Negative
Tissue	20	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Cheryl Cecconi Verified by: Melissa Trapp on 08/28/08 @ 11:56 AM

Specimen	ID	Isolate	Result	Level
Tissue	6804-11		No viruses isolated	
Tissue	12		No viruses isolated	
Tissue	13		No viruses isolated	
Tissue	14		No viruses isolated	
Tissue	15		No viruses isolated	
Tissue	16		No viruses isolated	
Tissue	17		No viruses isolated	
Tissue	18		No viruses isolated	
Tissue	19		No viruses isolated	
Tissue	20		No viruses isolated	

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

END OF REPORT

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Final Report AHC Case: 08-3090

Last Updated: 08/13/08 12:17 PM
Pathologist: Gary D. Marty
Received Date: 08/08/08
Collected Date: 08/08/08
Client Ref No: PO 11898

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **Zarah Vansnick**
Phone:
Fax:
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 8 cassettes for histology.

Fish: A-1, B-2, C-3, D-5, E-6, F-8, G-9.

See attached sheet for more details.

Final Diagnosis

Lesions most likely to have caused death of these fish:

1. Liver: hepatitis, granulomatous, multifocal, coalescing, with multinucleate giant cells and amorphous foreign material (probable vaccine reaction), severe (fish #s 5, 6, and 11)

2. Heart: Epicarditis, histiocytic, multifocal, coalescing (probable vaccine reaction), moderate (fish #6)

3. Heart: endothelial cell hypertrophy, diffuse (probable vaccine reaction), moderate (fish #6)

Final Comment: The most severe lesions in these fish could all be explained by an excessive reaction to a vaccine. Details for this case are included on an Excel spreadsheet (2008- 3090.xls); note that the spreadsheet is not included with the official final report generated by the Animal Health Centre's VADDS database because the database cannot handle spreadsheets. The "Abbreviation" worksheet on the spreadsheet includes comments about each lesion. Specific comments on significant lesions in these fish follow:

Fish #6 has two significant lesions in the heart, both of which might have the same cause. Histiocytic epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Endothelial cell hypertrophy is evidence of systemic immune stimulation: probably resulting from inflammatory cell mediators released into the circulation (e.g., during a bacterial infection, viral infection, or reaction to a vaccine).

Several fish have disseminated granulomatous inflammation, and lesions in fish #s 5, 6, and 11 are sufficient to have killed the affected fish. With these fish, a vaccine reaction is the most likely cause for the granulomatous inflammation. Foci of radiating globular material within foci of granulomatous inflammation are characteristic of severe vaccine reactions in fish. Differentials include a chronic bacterial disease (e.g., *Yersinia ruckeri* or *Renibacterium salmoninarum* infection); however, lack of bacteria on the Gram stain of tissues from fish #11 makes bacteria highly unlikely in this case.

Lesions in fish 1, 2, 3, 8, and 9 are not of sufficient severity to explain their death. Scores for other lesions are included on the spreadsheet.

Value of brain histopathology for determining cause of death - I recommend sampling of brain (including brainstem) in cases like this one where the cause of death is unknown. At the beginning of 2007, brain was added to the list of organs to sample for histopathology as part of the BC Provincial government's Fish Health Auditing and Surveillance Program. Of the 168 Atlantic salmon examined during the first quarter of 2007, liver, kidney, and heart had lesions sufficient to explain the cause of death in 27% of the cases. The addition of brain histopathology allowed me to determine the cause of death of another 20% of the fish. Spleen, intestine, and mesenteric adipose tissue did not provide any unique information for determining the cause of death. Gill and mouth, selected only from fish with lesions in these structures, added a cause of death to another 4% of the fish.

Histopathology

Formalin-fixed tissues were submitted in 8 cassettes for histopathology; slide numbers correspond to fish numbers. All organs were examined. A Twort's Gram stain was done on sections from the same paraffin block as slide 11. The scoring system used for these fish is the same as that used for the BC Provincial Government's Fish Health Auditing and Surveillance Program. All scored lesions are included, even though many of the lesions did not occur in this group of fish.

Quality Control: Details are included on the spreadsheet (2008-3090.xls).

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

END OF REPORT

BRITISH COLUMBIA
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Animal Health Centre

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Final Report AHC Case: 08-3153

Last Updated: 09/05/08 2:52 PM
Pathologist: Gary D. Marty
Received Date: 08/14/08
Collected Date: 08/14/08
Client Ref No: 6818

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **D. Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for PCR for IHN and VHS.

Routine sample during lice count; no mortality; low DO's. Three samples for PCR for IHN and VHS. Sample labeled "pen 5" is a pooled sample of 3 fish. Samples "pen 7" and "pen 9" are single fish samples.

Saltwater entry 2007 S1, euthanized TMS.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: A Scouras on 08/15/08 @ 3:27 PM

Specimen	ID	Test	Result
Tissue	6818 Pen 5	PCR - IHNV	Negative
Tissue	6818 Pen 7	PCR - IHNV	Negative
Tissue	6818 Pen 9	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: A Scouras on 08/15/08 @ 3:27 PM

Specimen	ID	Test	Result
Tissue	6818 Pen 5	PCR - VHSV	Negative
Tissue	6818 Pen 7	PCR - VHSV	Negative
Tissue	6818 Pen 9	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 09/05/08 @ 2:52 PM

Specimen	ID	Isolate	Result	Level

Tissue	6818 Pen 5	No viruses isolated
Tissue	6818 Pen 7	No viruses isolated
Tissue	6818 Pen 9	No viruses isolated

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

END OF REPORT

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Ministry of Agriculture and Lands

Animal Health Centre

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Ministry of Agriculture and Lands
1767 Angus Campbell Road
Abbotsford BC V3G 2M3
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Final Report AHC Case: 08-3239

Last Updated: 09/11/08 4:18 PM
Pathologist: Gary D. Marty
Received Date: 08/21/08
Collected Date: 08/21/08
Client Ref No: 6832

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh and formalized fish tissue for histopathology, viral culture, and PCR for IHN and VHS.

Fish had mild hemorrhage on belly, but appeared normal internally.

Euthanized TMS, vaccinated.

ID: 6832.

Final Diagnosis

1. Swimbladder: aerocystitis, granulomatous, diffuse, severe (slide 3B)

2. Gill: lamellar epithelial hyperplasia, multifocal, moderate (slide 2A)

3. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 1B)

4. Heart: epicarditis, focal, lymphohistiocytic, mild (slide 1B)

5. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 1B)

6. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1B, 2B, 3B)

Final Comment: Fish #3 has a severe case of granulomatous inflammation of the swimbladder adjacent to the head kidney. Inflammation extends from the epithelium into the lumen, and includes two vacuoles that are 20 - 35 µm in diameter. Twort's Gram stain highlights small numbers of eosinophilic granular cells among the inflammation, but no bacteria. Swimbladder inflammation might have resulted from aspiration of food or other particles into the swimbladder through the pneumatic duct. It is very difficult for fish to clear foreign material from their pneumatic duct, so the prognosis for this fish was poor. If the changes in this fish are representative of a significant proportion of the population, the frequency of aspiration aerocystitis might be decreased by keeping feed and foreign material off the surface of the water; otherwise, though, this lesion does not represent an infectious threat to other fish.

Other changes in these fish are fairly common among cultured salmon in British Columbia. Specific comments follow:

Hyperplasia of the epithelium lining gill lamellae is a nonspecific response to irritation. Inciting causes include parasites, bacteria, viruses, and toxins. The sections have no obvious organisms.

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.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Histopathology

Formalin -fixed tissues were submitted in 6 cassettes for histopathology.

Slide 1A (6832-1 8/19/08), Slide 2A (6832-2 8/19/08) and Slide 3A (6832-3 8/19/08) - gill

Slide 1B (6832-1 8/19/08), Slide 2B (6832-2 8/19/08) and Slide 3B (6832-3 8/19/08) -heart, spleen, liver, intestine, head kidney, trunk kidney, skin/skeletal muscle (2 pieces), mesenteric adipose tissue; a Twort's Gram stain was done on a section of block 3B.

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

Quality control: Liver autolysis: none (slides 1B, 2B, 3B). Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/25/08 @ 11:51 AM

Specimen	ID	Test	Result
Tissue	6832-1	PCR - IHNV	Negative
Tissue	6832-2	PCR - IHNV	Negative
Tissue	6832-3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/25/08 @ 11:52 AM

Specimen	ID	Test	Result
Tissue	6832-1	PCR - VHSV	Negative
Tissue	6832-2	PCR - VHSV	Negative
Tissue	6832-3	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Cheryl Cecconi Verified by: Dr. J. Robinson on 09/11/08 @ 4:18 PM

Specimen	ID	Isolate	Result	Level

Tissue	6832-1	No viruses isolated
Tissue	6832-2	No viruses isolated
Tissue	6832-3	No viruses isolated

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

END OF REPORT

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Ministry of Agriculture and Lands

Animal Health Centre

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Ministry of Agriculture and Lands
1767 Angus Campbell Road
Abbotsford BC V3G 2M3
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Final Report AHC Case: 08-3240

Last Updated: 09/19/08 2:55 PM
Pathologist: Gary D. Marty
Received Date: 08/21/08
Collected Date: 08/21/08
Client Ref No: 6820

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fish tissue for viral culture and PCR - IHN and VHS.

Samples taken from poor performers, 3 fish pools.

Vaccinated.

ID: 6820.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/25/08 @ 11:53 AM

Specimen	ID	Test	Result
Tissue	6820-1	PCR - IHNV	Negative
Tissue	6820-2	PCR - IHNV	Negative
Tissue	6820-3	PCR - IHNV	Negative
Tissue	6820-4	PCR - IHNV	Negative
Tissue	6820-5	PCR - IHNV	Negative
Tissue	6820-6	PCR - IHNV	Negative
Tissue	6820-7	PCR - IHNV	Negative
Tissue	6820-8	PCR - IHNV	Negative
Tissue	6820-9	PCR - IHNV	Negative
Tissue	6820-10	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/25/08 @ 11:53 AM

Specimen	ID	Test	Result
Tissue	6820-1	PCR - VHSV	Negative

Tissue	6820-2	PCR - VHSV	Negative
Tissue	6820-3	PCR - VHSV	Negative
Tissue	6820-4	PCR - VHSV	Negative
Tissue	6820-5	PCR - VHSV	Negative
Tissue	6820-6	PCR - VHSV	Negative
Tissue	6820-7	PCR - VHSV	Negative
Tissue	6820-8	PCR - VHSV	Negative
Tissue	6820-9	PCR - VHSV	Negative
Tissue	6820-10	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 09/19/08 @ 2:55 PM

Specimen	ID	Isolate	Result	Level
Tissue	6820-1		No viruses isolated	
Tissue	6820-2		No viruses isolated	
Tissue	6820-3		No viruses isolated	
Tissue	6820-4		No viruses isolated	
Tissue	6820-5		No viruses isolated	
Tissue	6820-6		No viruses isolated	
Tissue	6820-7		No viruses isolated	
Tissue	6820-8		No viruses isolated	
Tissue	6820-9		No viruses isolated	
Tissue	6820-10		No viruses isolated	

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

END OF REPORT

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Final Report AHC Case: 08-3242

Last Updated: 09/11/08 4:18 PM
Pathologist: Gary D. Marty
Received Date: 08/21/08
Collected Date: 08/21/08
Client Ref No: 6830

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh and formalized fish tissue for histology, viral culture and PCR for IHN and VHS.

Three fresh morts with petechial hemorrhage in liver. No other disease signs.

Vaccinated.

ID: 6830

Final Diagnosis

1a. Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical amphophilic inclusions, acute, multifocal, mild (slides 1, 3), moderate (slide 2)

1b. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 1)

1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 3), moderate (slide 2)

2a. Heart: epicarditis, multifocal, lymphohistiocytic, mild (slides 1, 2)

2b. Heart: myocardial karyomegaly, multifocal, mild (slide 3)

3a. Trunk kidney: nephritis, interstitial, neutrophilic, focal (~300 µm in diameter), mild (slide 3)

3b. Trunk kidney, head kidney: interstitial vascular congestion, diffuse, mild (slide 1)

3b. Trunk kidney: renal tubular epithelial necrosis, focal, acute, mild (slide 2)

4a. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slide 2)

4b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 2)

5. Skeletal muscle: myonecrosis, acute, bifocal (2 myofibres), mild (slide 2)

Final Comment: These fish have several lesions that are fairly common among cultured Atlantic salmon in British Columbia. No specific cause emerges, but the lesions might provide clues to rule in or out differentials. PCR results rule out VHSV as a differential in these fish. Specific comments follow:

In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of circulating vasodilators. I have seen it associated with viral hemorrhagic septicemia virus and *Listonella anguillarum* infection. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. 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 congested foci, but nowhere else in the sections, are evidence that the congested foci were acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion. Interestingly, the foci of congestion are the only regions of the liver in slide 1 with only mild autolysis: evidence that the focal acidosis decreased the activity of autolytic enzymes.

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; differentials include a low grade bacterial infection and reaction to a vaccine.

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.1% of the 1142 Atlantic salmon hearts sampled as part of the province's Fish Health Auditing and Surveillance Program during 2006 and 2007). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. 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 focus of neutrophils separating the hematopoietic tissues of the trunk kidney in slide 3 is evidence of acute immune stimulation. The most likely differential is a focal bacterial infection, but the section contains no obvious organisms.

Renal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV.

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Renal tubular epithelial necrosis was fairly common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 8.4%; n = 643) and Pacific salmon (prevalence = 4.2%; n = 120); the cause was not determined in many cases. Differentials include viral hemorrhagic septicemia virus (VHSV) and exposure to toxins (e.g., bacterial toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin).

The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Skeletal muscle necrosis 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.

Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology.

Slide 1 (6830-1 08 18 08), Slide 2 (6830-2 Glacier 2) and Slide 3 (6830-3 Glacier) - heart, spleen, liver, intestine, head kidney, trunk kidney, skeletal muscle, and mesenteric adipose tissue

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

Quality control: Liver autolysis: mild (slides 2, 3), severe but readable (slide 1). Large foci of erythrocytes (e.g., foci of congestion in liver, slide 1) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with bloody pieces of tissue). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/25/08 @ 11:53 AM

Specimen	ID	Test	Result
Tissue	6830-1	PCR - IHNV	Negative
Tissue	6830-2	PCR - IHNV	Negative
Tissue	6830-3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/25/08 @ 11:53 AM

Specimen	ID	Test	Result
Tissue	6830-1	PCR - VHSV	Negative
Tissue	6830-2	PCR - VHSV	Negative
Tissue	6830-3	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Cheryl Cecconi Verified by: Dr. J. Robinson on 09/11/08 @ 4:18 PM

Specimen	ID	Isolate	Result	Level
Tissue	6830-1		No viruses isolated	
Tissue	6830-2		No viruses isolated	
Tissue	6830-3		No viruses isolated	

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

END OF REPORT

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Final Report AHC Case: 08-3243

Last Updated: 09/18/08 11:44 AM
Pathologist: Gary D. Marty
Received Date: 08/21/08
Collected Date: 08/21/08
Client Ref No: 6831

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
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Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh fish tissue for viral culture and PCR for IHN and VHS.

Five morts. Disease signs and culture suggest A.S but want to rule out viral agent as well.

Vaccinated.

ID: 6831

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 08/27/08 @ 11:47 AM

Specimen	ID	Test	Result
Tissue	6831-1	PCR - IHNV	Negative
Tissue	6831-2	PCR - IHNV	Negative
Tissue	6831-3	PCR - IHNV	Negative
Tissue	6831-4	PCR - IHNV	Negative
Tissue	6831-5	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 08/27/08 @ 11:47 AM

Specimen	ID	Test	Result
Tissue	6831-1	PCR - VHSV	Negative
Tissue	6831-2	PCR - VHSV	Negative
Tissue	6831-3	PCR - VHSV	Negative
Tissue	6831-4	PCR - VHSV	Negative
Tissue	6831-5	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 09/18/08 @ 11:44 AM

Specimen	ID	Isolate	Result	Level
Tissue	6831-1		No viruses isolated	
Tissue	6831-2		No viruses isolated	
Tissue	6831-3		No viruses isolated	
Tissue	6831-4		No viruses isolated	
Tissue	6831-5		No viruses isolated	

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

END OF REPORT

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Final Report AHC Case: 08-3244

Last Updated: 09/18/08 11:44 AM
Pathologist: Gary D. Marty
Received Date: 08/21/08
Collected Date: 08/21/08
Client Ref No: 6819

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
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Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh fish tissue for viral culture and PCR for IHN and VHS.

Poor performers sampled for disease screen. 3 fish pools.

Euthanized TMS.

ID: 6819

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 08/27/08 @ 11:47 AM

Specimen	ID	Test	Result
Tissue	6819-1	PCR - IHNV	Negative
Tissue	6819-2	PCR - IHNV	Negative
Tissue	6819-3	PCR - IHNV	Negative
Tissue	6819-4	PCR - IHNV	Negative
Tissue	6819-5	PCR - IHNV	Negative
Tissue	6819-6	PCR - IHNV	Negative
Tissue	6819-7	PCR - IHNV	Negative
Tissue	6819-8	PCR - IHNV	Negative
Tissue	6819-9	PCR - IHNV	Negative
Tissue	6819-10	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 08/27/08 @ 11:47 AM

Specimen	ID	Test	Result
Tissue	6819-1	PCR - VHSV	Negative

Tissue	6819-2	PCR - VHSV	Negative
Tissue	6819-3	PCR - VHSV	Negative
Tissue	6819-4	PCR - VHSV	Negative
Tissue	6819-5	PCR - VHSV	Negative
Tissue	6819-6	PCR - VHSV	Negative
Tissue	6819-7	PCR - VHSV	Negative
Tissue	6819-8	PCR - VHSV	Negative
Tissue	6819-9	PCR - VHSV	Negative
Tissue	6819-10	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 09/18/08 @ 11:44 AM

Specimen	ID	Isolate	Result	Level
Tissue	6819-1		No viruses isolated	
Tissue	6819-2		No viruses isolated	
Tissue	6819-3		No viruses isolated	
Tissue	6819-4		No viruses isolated	
Tissue	6819-5		No viruses isolated	
Tissue	6819-6		No viruses isolated	
Tissue	6819-7		No viruses isolated	
Tissue	6819-8		No viruses isolated	
Tissue	6819-9		No viruses isolated	
Tissue	6819-10		No viruses isolated	

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

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Final Report AHC Case: 08-3246

Last Updated: 09/18/08 11:45 AM
Pathologist: Gary D. Marty
Received Date: 08/21/08
Collected Date: 08/21/08
Client Ref No: 6835

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
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Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh fish tissue for viral culture and PCR for IHN and VHS.

Three fresh morts sampled for viral screen. No disease signs.

ID: 6835

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 08/27/08 @ 11:48 AM

Specimen	ID	Test	Result
Tissue	6835-1	PCR - IHNV	Negative
Tissue	6835-2	PCR - IHNV	Negative
Tissue	6835-3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 08/27/08 @ 11:48 AM

Specimen	ID	Test	Result
Tissue	6835-1	PCR - VHSV	Negative
Tissue	6835-2	PCR - VHSV	Negative
Tissue	6835-3	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 09/18/08 @ 11:45 AM

Specimen	ID	Isolate	Result	Level
Tissue	6835-1		No viruses isolated	

Tissue	6835-2	No viruses isolated
Tissue	6835-3	No viruses isolated

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

END OF REPORT

Case: 08-3246

BRITISH COLUMBIA
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Final Report AHC Case: 08-3247

Last Updated: 09/18/08 11:45 AM
Pathologist: Gary D. Marty
Received Date: 08/21/08
Collected Date: 08/21/08
Client Ref No: 6834

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
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Submitter: **Marine Harvest**
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Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh fish tissue for viral culture and PCR for IHN and VHS.

Three fresh morts sampled for viral screen. No disease signs.

ID: 6834

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 08/27/08 @ 11:48 AM

Specimen	ID	Test	Result
Tissue	6834-1	PCR - IHNV	Negative
Tissue	6834-2	PCR - IHNV	Negative
Tissue	6834-3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 08/27/08 @ 11:48 AM

Specimen	ID	Test	Result
Tissue	6834-1	PCR - VHSV	Negative
Tissue	6834-2	PCR - VHSV	Negative
Tissue	6834-3	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 09/18/08 @ 11:45 AM

Specimen	ID	Isolate	Result	Level
Tissue	6834-1		No viruses isolated	

Tissue	6834-2	No viruses isolated
Tissue	6834-3	No viruses isolated

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

END OF REPORT

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Final Report AHC Case: 08-3302

Last Updated: 08/28/08 1:28 PM
Pathologist: Gary D. Marty
Received Date: 08/26/08
Collected Date: 08/26/08
Client Ref No: 8-2519

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **Tim Hewison - Microtek**
Phone:
Fax:
Owner: **Microtek International In**
Phone:
Fax:(250) 652-4802

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted two salmo salar tissues (gill, head kidney, trunk kidney, liver & spleen) for histo.

Mortality spike. DOD: Aug 21, 08.

Final Diagnosis

1. Trunk kidney: nephritis, interstitial, fibrinous, multifocal, mild (slides 1A, 2A)

2. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 2A)

Final Comment: Fibrinous nephritis is evidence of acute inflammation in the kidney. The Gram stain reveals no organisms. Although acute forms of *Renibacterium salmoninarum* are sometimes associated with fibrinous exudate, this case has very small numbers of fibrin foci limited to the kidney: not a pattern I have seen with *Renibacterium salmoninarum.* To better characterize the cause of mortality, consider bacteriology and virology, if not already done.

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

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology. For both cassettes, gills were removed from the original (A) cassette and placed in a separate (B) cassette.

Slide 1A (Case File 8-2519 Fish #10) - spleen, liver, head kidney, trunk kidney, stomach

Slide 2A (Case File 8-2519 Fish #11) - spleen, liver, trunk kidney, head kidney; a Twort's Gram stain was done on a section from the same paraffin block.

Slide 1B (Case File 8-2519 Fish #10) and Slide 2B (Case File 8-2519 Fish #11) - gill

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

Quality control: Liver autolysis: moderate (slide 2A), severe (slide 1A). Organs have no postfixation dehydration and no acid hematin deposits.

Measures of physiologic condition:

Hepatocellular glycogen: none (slides 1A, 2A)

Mesenteric adipose tissue: abundant (slide 1A; not present for analysis on slide 2A)

These measures of physiologic condition are consistent fish that had been healthy but were not eating normally for a couple days before they died.

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

END OF REPORT

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Final Report AHC Case: 08-3362

Last Updated: 09/19/08 2:55 PM
Pathologist: Gary D. Marty
Received Date: 08/29/08
Collected Date: 08/29/08
Client Ref No:

Veterinarian: **Dr. Peter McKenzie**
Clinic: **Mainstream Canada**
Phone: (250) 286-0022
Fax: (250) 286-0042

Submitter: **Nathan Cassan - Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 4 fresh tissues for virology and 4 cassettes for histology testing.

Saltwater. Vaccinated.

PO# 11648.

Final Diagnosis

1. Brain: meningitis and ependymitis, multifocal, with intralesional intracellular organisms (presporogonic myxosporean?), severe (slide 1A)

2a. Liver: hepatic necrosis, acute, multifocal, moderate (slide 2A)

2b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 1A, 2A, 4A)

3a. Trunk kidney: moderate numbers of interstitial intracytoplasmic eosinophilic granules, diffuse (slide 2A)

3b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 1A, 3A)

4a. Heart: endocarditis, multifocal, with endothelial cell hypertrophy and scattered lymphocytes and macrophages, moderate (slide 3A)

4b. Heart: epicarditis, multifocal, lymphoplasmacytic, mild (slides 1A, 3A)

5. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate (slide 2A)

6. Stomach: peritonitis, granulomatous, regionally diffuse, mild (slide 2A)

Final Comment: Two fish died of complications related to meningitis (fish #1) or hepatic necrosis (fish #2), but the other two fish do not have an obvious cause of death. However, brain was not included for examination from fish #s 3 and 4, and microscopic findings in these fish were similar to the nonbrain lesions in fish #1. Comments of specific lesions follow:

An unidentified organism, tentatively classified as a presporogonic myxosporean, is sporadically associated with meningitis in Atlantic salmon [I have seen it in Audit samples from areas 2.3, 3.3, and 3.2]. Organisms are most common in macrophages and ependymal cells. Organisms vary from 2.5 × 3 µm to 3 × 4 µm; they seem to be intracytoplasmic and unicellular, but some are either bi- or tri-nucleate or have deeply lobed nuclei. Parasites do not stain with Twort's Gram of ZN acid-fast stains. Some parasites seem to be surrounded by host nuclei, but I think this is just an artefact associated with the thickness of the section. Most of the parasites occur within macrophages, including melanophages, but some occur in ependymal cells. Inflammation is common and mostly granulomatous, but sometimes includes lymphocytes. Significant inflammation associated with the parasite is evidence that it is the cause of death in most affected fish. It has not been found in Pacific salmon.

Hepatocellular necrosis can be caused by inadequate vascular perfusion (e.g., as occurs with harmful algal blooms or hypoxia) or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Renibacterium salmoninarum* , or *Piscirickettsia salmonis*); the cause is not determined in most cases. 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. Hepatic necrosis is somewhat common in salmon that die in marine net pens, affecting 11% of the 645 Atlantic salmon and 6% of the 119 Pacific salmon examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program.

Because this parasite has not yet been described in Atlantic salmon, it is a new disease of unknown pathogenicity for wild salmon. Inflammation associated with this parasite might simply be an unusual manifestation of infection with a brain myxosporean common in Pacific salmon (e.g., *Myxobolus arcticus*), but this hypothesis needs to be confirmed with PCR.

To clarify this issue, I would like to request that brains be removed from several "fresh silvers" at this site, cut mid-sagittally, and submitted as follows for each fish [You will not be charged for these analysis.]:

1. half of the brain on ice (not fixed) for DNA analysis;

2. half of the brain fixed in 10% neutral buffered formalin for histopathology (I want to confirm that any PCR-positive fish also have lesions consistent with "presporogonic myxosporean").

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

Accumulation of eosinophilic granules in the cytoplasm of cells lining small vessels in the kidney is a distinctive finding in Atlantic salmon. These granules probably accumulate in response to systemic immune stimulation. The granules might be part of eosinophilic granular cells or endothelial cells; granule size seems too large and variable for eosinophilic granular cells. I have seen these granules associated with *Piscirickettsia* salmonis infection and with severe cerebral *Renibacterium salmoninarum* (in at least one case, the affected fish had no granulomatous inflammation in the kidney), but other cases (like this one) have no known cause. Renal eosinophilic granules have also been described in Atlantic salmon naturally infected with chronic pancreas disease in Norway (Salmonid alphavirus subtype 3, SAV3; McLoughlin and Graham 2007), but SAV3 has not been identified in BC salmon.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

The pattern of inflammation in the heart (slide 3A) is consistent with systemic immune stimulation; differentials include a bacterial or viral infection. Inflammatory cells lining the endocardial surface in foci of inflammation are rarely more than 2 cell layers thick. This pattern of inflammation has also been described with Heart and Skeletal Muscle Inflammation in Atlantic salmon reared in Europe, but this disease has not been identified in BC salmon.

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

Splenic and gastric peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe). Vacuoles probably are a result of vaccine material lost during tissue processing.

Literature Cited:

McLoughlin, M.F. and D.A. Graham. 2007. Alphavirus infections in salmonids ¿ a review. Journal of Fish Diseases. 30(9):511-531.

Histopathology

Case: 08-3362

Formalin-fixed tissues were submitted in 4 cassettes for histopathology. Gills were removed from the original cassette (A) and placed in a second cassette (B) before being immersed in decalcifier and then processing into paraffin.

Slide 1A (W170808) - brain, heart, liver (2 pieces), intestine, trunk kidney (2 pieces); special stains on sections from the same block include Twort's Gram and ZN acid-fast.

Slide 2A (W270808) - brain, spleen, liver (2 pieces), stomach (2 pieces), trunk kidney (2 pieces), intestinal ceca, mesenteric adipose tissue

Slide 3A (W270808) - heart (2 pieces), liver (2 pieces), stomach (2 pieces), intestine, trunk kidney (2 pieces)

Slide 4A (W270808) - heart, spleen, liver (2 pieces), head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide 1B (W170808), Slide 2B (W270808), Slide 3B (W270808) and Slide 4B (W270808) - gill

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

Quality control: Liver autolysis: mild (slide 2A), moderate (slides 1A, 3A), severe (slide 4A). Decalcification is complete. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: A Scouras on 09/04/08 @ 9:17 AM

Specimen	ID	Test	Result
Tissue	W270808-A	PCR - IHNV	Negative
Tissue	W270808-B	PCR - IHNV	Negative
Tissue	W270808-C	PCR - IHNV	Negative
Tissue	W270808-D	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: A Scouras on 09/04/08 @ 9:17 AM

Specimen	ID	Test	Result
Tissue	W270808-A	PCR - VHSV	Negative
Tissue	W270808-B	PCR - VHSV	Negative
Tissue	W270808-C	PCR - VHSV	Negative
Tissue	W270808-D	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 09/19/08 @ 2:55 PM

Specimen	ID	Isolate	Result	Level
Tissue	W270808-A		No viruses isolated	
Tissue	W270808-B		No viruses isolated	
Tissue	W270808-C		No viruses isolated	
Tissue	W270808-D		No viruses isolated	

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

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Final Report AHC Case: 08-3411

Last Updated: 09/25/08 2:52 PM
Pathologist: Gary D. Marty
Received Date: 09/03/08
Collected Date: 09/03/08
Client Ref No: 6824

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Diane Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for PCR for IHN and VHS.

Routine sampling of morts. One fish with erosion from net and bloat. Internally fish had hemorrhage on the swim bladder. Sample taken for PCR for IHN and VHS.

Client ID: 6824. Vaccinated. Saltwater entry 2007.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/05/08 @ 11:45 AM

Specimen	ID	Test	Result
Tissue	6824-1	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/05/08 @ 11:45 AM

Specimen	ID	Test	Result
Tissue	6824-1	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 09/25/08 @ 2:52 PM

Specimen	ID	Isolate	Result	Level
Tissue	6824-1		No viruses isolated	

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

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Final Report AHC Case: 08-3412

Last Updated: 09/25/08 2:53 PM
Pathologist: Gary D. Marty
Received Date: 09/03/08
Collected Date: 09/03/08
Client Ref No: 6822

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Diane Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for PCR for IHN and VHS.

One pooled sample from two non symptomatic fish taken from site with VHS. Bloated previously (ie AHC 2816 and 3070)

Client ID: 6822. Vaccinated. Saltwater entry 2008.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/05/08 @ 11:45 AM

Specimen	ID	Test	Result
Tissue	6822	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/05/08 @ 11:45 AM

Specimen	ID	Test	Result
Tissue	6822	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 09/25/08 @ 2:53 PM

Specimen	ID	Isolate	Result	Level
Tissue	6822		No viruses isolated	

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

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Final Report AHC Case: 08-3413

Last Updated: 09/25/08 2:53 PM
Pathologist: Gary D. Marty
Received Date: 09/03/08
Collected Date: 09/03/08
Client Ref No: 6837

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Diane Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for PCR for IHN and VHS.

8 samples for routine PCR and IHN and VHS. Samples collected during broodstock sort, no visible lesions noted.

Client ID: 6837. Saltwater entry 2005.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/05/08 @ 11:46 AM

Specimen	ID	Test	Result
Tissue	6837-1	PCR - IHNV	Negative
Tissue	6837-2	PCR - IHNV	Negative
Tissue	6837-3	PCR - IHNV	Negative
Tissue	6837-4	PCR - IHNV	Negative
Tissue	6837-5	PCR - IHNV	Negative
Tissue	6837-6	PCR - IHNV	Negative
Tissue	6837-7	PCR - IHNV	Negative
Tissue	6837-8	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/05/08 @ 11:46 AM

Specimen	ID	Test	Result
Tissue	6837-1	PCR - VHSV	Negative
Tissue	6837-2	PCR - VHSV	Negative
Tissue	6837-3	PCR - VHSV	Negative
Tissue	6837-4	PCR - VHSV	Negative
Tissue	6837-5	PCR - VHSV	Negative

Tissue	6837-6	PCR - VHSV	Negative
Tissue	6837-7	PCR - VHSV	Negative
Tissue	6837-8	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 09/25/08 @ 2:53 PM

Specimen	ID	Isolate	Result	Level
Tissue	6837-1		No viruses isolated	
Tissue	6837-2		No viruses isolated	
Tissue	6837-3		No viruses isolated	
Tissue	6837-4		No viruses isolated	
Tissue	6837-5		No viruses isolated	
Tissue	6837-6		No viruses isolated	
Tissue	6837-7		No viruses isolated	
Tissue	6837-8		No viruses isolated	

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

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Final Report AHC Case: 08-3414

Last Updated: 09/25/08 2:53 PM
Pathologist: Gary D. Marty
Received Date: 09/03/08
Collected Date: 09/03/08
Client Ref No: 6836

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Diane Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for PCR for IHN and VHS.

5 samples for routine PCR and IHN and VHS. Increase in mortality in pen 14. Samples taken from 10 of 160 dead fish. No signs of disease in sampled fish. DO's were low during feeding on previous day.

Client ID: 6836. Vaccinated. Saltwater entry 2007.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/05/08 @ 11:46 AM

Specimen	ID	Test	Result
Tissue	6836-1	PCR - IHNV	Negative
Tissue	6836-2	PCR - IHNV	Negative
Tissue	6836-3	PCR - IHNV	Negative
Tissue	6836-4	PCR - IHNV	Negative
Tissue	6836-5	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/05/08 @ 11:46 AM

Specimen	ID	Test	Result
Tissue	6836-1	PCR - VHSV	Negative
Tissue	6836-2	PCR - VHSV	Negative
Tissue	6836-3	PCR - VHSV	Negative
Tissue	6836-4	PCR - VHSV	Negative
Tissue	6836-5	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 09/25/08 @ 2:53 PM

Specimen	ID	Isolate	Result	Level
Tissue	6836-1		No viruses isolated	
Tissue	6836-2		No viruses isolated	
Tissue	6836-3		No viruses isolated	
Tissue	6836-4		No viruses isolated	
Tissue	6836-5		No viruses isolated	

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

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Final Report AHC Case: 08-3415

Last Updated: 09/25/08 2:53 PM
Pathologist: Gary D. Marty
Received Date: 09/03/08
Collected Date: 09/03/08
Client Ref No: 6838

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Diane Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for PCR for IHN and VHS.

3 samples from morts for PCR for IHN and VHS. Routine samples, NVL internally.

Client ID: 6838. Saltwater entry 2006.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/05/08 @ 11:46 AM

Specimen	ID	Test	Result
Tissue	6838-1	PCR - IHNV	Negative
Tissue	6838-2	PCR - IHNV	Negative
Tissue	6838-3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/05/08 @ 11:46 AM

Specimen	ID	Test	Result
Tissue	6838-1	PCR - VHSV	Negative
Tissue	6838-2	PCR - VHSV	Negative
Tissue	6838-3	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 09/25/08 @ 2:53 PM

Specimen	ID	Isolate	Result	Level
Tissue	6838-1		No viruses isolated	

Tissue	6838-2	No viruses isolated
Tissue	6838-3	No viruses isolated

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

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Final Report AHC Case: 08-3589

Last Updated: 10/09/08 4:20 PM
Pathologist: Gary D. Marty
Received Date: 09/17/08
Collected Date: 09/17/08
Client Ref No: 6852

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Diane Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for PCR for IHN and VHS.

Routine fish health visit. Site experiencing low DO's. Sampled one fish from pen 4 with hemorrhaging on the liver, rash on anterior belly and damage to right pectoral fin.

6852. 2206 S0, Vaccinated.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/19/08 @ 11:44 AM

Specimen	ID	Test	Result
Tissue	6852-1	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/19/08 @ 11:44 AM

Specimen	ID	Test	Result
Tissue	6852-1	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Cheryl Cecconi on 10/09/08 @ 4:20 PM

Specimen	ID	Isolate	Result	Level
Tissue	6852-1		No viruses isolated	

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

END OF REPORT

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Animal Health Centre

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Final Report AHC Case: 08-3590

Last Updated: 10/09/08 4:20 PM
Pathologist: Gary D. Marty
Received Date: 09/17/08
Collected Date: 09/17/08
Client Ref No: 6856

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Diane Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for PCR for IHN and VHS. Samples submitted for histology (5).

Routine fish health check on population prior to move. 3 pooled samples for PCR for IHN and VHS. Histo collected from five fish.

6856, 2008

Final Diagnosis

1. Liver: hepatocellular karyomegaly and megalocytosis, focal (2 cells, slide 4), multifocal (slide 5), mild

Final Comment: These are among the healthiest Atlantic salmon I have ever examined. They have only one lesion-- mild hepatocellular karyomegaly-- but this lesion is of no concern for moving the fish.

Hepatocellular karyomegaly has been described in pink salmon larvae (Marty et al. 1997); it is often associated with cytomegaly. Karyomegaly was thought to be related to osmotic shock during the first minutes to hours after fertilization, but this hypothesis has not been validated under controlled conditions. Hepatic megalocytosis can also result from exposure to several types of toxins, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. Based on the presence of abundant hepatocellular glycogen in affected fish, hepatocellular karyomegaly is probably of little significance to the health of these Atlantic salmon. The main concern is that if megalocytosis is not a result of something that happened near the time of fertilization, it is evidence of toxins in the water or feed.

Literature cited:

Marty, G.D., R. Heintz, and D.E. Hinton. 1997. Histology and teratology of pink salmon larvae near the time of emergence from gravel substrate in the laboratory. Can. J. Zool. 75:978-988.

Histopathology

Formalin-fixed tissues were submitted in 5 cassettes for histopathology.

Slide 1 (6856-1) - brain, liver, heart, spleen, trunk kidney, intestine, intestinal ceca and mesenteric adipose tissue

Slide 2 (6856-2) - brain, liver, heart, spleen, trunk kidney, stomach, intestine, intestinal ceca and mesenteric adipose tissue

Slide 3 (6856-3) - brain, liver, heart, spleen, trunk kidney, testis, intestine, intestinal ceca and mesenteric adipose tissue

Slide 4 (6856-4) - brain, liver, heart, spleen, head kidney, trunk kidney, stomach, intestine, intestinal ceca and mesenteric adipose tissue

Slide 5 (6856-5) - brain, liver, heart, spleen, head kidney, trunk kidney, intestine, intestinal ceca and mesenteric adipose tissue

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

Quality control: Tissue preservation is excellent for most organs; some sections of intestine have mild autolysis (common in immersion-fixed tissues). Organs have no postfixation dehydration and no acid hematin accumulation.

Measures of physiologic condition

Hepatocellular glycogen - abundant (slides 1, 2, 3, 4, 5)

Lipid in mesenteric adipose tissue - abundant (slides 1, 2, 3, 4, 5)

This pattern in the measures of physiologic condition is consistent with healthy growing fish.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/19/08 @ 11:45 AM

Specimen	ID	Test	Result
Tissue	6856-1	PCR - IHNV	Negative
Tissue	6856-2	PCR - IHNV	Negative
Tissue	6856-3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/19/08 @ 11:45 AM

Specimen	ID	Test	Result
Tissue	6856-1	PCR - VHSV	Negative
Tissue	6856-2	PCR - VHSV	Negative
Tissue	6856-3	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Cheryl Cecconi on 10/09/08 @ 4:20 PM

Specimen	ID	Isolate	Result	Level
Tissue	6856-1		No viruses isolated	
Tissue	6856-2		No viruses isolated	
Tissue	6856-3		No viruses isolated	

Gary D. Marty
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Final Report AHC Case: 08-3591

Last Updated: 10/09/08 4:19 PM
Pathologist: Gary D. Marty
Received Date: 09/17/08
Collected Date: 09/17/08
Client Ref No: 6861

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Brad Boyce**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for PCR for IHN and VHS, viral culture and histology.

Mass mortality from *Chatonella* bloom. Samples from moribund fish (8). This will be an insurance claim - rule out other pathogens and confirm damage caused by plankton.

6861. 2006 SO.

Final Diagnosis

Most significant lesions in these fish:

1. Liver: hepatic necrosis, acute, multifocal, moderate (slides 4A, 7A, 8A)

2. Gill: lamellar capillary thrombosis, multifocal, acute, mild (slides 2G, 3G, 4G, 5G, 6G, 7G, 8G), or moderate (slide 1G)

Final Comment: The most significant lesions in these fish--hepatocellular necrosis and gill lamellar thrombosis-- are consistent with the clinical history of morbidity due to exposure to algal toxins. Details for this case are included on an Excel spreadsheet (2008-3591.xls) that is not included with the official final report generated by the Animal Health Centre's VADDS database (the database cannot handle spreadsheets). Lesions scored on the spreadsheet are the same as those included in the histopathology part of the BC Fish Health Auditing and Surveillance Program. Therefore, the spreadsheet includes many lesions not present in this case. Specific comments on significant lesions in these fish follow:

Hepatocellular necrosis can be caused by inadequate vascular perfusion (e.g., as occurs with hypoxia or some types of harmful algal blooms) or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Renibacterium salmoninarum* , or *Piscirickettsia salmonis*) or aqueous toxins (e.g., other types of toxic algae blooms). 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. Hepatic necrosis is somewhat common in salmon that die in marine net pens, affecting 11% of the 645 Atlantic salmon and 6% of the 119 Pacific salmon examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program.

All fish had several gill lamellar capillaries that were obstructed by occlusive thrombi. Thrombosis in the gill is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection, or exposure to toxins from harmful algal blooms.

For comments on other lesions, see the "Abbreviations" worksheet in the spreadsheet.

Histopathology

Formalin-fixed tissues were submitted in 16 cassettes for histopathology. Cassettes containing gills ("G" cassettes) were immersed in Protocol B (hydrochloric acid solution) for 2 hours, rinsed in water, and processed routinely with other "A" cassettes into paraffin.

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

Quality control: Details are included on the spreadsheet (2008-3591.xls). Large foci of erythrocytes in the gill in slide 4G have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue, or with acid decalcification--most likely in this case); other sections have no acid hematin deposits. Tissues have no postfixation dehydration. Mild artifact is normal for paraffin-embedded sections.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/19/08 @ 11:45 AM

Specimen	ID	Test	Result
Tissue	6861-1	PCR - IHNV	Negative
Tissue	6861-2	PCR - IHNV	Negative
Tissue	6861-3	PCR - IHNV	Negative
Tissue	6861-4	PCR - IHNV	Negative
Tissue	6861-5	PCR - IHNV	Negative
Tissue	6861-6	PCR - IHNV	Negative
Tissue	6861-7	PCR - IHNV	Negative
Tissue	6861-8	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/19/08 @ 11:45 AM

Specimen	ID	Test	Result
Tissue	6861-1	PCR - VHSV	Negative
Tissue	6861-2	PCR - VHSV	Negative
Tissue	6861-3	PCR - VHSV	Negative
Tissue	6861-4	PCR - VHSV	Negative
Tissue	6861-5	PCR - VHSV	Negative
Tissue	6861-6	PCR - VHSV	Negative
Tissue	6861-7	PCR - VHSV	Negative
Tissue	6861-8	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Cheryl Cecconi on 10/09/08 @ 4:19 PM

Specimen	ID	Isolate	Result	Level
Tissue	6861-1		No viruses isolated	
Tissue	6861-2		No viruses isolated	
Tissue	6861-3		No viruses isolated	

Tissue	6861-4	No viruses isolated
Tissue	6861-5	No viruses isolated
Tissue	6861-6	No viruses isolated
Tissue	6861-7	No viruses isolated
Tissue	6861-8	No viruses isolated

Gary D. Marty
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END OF REPORT

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Final Report AHC Case: 08-3643

Last Updated: 10/14/08 3:02 PM
Pathologist: Gary D. Marty
Received Date: 09/19/08
Collected Date: 09/19/08
Client Ref No: 6868

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner:
Phone:
Fax:

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted salmon tissues for viral culture, PCR - IHNV, VHSV.

Still seeing moribund's in 2 pens. Samples taken from 1 fish with hem of pyloric & fat. No growth on blood agar or BA. Saltwater: 2006. DOD: Sept 16/ 08.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/22/08 @ 4:18 PM

Specimen	ID	Test	Result
Tissue	6868	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/22/08 @ 4:18 PM

Specimen	ID	Test	Result
Tissue	6868	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 10/14/08 @ 3:02 PM

Specimen	ID	Isolate	Result	Level
Tissue	6868		No viruses isolated	

Gary D. Marty
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Final Report AHC Case: 08-3644

Last Updated: 09/22/08 4:18 PM
Pathologist: Gary D. Marty
Received Date: 09/19/08
Collected Date: 09/19/08
Client Ref No: 6866

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest Canada**
Phone:
Fax:
Owner:
Phone:
Fax:

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted salmon tissues for PCR for IHN and VHS.

Routine site visit to sample fish showing signs of septicemia. DOD: Setp 15, 08. Saltwater entry 2006 S0.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/22/08 @ 4:18 PM

Specimen	ID	Test	Result
Tissue	6866-1	PCR - IHNV	Negative
Tissue	6866-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/22/08 @ 4:18 PM

Specimen	ID	Test	Result
Tissue	6866-1	PCR - VHSV	Negative
Tissue	6866-2	PCR - VHSV	Negative

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

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Final Report AHC Case: 08-3761

Last Updated: 10/22/08 4:09 PM
Pathologist: Gary D. Marty
Received Date: 09/30/08
Collected Date: 09/30/08
Client Ref No: 6884

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Diane Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

15 fresh dead fish sampled during routine fish health visit. All fish were a mix of culls, matures and silvers. Two fish had suspect BKD, all others had no signs of disease. 10 samples for PCR for IHN and VHS, samples for histology.

ID: 6884.

Final Diagnosis

1a. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 1)

1b. Liver: yellow-brown to yellow-green pigmented (lipofuscin and hemosiderin?) macrophage aggregates and sinusoidal macrophages, disseminated, mild (slides 1, 3, 4), moderate (slides 2, 5)

1c. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slides 1, 3), moderate (slide 5)

1d. Liver: hepatocellular intracytoplasmic spherical golden to amphophilic inclusions, acute, focal, small numbers (slide 1)

1e. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 2)

1f. Liver: sinusoidal congestion, multifocal, moderate (slide 3)

2. Liver, head kidney, trunk kidney, spleen: granulomatous inflammation, multifocal, moderate (slide 4)

3. Heart: myocardial karyomegaly, multifocal, mild (slides 1, 2, 3, 5)

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

5. Spleen: peritonitis, granulomatous, regionally diffuse, with occasional fine fibrocellular fronds, mild (slide 5), moderate (slide 4)

6. Intestinal ceca and mesenteric adipose tissue: peritonitis, granulomatous, focal (slide 5), multifocal (slide 4), with occasional fine fibrocellular fronds, moderate

Final Comment: These fish have several lesions that are fairly common in older fish reared in marine net pens in British Columbia. Because I do not know which fish are culls, matures, or silvers, I am not able comment on the relation of the microscopic lesions to clinical mortality classification. General comments on each lesion follow:

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 probably is lipofuscin, and it might also include hemosiderin. 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; it is more common in older fish. 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 might result from increased turnover of red blood cells.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These types of vacuoles were rare before 2007 and might be related to the significant increase in the proportion of plant-based components in commercial feeds that has occurred since 2006. The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown.

Golden to amphophilic cytoplasmic inclusions in hepatocytes vary from ½ to 2× 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).

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

Sinusoidal congestion in the liver is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.

Granulomas in several organs of the fish in slide 4 are evidence of persistent antigen. A vaccine reaction the most likely differential (no organisms are highlighted on the Twort's Gram stain).

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.1% of the 1142 Atlantic salmon hearts sampled as part of the province's Fish Health Auditing and Surveillance Program during 2006 and 2007). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. 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 might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Histopathology

Formalin-fixed tissues were submitted in 5 cassettes for histopathology.

Slide 1 (6884-29), Slide 2 (6884-21), Slide 3 (6884- 23), Slide 4 (6884-17) and Slide 5 - heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

Twort's Gram stain was done on a section from the same paraffin block as slide 4. All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Liver autolysis: none (slides 1, 4, 5), mild (slide 3), moderate (slide 2). Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/02/08 @ 10:48 AM

Specimen	ID	Test	Result
Tissue	6884-1	PCR - IHNV	Negative
Tissue	6884-2	PCR - IHNV	Negative
Tissue	6884-3	PCR - IHNV	Negative
Tissue	6884-4	PCR - IHNV	Negative
Tissue	6884-5	PCR - IHNV	Negative
Tissue	6884-6	PCR - IHNV	Negative
Tissue	6884-7	PCR - IHNV	Negative
Tissue	6884-8	PCR - IHNV	Negative
Tissue	6884-9	PCR - IHNV	Negative
Tissue	6884-10	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/02/08 @ 10:48 AM

Specimen	ID	Test	Result
Tissue	6884-1	PCR - VHSV	Negative
Tissue	6884-2	PCR - VHSV	Negative
Tissue	6884-3	PCR - VHSV	Negative
Tissue	6884-4	PCR - VHSV	Negative
Tissue	6884-5	PCR - VHSV	Negative
Tissue	6884-6	PCR - VHSV	Negative
Tissue	6884-7	PCR - VHSV	Negative
Tissue	6884-8	PCR - VHSV	Negative
Tissue	6884-9	PCR - VHSV	Negative
Tissue	6884-10	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Cheryl Cecconi on 10/22/08 @ 4:09 PM

Specimen	ID	Isolate	Result	Level
Tissue	6884-1		No viruses isolated	
Tissue	6884-2		No viruses isolated	
Tissue	6884-3		No viruses isolated	
Tissue	6884-4		No viruses isolated	
Tissue	6884-5		No viruses isolated	
Tissue	6884-6		No viruses isolated	
Tissue	6884-7		No viruses isolated	
Tissue	6884-8		No viruses isolated	
Tissue	6884-9		No viruses isolated	
Tissue	6884-10		No viruses isolated	

Gary D. Marty
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Final Report AHC Case: 08-3770

Last Updated: 10/02/08 3:08 PM
Pathologist: Gary D. Marty
Received Date: 09/30/08
Collected Date: 09/30/08
Client Ref No:

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Mainstram Canada**
Phone:
Fax:
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 3 cassettes for histology. Mouth rot-associated mortality on fish recovered by divers was 7,000 (22 Sep), 4,400 (23 Sep), and 12.500 (25 Sept.).

Final Diagnosis

Diagnoses:

1. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 2), moderate (slides 1, 3)

2. Brain: capillary (vascular) congestion, diffuse, mild (slide 3), moderate (slide 1)

3. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 2, 3)

4. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate (slide 3)

5. Intestinal ceca: peritonitis, granulomatous, focal, mild (slide 3)

Final Comment: Microscopic changes in these fish are consistent with the clinical history of fish that have been vaccinated and are suffering from mouthrot. The sections have no lesions that might have predisposed the fish to developing mouthrot. Comments on specific lesions follow:

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

Congestion of brain capillaries, including the meninges, is nonspecific evidence of circulating vasodilators. Capillary congestion is commonly associated with bacterial infections (e.g., mouthrot in smolts), but it is not common with VHSV. [The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section contain erythrocytes.]

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish

sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe). Vacuoles probably are a result of vaccine material lost during tissue processing.

Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology.

Slide 1 (Sept 23/08 Ross Fish 1) and Slide 3 (Sept 23/08 Ross Fish 3) - brain, heart, spleen, liver, intestinal ceca, head kidney, trunk kidney

Slide 2 (Sept 23/08 Ross Fish 2) - brain, heart, spleen, liver, trunk kidney, mesenteric adipose tissue

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

Quality control: Liver autolysis: moderate (slide 3), severe (slides 1, 2). Organs have no postfixation dehydration and no acid hematin deposits.

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

END OF REPORT

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Final Report AHC Case: 08-3785

Last Updated: 10/24/08 2:36 PM
Pathologist: Gary D. Marty
Received Date: 10/01/08
Collected Date: 10/01/08
Client Ref No: 6886

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Gerry Barry**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 10 samples (3 fish/pool) for IHN and VHS PCR and histology taken from 3 fish showing reddening in the pyloric caeca and lower intestine.

Routine site visit and sampling prior to moves. DO's low.

Final Diagnosis

1a. Liver: hepatic necrosis, acute, multifocal, moderate (slide 3)

1b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate (slide 1)

1c. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages (lipofuscin and hemosiderin?), disseminated, mild (slide 2), moderate (slide 3)

1d. Liver: sinusoidal congestion, multifocal, mild (slide 2)

2a. Brain: capillary (vascular) congestion, diffuse, mild (slide 2)

2b. Brain: meningoencephalitis, multifocal, lymphocytic, with gliosis, mild (slide 1)

3. Heart: myocardial karyomegaly, multifocal, mild (slide 1)

4. Mesenteric adipose tissue: capillary congestion, diffuse, moderate (slide 1)

5. Intestine: peritonitis, chronic, focal, with fibrocellular fronds, moderate(slide 3)

6. Trunk kidney: nephritis, interstitial, granulomatous, focal, moderate (slide 3)

7. Head kidney: nephritis, interstitial, granulomatous, bifocal, with a multinucleate giant cell, mild (slide 3)

Final Comment: These fish have a number of mild to moderate lesions, most of which are fairly common among older Atlantic salmon that die

in marine net pens in British Columbia, particularly when death is associated with chronic hypoxia.

Hepatocellular necrosis can be caused by inadequate vascular perfusion (e.g., as occurs with harmful algal blooms or hypoxia) or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Renibacterium salmoninarum* , or *Piscirickettsia salmonis*); the cause is not determined in most cases. The history in this case is consistent with hypoxia as the cause (the sections have no obvious organisms). Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Hepatic necrosis is somewhat common in salmon that die in marine net pens, affecting 11% of the 645 Atlantic salmon examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These types of vacuoles were rare before 2007 and might be related to the significant increase in the proportion of plant-based components in commercial feeds that has occurred since 2006. The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown.

Pigment in the liver probably is lipofuscin, and it might also include hemosiderin. The liver in slide 2 has a unusually large focus of pigmented macrophages (400 × 200 µm). 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; it is more common in older fish. 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 might result from increased turnover of red blood cells.

Vascular congestion in the liver and brain is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV.

Meningoencephalitis is evidence of immune stimulation. Lymphocytic inflammation with gliosis is most consistent with a virus, but consider bacteria as a possible differential. Meningoencephalitis of unknown cause occurs occasionally in fish sampled as part of the BC Fish Health Auditing and Surveillance Program, affecting 7% of the 644 Atlantic salmon examined in 2007 (5.9% were mild, and 1.1% were moderate or severe).

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.1% of the 1142 Atlantic salmon hearts sampled as part of the province's Fish Health Auditing and Surveillance Program during 2006 and 2007). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Distension of capillaries in the mesenteric adipose tissue is often part of the inflammatory response to many infectious diseases. In British Columbia, mesenteric congestion and hemorrhage is most commonly associated with VHSV and bacterial infections, and sometimes it seems to be associated with a vaccine reaction.

Intestinal peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Slide 3 has a single focus of granulomatous inflammation that is ~700 µm in diameter. Many of the macrophages contain anisomorphic eosinophilic granules, but no evidence of infectious organisms. The primary differential is a vaccine reaction. Among infectious causes, the most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease; chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.

Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology.

Slide 1 (6886-1) and Slide 2 (6886-2) - brain, heart, liver, intestine, head kidney, trunk kidney, mesenteric adipose tissue

Slide 3 (6886-3) - brain, heart liver, intestine, head kidney, trunk kidney (2 pieces), mesenteric adipose tissue

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

Quality control: Liver autolysis: mild (slides 1, 2, 3). Intestinal autolysis: moderate (slide 2), severe (slides 1, 3). Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/03/08 @ 3:00 PM

Specimen	ID	Test	Result
Tissue	6886-1	PCR - IHNV	Negative
Tissue	6886-2	PCR - IHNV	Negative
Tissue	6886-3	PCR - IHNV	Negative
Tissue	6886-4	PCR - IHNV	Negative
Tissue	6886-5	PCR - IHNV	Negative
Tissue	6886-6	PCR - IHNV	Negative
Tissue	6886-7	PCR - IHNV	Negative
Tissue	6886-8	PCR - IHNV	Negative
Tissue	6886-9	PCR - IHNV	Negative
Tissue	6886-10	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/03/08 @ 3:00 PM

Specimen	ID	Test	Result
Tissue	6886-1	PCR - VHSV	Negative
Tissue	6886-2	PCR - VHSV	Negative
Tissue	6886-3	PCR - VHSV	Negative
Tissue	6886-4	PCR - VHSV	Negative
Tissue	6886-5	PCR - VHSV	Negative
Tissue	6886-6	PCR - VHSV	Negative
Tissue	6886-7	PCR - VHSV	Negative
Tissue	6886-8	PCR - VHSV	Negative
Tissue	6886-9	PCR - VHSV	Negative
Tissue	6886-10	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 10/24/08 @ 2:36 PM

Specimen	ID	Isolate	Result	Level
Tissue	6886-1		No viruses isolated	
Tissue	6886-2		No viruses isolated	
Tissue	6886-3		No viruses isolated	
Tissue	6886-4		No viruses isolated	
Tissue	6886-5		No viruses isolated	
Tissue	6886-6		No viruses isolated	
Tissue	6886-7		No viruses isolated	
Tissue	6886-8		No viruses isolated	
Tissue	6886-9		No viruses isolated	
Tissue	6886-10		No viruses isolated	

Gary D. Marty
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Final Report AHC Case: 08-3790

Last Updated: 10/06/08 1:39 PM
Pathologist: Gary D. Marty
Received Date: 10/01/08
Collected Date: 10/01/08
Client Ref No:

Veterinarian: **Barry Milligan**
Clinic: **Grieg Seafoods BC Ltd.**
Phone: (250) 286-0838
Fax: (250) 286-1883

Submitter: **Jeanine Sumner**
Phone:
Fax:
Owner: **Grieg Seafoods BC Ltd.**
Phone: (250) 286-0838
Fax:(250) 286-1883

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted samples for histology. Increased mortality in brood. Possible water quality issue.

Final Diagnosis

Most significant lesions in these fish:

1. Liver: hepatic necrosis, acute, multifocal, mild (slides 3-1, 5-1), moderate (slides 1-1, 2-1, 6-1)

2. Mesenteric adipose tissue: capillary congestion, diffuse, moderate (slide GRB-1)

3. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, mild (slide GRB-2)

4. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, mild (slides GRB-3, 4-1A)

5. Gill: lamellar capillary thrombosis, multifocal, mild (slide 8-G)

6. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages (lipofuscin and hemosiderin?), disseminated, mild (slide 7-1)

Final Comment: Among the many "most significant lesions" in these fish, only moderate hepatocellular necrosis (slides 1-1, 2-1, and 6-1) is of sufficient severity to be considered a cause of death. Other "most significant lesions" provide clues to causes of morbidity. Details for this case are included on an Excel spreadsheet (2008-3790.xls) that is not included with the official final report generated by the Animal Health Centre's VADDS database (the database cannot handle spreadsheets). Most of the lesions scored on the spreadsheet are the same as those included in the histopathology part of the BC Fish Health Auditing and Surveillance Program. Therefore, the spreadsheet includes many lesions not present in this case. Specific comments on the most significant lesions in each fish follow (in alphabetical order):

GTH = Gill thrombosis. Blood clots in the vascular spaces of the gill are evidence of increased coagulability. GTH can result from endothelial damage related to virus (e.g., VHSV), bacterial, or parasitic infection, or exposure to toxins.

KGR = kidney, granulomatous inflammation. The most common causes of granulomatous inflammation in the kidney of farmed Atlantic salmon include a vaccine reaction and infection with *Renibacterium salmoninarum*, the cause of bacterial kidney disease. Chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation. Mild cases are probably not significant for the health the affected fish.

MCC = mesenteric capillary congestion. Distension of capillaries in the mesenteric adipose tissue is often part of the inflammatory response to many infectious diseases; hemorrhage sometimes occurs in severe cases. In British Columbia, mesenteric congestion and hemorrhage is most commonly associated with VHSV and bacterial infections, and sometimes it seems to be associated with a vaccine reaction.

LFN = Hepatocellular necrosis. Hepatocellular necrosis can be caused by inadequate vascular perfusion (e.g., as occurs with hypoxia or some types of harmful algal blooms) or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Renibacterium salmoninarum*, or *Piscirickettsia salmonis*) or aqueous toxins (e.g., other types of harmful algae blooms). This case has no obvious organisms (including on the Twort's Gram stain of slide 2-1). Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Hepatic necrosis is somewhat common in salmon that die in marine net pens, affecting 11% of the 645 Atlantic salmon and 6% of the 119 Pacific salmon examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program.

PMP = Pigmented macrophages (liver). Pigment in the liver probably is lipofuscin, and it might also include hemosiderin. 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; it is more common in older fish. 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 might result from increased turnover of red blood cells.

RTN = Renal tubular epithelial necrosis. Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Renal tubular epithelial necrosis was fairly common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 8.4%; n = 643) and Pacific salmon (prevalence = 4.2%; n = 120); the cause was not determined in many cases. Differentials include viral hemorrhagic septicemia virus (VHSV) and exposure to toxins (e.g., bacterial toxins, algal toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin). Consider bacteriology and virology, if not already done.

For comments on other scored lesions, see the "Abbreviations" worksheet in the spreadsheet.

Histopathology

Formalin-fixed tissues from Atlantic salmon were received for histopathology.

Slide GRB-1, Slide 1-1, Slide 2-1, Slide 3-1, Slide 4- 1, Slide 6-1, Slide 7-1 and Slide 8-1 - spleen, liver, intestine, trunk kidney, mesenteric adipose tissue

Slide GRB-2 and Slide GRB-3 - gill, spleen, liver, intestine, trunk kidney, mesenteric adipose tissue

Slide 5-1 - spleen, liver, intestine, mesenteric adipose tissue

Slide GRB-1G, Slide 1-G, Slide 2-G, Slide 3-G, Slide 4-1B, Slide 4-G, Slide 5-G, Slide 6-G, Slide 7-G and Slide 8-G - gill

All organs on each slide were examined. A Twort's Gram stain was done on a section from the same paraffin block as slide 2- 1. Organs not listed elsewhere have no significant lesions.

Quality control: Details are included on the spreadsheet (2008-3790.xls). The gills were not decalcified; however, because only the tips of filaments were submitted, most gill sections have very little (if any) mineral, and section quality is not significantly impaired. Tissues have no postfixation dehydration and no acid hematin deposits. Mild artifact is normal for paraffin- embedded sections.

Gary D. Marty

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Final Report AHC Case: 08-3797

Last Updated: 10/09/08 12:33 PM
Pathologist: Gary D. Marty
Received Date: 10/01/08
Collected Date: 09/29/08
Client Ref No:

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Zarah Vansnick**
Phone:
Fax:
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted tissues for PCR and a plate for bacteriology.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 10/09/08 @ 11:15 AM

Specimen	ID	Isolate	Result	Level
Isolate	Pen 2 (1,2,3,4,5)		No Bacteria Isolated	
Isolate	Pen 107 (1)	Vibrio sp.	Positive	
**: Identification based on DNA sequencing Sensitivities unable to be performed due to poor growth of isolate				
Isolate	Pen 107 (2)		No Bacteria Isolated	
Isolate	Pen 107 (3)		No Bacteria Isolated	
Isolate	Pen 107 (4)	Vibrio lentus	Positive	
**: Sensitivities unable to be performed due to poor growth of isolate				
Isolate	Pen 107 (4)	Vibrio sp.	Positive	
Isolate	Pen 107 (5)		No Bacteria Isolated	

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/03/08 @ 3:00 PM

Specimen	ID	Test	Result
Tissue	A Fish 1 Pen 102	PCR - IHNV	Negative
Tissue	B Fish 2 Pen 102	PCR - IHNV	Negative
Tissue	C Fish 3 Pen 102	PCR - IHNV	Negative
Tissue	D Fish 4 Pen 102	PCR - IHNV	Negative
Tissue	E Fish 5 Pen 102	PCR - IHNV	Negative

Tissue	F Fish 6 Pen 107	PCR - IHNV	Negative
Tissue	G Fish 7 Pen 107	PCR - IHNV	Negative
Tissue	H Fish 8 Pen 107	PCR - IHNV	Negative
Tissue	I Fish 9 Pen 107	PCR - IHNV	Negative
Tissue	J Fish 10 Pen 107	PCR - IHNV	Negative
Tissue	K Fish 14 Pen 104	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/03/08 @ 3:00 PM

Specimen	ID	Test	Result
Tissue	A Fish 1 Pen 102	PCR - VHSV	Negative
Tissue	B Fish 2 Pen 102	PCR - VHSV	Negative
Tissue	C Fish 3 Pen 102	PCR - VHSV	Negative
Tissue	D Fish 4 Pen 102	PCR - VHSV	Negative
Tissue	E Fish 5 Pen 102	PCR - VHSV	Negative
Tissue	F Fish 6 Pen 107	PCR - VHSV	Negative
Tissue	G Fish 7 Pen 107	PCR - VHSV	Negative
Tissue	H Fish 8 Pen 107	PCR - VHSV	Negative
Tissue	I Fish 9 Pen 107	PCR - VHSV	Negative
Tissue	J Fish 10 Pen 107	PCR - VHSV	Negative
Tissue	K Fish 14 Pen 104	PCR - VHSV	Negative

Gary D. Marty
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Final Report AHC Case: 08-3831

Last Updated: 10/08/08 3:16 PM
Pathologist: Gary D. Marty
Received Date: 10/06/08
Collected Date: 10/06/08
Client Ref No: D096

Veterinarian:
Clinic:
Phone:
Fax:

Submitter: **BC Centre for Aquatic Health**
Phone:
Fax:
Owner: **BC Centre Aquatic Hth**
Phone:
Fax:(250) 286-6103

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted one plate with 2 fish for bacti id and sensitivity, 4 cassettes for histology, and 4 whirl paks for PCR.

D-096

Final Diagnosis

1. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute (viral hemorrhagic septicemia virus, VHSV), mild (slide 2), moderate (slides 1, 3)

2a. Liver: sinusoidal congestion, focal, moderate (slide 2)

2b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 3, 4), moderate (slide 1)

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1)

Final Comment: These fish have lesions consistent with viral hemorrhagic septicemia virus, VHSV. Some stains of *Vibrio splendidus* kill oysters and larval fish under controlled laboratory conditions (Le Roux et al. 2002, Thomson et al. 2005), but *Vibrio splendidus* has not been shown to kill salmonids. *Pseudomonas fragi* is not considered a salmonid pathogen.

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Renal tubular epithelial necrosis was fairly common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 8.4%; n = 643) and Pacific salmon (prevalence = 4.2%; n = 120); the cause was not determined in many cases. This case is probably associated with viral hemorrhagic septicemia virus (tissues are PCR+ for VHSV). General differentials include exposure to toxins (e.g., bacterial toxins, algal toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin).

Sinusoidal congestion in the liver is evidence of circulating vasodilators. This case is probably associated with VHSV. General differentials include substances released from inflammatory cells or bacteria. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.

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

with ulcers.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Literature cited:

Le Roux, F., M. Gay, C. Lambert, M. Waechter, S. Poubalanne, B. Chollet, J.L. Nicolas, and F. Berthe. 2002. Comparative analysis of *Vibrio splendidus* -related strains isolated during *Crassostrea gigas* mortality events. Aquat. Living Resour. 15:251¿258.

Thomson, R., Macpherson, H.L., Riaza, A., and T.H. Birkbeck, 2005. *Vibrio splendidus* biotype 1 as a cause of mortalities in hatchery-reared larval turbot, *Scophthalmus maximus* (L.). Journal of Applied Microbiology [J. Appl. Microbiol.] 99(2):243- 250.

Histopathology

Formalin -fixed tissues were submitted in 4 cassettes for histopathology.

Slides 1 (WB #1), 2 (WB #2), 3 (WB #3) and 4 (WB #4) - spleen, liver, trunk kidney

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

Quality control: Liver autolysis: moderate (slide 1), severe (slides 2, 3, 4). Organs have no postfixation dehydration and no acid hematin deposits.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 10/08/08 @ 10:44 AM

Specimen	ID	Isolate	Result	Level
Isolate	Fish 1	Pseudomonas sp.	Positive	
**: Pseudomonas identified as Pseudomonas fragi				
Isolate	Fish 1	Vibrio splendidus	Positive	
Isolate	Fish 2	Pseudomonas sp.	Positive	
**: Pseudomonas identified as Pseudomonas fragi				
Isolate	Fish 2	Vibrio splendidus	Positive	

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 10/08/08 @ 10:44 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Pseudomonas sp.	Fish 1	r	r	r	s	r	s
Vibrio splendidus	Fish 1	r	s	s	r	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline							

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 10/08/08 @ 10:15 AM

Specimen	ID	Test	Result

Tissue	organs#1	PCR - IHNV	Negative
Tissue	organs#2	PCR - IHNV	Negative
Tissue	organs#3	PCR - IHNV	Negative
Tissue	organs#4	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 10/08/08 @ 9:16 AM

Specimen	ID	Test	Result
Tissue	organs#1	PCR - VHSV	Positive
Tissue	organs#2	PCR - VHSV	Positive
Tissue	organs#3	PCR - VHSV	Positive
Tissue	organs#4	PCR - VHSV	Positive

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

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Final Report AHC Case: 08-3839

Last Updated: 10/08/08 2:29 PM
Pathologist: Gary D. Marty
Received Date: 10/06/08
Collected Date: 09/29/08
Client Ref No: PO 12133

Veterinarian: **Dr. Peter McKenzie**
Clinic: **Mainstream Canada-T**
Phone: (250) 725-1255
Fax: (250) 725-1250

Submitter: **Mainstream Canada**
Phone:
Fax:
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted formalized tissue for histology (sampled on 29 Sept 2008).

Duration of illness: 14 days. Saltwater. Vaccinated.

PO# 12133

Final Diagnosis

1a. Gill: filament necrosis, with abundant filamentous bacteria and moderate numbers of intralesional diatom cell bodies and spines/setae (*Chaetoceros sp.*?), multifocal, severe (slides 9B, 14B)

1b. Gill: lamellar necrosis, with filamentous bacteria and intralesional diatom spines/setae (*Chaetoceros sp.*?), multifocal, mild (slide 11), moderate (slide 10B)

1c. Gill: lamellar epithelial hyperplasia and fusion, multifocal, with intralesional diatom spines/setae and filamentous bacteria, moderate (slides 12, 13)

2a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slides 9A, 10A, 14A)

2b. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 10A)

3a. Trunk kidney: renal tubular epithelial necrosis, with luminal dilation, focal, acute, mild (slide 10A)

3b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 14A), moderate (slide 9A)

4. Brain: capillary (vascular) congestion, diffuse, moderate (slide 14A)

5. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 10A, 14A)

6. Intestinal ceca: peritonitis, granulomatous, multifocal, mild (slide 14A)

Final Comment: Microscopic changes in these fish are consistent with vaccinated fish that are suffering from harmful algae and associated filamentous bacteria in the gills. The previous case from this site (2008-3770) did not include gill; therefore, analysis for the presence of harmful algae could not be done on that case. Comments on specific lesions follow:

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, in marine waters *Tenacibaculum maritimum* (one cause of necrotizing branchitis) is most common. Infections are usually associated with crowding, poor water quality, or stress. In slide 14B, the epithelium of the gill rakers is also ulcerated and covered by filamentous bacteria.

The diatoms *Chaetoceros concavicornis*, *C. convolutus*, and a *Corethron sp*. have been associated with mortality of salmon reared in seawater netpens at numbers as low as 5/mL (Taylor and Harrison 2002). The space between the base of gill filaments often contain moderate numbers of unstained structures that are consistent with the diatom *Chaetoceros*. Chains of cell bodies are about 25 µm in diameter (none in section 10B), and individual spines/setae are about 3.5 µm in diameter. Mortality due to *Chaetoceros* can occur within a few days of exposure, as a result of physical damage to the gills and the resultant inflammatory response. Affected fish are also more vulnerable to *Vibrio* infections. Larger fish tend to be more susceptible than small fish. Characteristic gill lesions (e.g., slide 12) include mucous cell hyperplasia, lamellar epithelial cell hyperplasia and necrosis, and variable numbers of neutrophils. Diatoms and their processes are on the surface of the gill epithelium and sometimes entrapped by multinucleate giant cells. [Source: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.] Lesions due to *Corethron* sp. are similar (Spear et al. 1989).

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

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.

Congestion of brain capillaries, including the meninges, is nonspecific evidence of circulating vasodilators. Capillary congestion is commonly associated with bacterial infections (e.g., mouthrot in smolts), but it is not common with VHSV. [The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section contain erythrocytes.]

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Renal tubular epithelial necrosis was fairly common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 8.4%; n = 643) and Pacific salmon (prevalence = 4.2%; n = 120); the cause was not determined in many cases. Differentials include viral hemorrhagic septicemia virus (VHSV) and exposure to toxins (e.g., bacterial toxins, algal toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin). In this case, the toxins could be coming from the filamentous bacteria on the gill.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). In wild juvenile pink salmon sampled in March 2008, they did not occur in fish sampled from fresh water (n = 20), but they occurred in half the fish sampled from salt water (n = 45): evidence of stress associated with the transition from fresh to salt water. Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Literature cited:

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

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

Histopathology

Formalin-fixed tissues were submitted in 6 cassettes for histopathology. After processing into paraffin, gills were removed from cassettes 9A, 10A and 14A and placed in separate (B) cassettes. The notes written by the client on the cassettes came off during processing into paraffin.

Slide 9A - brain, liver, intestinal ceca, trunk kidney, and mesenteric adipose tissue

Slide 10A - brain, spleen, liver, intestinal ceca, trunk kidney

Slide 14A - brain, spleen, liver, intestinal ceca, trunk kidney, mesenteric adipose tissue

Slide 9B, 10B, 11, 12, 13 and 14B - gill

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

Quality control: Liver autolysis: mild (slide 10A), moderate (slide 9A), severe (slide 14A). Gill autolysis: mild (slides 10B, 11, 12, 13), moderate (slide 9B), severe (slide 14B). Organs have no postfixation dehydration and no acid hematin deposits.

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

END OF REPORT

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Final Report AHC Case: 08-3859

Last Updated: 10/09/08 12:08 PM
Pathologist: Gary D. Marty
Received Date: 10/07/08
Collected Date: 10/07/08
Client Ref No: 6898

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Diane Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 5 fresh salmon tissues for PCR for IHN and VHS.

Vaccinated. Saltwater yr: 2008, S1. Routine site visit. DOD: Oct 1, 08. Ref# 6898.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 10/09/08 @ 12:07 PM

Specimen	ID	Test	Result
Tissue	#1	PCR - IHNV	Negative
Tissue	#2	PCR - IHNV	Negative
Tissue	#3	PCR - IHNV	Negative
Tissue	#4	PCR - IHNV	Negative
Tissue	#5	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 10/09/08 @ 12:08 PM

Specimen	ID	Test	Result
Tissue	#1	PCR - VHSV	Positive
Tissue	#2	PCR - VHSV	Positive
Tissue	#3	PCR - VHSV	Positive
Tissue	#4	PCR - VHSV	Positive
Tissue	#5	PCR - VHSV	Positive

Gary D. Marty

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

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Final Report AHC Case: 08-3860

Last Updated: 10/09/08 12:08 PM
Pathologist: Gary D. Marty
Received Date: 10/07/08
Collected Date: 10/07/08
Client Ref No: 6897

Veterinarian: **Diane Morrison**
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Fax: (250) 850-3275

Submitter: **Diane Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh salmon tissues for PCR for IHN and VHS.

Routine site visit. DOD: Oct 1, 08. Vaccinated. Saltwater yr: 2008, S1. Ref# 6897.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 10/09/08 @ 12:08 PM

Specimen	ID	Test	Result
Tissue	#1	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 10/09/08 @ 12:08 PM

Specimen	ID	Test	Result
Tissue	#1	PCR - VHSV	Negative

Gary D. Marty
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Final Report AHC Case: 08-3861

Last Updated: 10/09/08 4:27 PM
Pathologist: Gary D. Marty
Received Date: 10/07/08
Collected Date: 10/02/08
Client Ref No: 6895

Veterinarian: **Diane Morrison**
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Submitter: **Diane Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 10 salmon tissues for PCR for IHN and VHS and 5 for histo.

General health on fish prior to movement to new site. Histo #1 - heart deformity. #18 - n.v.l. #20 - dark liver, reddened p.c. #21- n.v.l. #22 - white deposits in liver and heart.

Fresh morts. Vaccinated. Saltwater yr: 2007, S1. Routine site visit. DOD: Oct 2, 08. Ref# 6895.

Final Diagnosis

1a. Liver: hepatic necrosis, acute, focal, mild (slide 2); multifocal, mild (slide 3)

1b. Liver: sinusoidal congestion, multifocal, mild (slides 1, 2, 3)

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

1d. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 5), moderate (slides 1, 4)

1e. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 1)

1f. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slide 3)

1g. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages (lipofuscin and hemosiderin?), disseminated, mild (slides 4, 5)

1h. Liver: granulomatous hepatitis and hepatic necrosis, multifocal, with intralesional short bacilli characteristic of *Renibacterium salmoninarum*, severe (slide 5)

2a. Brain: meningoencephalitis, granulomatous, multifocal, with intralesional short bacilli characteristic of *Renibacterium salmoninarum*, severe (slide 5)

2b. Brain: capillary (vascular) congestion, diffuse, mild (slide 3), moderate (slides 1, 2, 4)

2c. Brain: meningeal and neuropil hemorrhage, multifocal, mild (slide 1)

3. Spleen and surrounding mesenteric adipose tissue: peritonitis, lymphocytic, focal, with fibrocellular fronds, mild (slide 1)

4. Intestine: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 4)

5a. Heart: epicarditis and myocarditis, multifocal, granulomatous (probable *Renibacterium salmoninarum* infection, moderate (slide 5)

5b. Heart: epicarditis, focal, lymphoplasmacytic, mild (slide 2)

6. Head kidney: interstitial vascular congestion, diffuse, mild (slide 4)

7. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate (slide 5)

Final Comment: Among the many lesions in these fish, the only ones that I can be fairly confident killed the fish were those associated with *Renibacterium salmoninarum* infection in the brain, heart, and liver of slide 5. Comments on other lesions follow:

Hepatocellular necrosis can be caused by inadequate vascular perfusion (e.g., as occurs with harmful algal blooms or hypoxia) or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Renibacterium salmoninarum* , or *Piscirickettsia salmonis*); the cause is not determined in most cases (all fish tested from this case are VHSV-negative by PCR). 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. Hepatic necrosis is somewhat common in salmon that die in marine net pens, affecting 11% of the 645 Atlantic salmon and 6% of the 119 Pacific salmon examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program.

Sinusoidal congestion in the liver is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.

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 fish it might be related to increased protein needed as part of an inflammatory response. It is common in juvenile fish with ulcers.

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 cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These types of vacuoles were rare before 2007 and might be related to the significant increase in the proportion of plant-based components in commercial feeds that has occurred since 2006. The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown.

Pigment in the liver probably is lipofuscin, and it might also include hemosiderin. 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; it is more common in older fish. 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 might result from increased turnover of red blood cells.

Congestion of brain capillaries, including the meninges, is nonspecific evidence of circulating vasodilators or a mass-occupying intracranial lesion; hemorrhage sometimes occurs in severe cases. Capillary congestion can be associated with bacterial infections (e.g., mouthrot in smolts), but it also results when venous return is blocked (e.g., with thrombi and massive intracranial hemorrhage or inflammation). Congestion of brain capillaries is not common with VHSV. [The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section contain erythrocytes.]

The most common cause of meningeal hemorrhage is trauma, but the small foci of hemorrhage in the neuropil of this fish might be a result of vascular damage (e.g., from a bacterial or viral infection). Trauma often results from fish running into something, including into other fish, and increased activity that leads to brain trauma might be associated with sea lice infestation, avoidance of predators, or stray voltage.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

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

Renal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). In fish #5, it might be related to concurrent *Renibacterium salmoninarum* infection. In wild juvenile pink salmon sampled in March 2008, they did not occur in fish sampled from fresh water (n = 20), but they occurred in half the fish sampled from salt water (n = 45): evidence of stress associated with the transition from fresh to salt water. Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

Histopathology

Formalin-fixed tissues were submitted in 5 cassettes for histopathology.

Slide 1 (6895-1), 2 (6895-2), 4 (6895-4) and 5(6895-5) ¿ brain, heart, spleen, liver, intestine/intestinal ceca, head kidney, trunk kidney (2 pieces) mesenteric adipose tissue

Slide 3 (6895-1) ¿ brain, heart, liver, intestine/intestinal ceca, head kidney, trunk kidney (2 pieces), mesenteric adipose tissue

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

Quality control: Liver autolysis: mild (slides 4, 5), moderate (slides 1, 2, 3). Large foci of erythrocytes in the spleen of slide 5 have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation. Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 10/09/08 @ 12:08 PM

Specimen	ID	Test	Result
Tissue	#1	PCR - IHNV	Negative
Tissue	#2	PCR - IHNV	Negative
Tissue	#3	PCR - IHNV	Negative
Tissue	#4	PCR - IHNV	Negative
Tissue	#5	PCR - IHNV	Negative
Tissue	#6	PCR - IHNV	Negative
Tissue	#7	PCR - IHNV	Negative
Tissue	#8	PCR - IHNV	Negative
Tissue	#9	PCR - IHNV	Negative
Tissue	#10	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 10/09/08 @ 12:08 PM

Specimen	ID	Test	Result
Tissue	#1	PCR - VHSV	Negative
Tissue	#2	PCR - VHSV	Negative
Tissue	#3	PCR - VHSV	Negative
Tissue	#4	PCR - VHSV	Negative
Tissue	#5	PCR - VHSV	Negative

Tissue	#6	PCR - VHSV	Negative
Tissue	#7	PCR - VHSV	Negative
Tissue	#8	PCR - VHSV	Negative
Tissue	#9	PCR - VHSV	Negative
Tissue	#10	PCR - VHSV	Negative

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Final Report AHC Case: 08-3862

Last Updated: 10/10/08 10:07 AM
Pathologist: Gary D. Marty
Received Date: 10/07/08
Collected Date: 10/07/08
Client Ref No: 6894

Veterinarian: **Diane Morrison**
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Fax: (250) 850-3275

Submitter: **Diane Morrison**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 2 salmon tissues for PCR for IHN and VHS and histo.

Fish health check prior to moving fish. Histo collected from 1 fresh dead fish. Minimal mortality at site except for one pen with predator mortality. Bacteriology from 6 fish resulted in no significant growth.

Vaccinated. Saltwater yr: 2007, S1. DOD: Oct 2, 08. Ref# 6894.

Final Diagnosis

1a. Liver: hepatic necrosis, acute, multifocal, severe

1b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate

Final Comment: This fish probably died of complications related to hepatic necrosis. Hepatocellular necrosis can be caused by inadequate vascular perfusion (e.g., as occurs with harmful algal blooms or hypoxia) or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Renibacterium salmoninarum*, or *Piscirickettsia salmonis*); the cause is not determined in most cases. 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. Hepatic necrosis is somewhat common in salmon that die in marine net pens, affecting 11% of the 645 Atlantic salmon and 6% of the 119 Pacific salmon examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These types of vacuoles were rare before 2007 and might be related to the significant increase in the proportion of plant-based components in commercial feeds that has occurred since 2006. The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown.

Histopathology

Formalin-fixed tissues were submitted in 1 cassette for histopathology.

Slide 1 (6894-1) - brain, heart, spleen, liver, intestine, head kidney, trunk kidney (2 pieces), mesenteric adipose tissue

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

Quality control: Liver autolysis: mild. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 10/10/08 @ 9:44 AM

Specimen	ID	Test	Result
Tissue	#1	PCR - IHNV	Negative
Tissue	#2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 10/10/08 @ 9:44 AM

Specimen	ID	Test	Result
Tissue	#1	PCR - VHSV	Negative
Tissue	#2	PCR - VHSV	Negative

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

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Final Report AHC Case: 08-3863

Last Updated: 10/10/08 11:05 AM
Pathologist: Gary D. Marty
Received Date: 10/07/08
Collected Date: 10/07/08
Client Ref No: 6892

Veterinarian: **Diane Morrison**
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Submitter: **Tiffany MacWilliam**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 10 fresh salmon tissues for PCR for IHN and VHS and histo.

Routine health check prior to moving fish. Some low D.O. mortality. Histo taken from 5 fresh dead - no visible lesions noted.

Vaccinated. Saltwater yr: 2007, S1. Ref# 6892.

Final Diagnosis

1a. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 1)

1b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slides 1, 2, 5)

1c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1, 2, 5)

1d. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages (lipofuscin and hemosiderin?), disseminated, mild (slides 2, 5)

1e. Liver: sinusoidal congestion, multifocal, mild (slide 5)

2a. Brain: hemorrhage, focal, mild (slide 1)

2b. Brain: capillary (vascular) congestion, diffuse, mild (slides 3, 5)

3. Heart: myocardial karyomegaly, multifocal, mild (slide 3)

4. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 5), moderate (slide 4)

5. Mesenteric adipose tissue: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 5)

Final Comment: These fish have several lesions that fairly common among Atlantic salmon that die in marine net pens in British Columbia. However, none of the lesions are of sufficient severity to explain the death of the affected fish. Comments on specific lesions follow:

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 cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These types of vacuoles were rare before 2007 and might be related to the significant increase in the proportion of plant-based components in commercial feeds that has occurred since 2006. The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown.

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

The most common cause of brain hemorrhage is trauma, but the small foci of hemorrhage in the neuropil of this fish might be a result of vascular damage (e.g., from a bacterial or viral infection). Trauma often results from fish running into something, including into other fish, and increased activity that leads to brain trauma might be associated with sea lice infestation, avoidance of predators, or stray voltage.

Pigment in the liver probably is lipofuscin, and it might also include hemosiderin. 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; it is more common in older fish. 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 might result from increased turnover of red blood cells.

Sinusoidal congestion in the liver is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV.

Congestion of brain capillaries, including the meninges, is nonspecific evidence of circulating vasodilators or a mass-occupying intracranial lesion; hemorrhage sometimes occurs in severe cases. Capillary congestion can be associated with bacterial infections (e.g., mouthrot in smolts), but it also results when venous return is blocked (e.g., with thrombi and massive intracranial hemorrhage or inflammation). [The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section contain erythrocytes.]

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.1% of the 1142 Atlantic salmon hearts sampled as part of the province's Fish Health Auditing and Surveillance Program during 2006 and 2007). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Histopathology

Formalin-fixed tissues were submitted in 5 cassettes for histopathology.

Slide 1 (6892-1) - brain, heart, spleen, liver, intestine, trunk kidney, skeletal muscle, mesenteric adipose tissue

Slide 2 (6892-2) - brain, heart, spleen, liver, intestinal ceca, skeletal muscle, skin, mesenteric adipose tissue

Slide 3 (6892-3) - brain, heart, spleen, intestinal ceca, trunk kidney with Corpuscle of Stannius, mesenteric adipose tissue

Slide 4 (6892-4) - heart, spleen, intestinal ceca, trunk kidney, mesenteric adipose tissue

Slide 5 (6892-5) - brain, heart, spleen, liver, intestinal ceca, trunk kidney, mesenteric adipose tissue

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

Quality control: Liver autolysis: mild (slides 1, 2, 5). In slides without liver (3 and 4), autolysis was mild for most organs. Organs have no postfixation dehydration and no acid hematin deposits.

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 10/09/08 @ 12:08 PM

Specimen	ID	Test	Result
Tissue	#1	PCR - IHNV	Negative
Tissue	#2	PCR - IHNV	Negative
Tissue	#3	PCR - IHNV	Negative
Tissue	#4	PCR - IHNV	Negative
Tissue	#5	PCR - IHNV	Negative
Tissue	#6	PCR - IHNV	Negative
Tissue	#7	PCR - IHNV	Negative
Tissue	#8	PCR - IHNV	Negative
Tissue	#9	PCR - IHNV	Negative
Tissue	#10	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 10/09/08 @ 12:09 PM

Specimen	ID	Test	Result
Tissue	#1	PCR - VHSV	Negative
Tissue	#2	PCR - VHSV	Negative
Tissue	#3	PCR - VHSV	Negative
Tissue	#4	PCR - VHSV	Negative
Tissue	#5	PCR - VHSV	Negative
Tissue	#6	PCR - VHSV	Negative
Tissue	#7	PCR - VHSV	Negative
Tissue	#8	PCR - VHSV	Negative
Tissue	#9	PCR - VHSV	Negative
Tissue	#10	PCR - VHSV	Negative

Gary D. Marty
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Final Report AHC Case: 08-3905

Last Updated: 10/14/08 11:38 AM
Pathologist: Gary D. Marty
Received Date: 10/09/08
Collected Date: 10/06/08
Client Ref No:

Veterinarian: **Dr. Peter McKenzie**
Clinic: **Mainstream Canada-T**
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Submitter: **Zarah Vansnick**
Phone: (250) 725-1255
Fax: (250) 725-1250
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted formalized tissue for histology.

Date fish died: Oct 6, 2008. Number in group: 55,000. Number dead: 30,000.

PO 12166.

Final Diagnosis

1 Gill: filament necrosis, with abundant filamentous bacteria and moderate numbers of intralesional diatom cell bodies and spines/setae (*Chaetoceros sp.*?), multifocal, moderate (slide 5), severe (slides 1, 4)

2 Gill: interfilament filamentous bacteria and diatom cell bodies and/or spines/setae (*Chaetoceros sp.*?), multifocal, mild (slides 2, 3), moderate (slides 9, 10)

3 Gill: interfilament lamellar fusion, with filamentous bacteria and diatom cell bodies and/or spines/setae (*Chaetoceros sp.*?), multifocal, moderate (slide 8)

4. Gill: interfilament filamentous bacteria, multifocal, mild (slide 6)

5. Gill: lamellar fusion, multifocal, moderate (slide 7)

Final Comment: Microscopic changes in these fish are consistent with that are suffering from harmful algae and associated filamentous bacteria in the gills. Findings are similar to a previous case from Mainstream (2008-3839). Comments on specific lesions follow:

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, in marine waters *Tenacibaculum maritimum* (one cause of necrotizing branchitis) is most common. Infections are usually associated with crowding, poor water quality, or stress.

The diatoms *Chaetoceros concavicornis*, *C. convolutus*, and a *Corethron sp*. have been associated with mortality of salmon reared in seawater netpens at numbers as low as 5/mL (Taylor and Harrison 2002). The space between the base of gill filaments often contains moderate numbers of unstained structures that are consistent with the diatom *Chaetoceros*. Chains of cell bodies are about 25 µm in diameter, and individual spines/setae are about 3.5 µm in diameter. Mortality due to *Chaetoceros* can occur within a few days of exposure, as a result of

physical damage to the gills and the resultant inflammatory response. Affected fish are also more vulnerable to *Vibrio* infections. Larger fish tend to be more susceptible than small fish. Characteristic gill lesions (e.g., slide 1) include lamellar epithelial cell hyperplasia and necrosis, and variable numbers of neutrophils. Diatoms and their processes are on the surface of the gill epithelium and sometimes entrapped by multinucleate giant cells. [Source: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.] Lesions due to *Corethron* sp. are similar (Spear et al. 1989).

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).

Literature cited:

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

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

Histopathology

Formalin-fixed gills (1 or 2 arches per cassette) were submitted in 10 cassettes for histopathology. The gills were decalcified in Protocol B (hydrochloric acid solution) for about 30 minutes before being rinsed in water and processed routinely into paraffin. The notes written by the client on the cassettes came off during processing into paraffin. Slide numbers assigned at the Animal Health Centre run consecutively 1 through 10. All gills were examined.

Quality control: Gill autolysis: mild (slides 2, 7, 8), moderate (slides 1, 3, 4, 5), severe (slides 6, 9, 10). Organs have no postfixation dehydration and no acid hematin deposits.

Gary D. Marty
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Final Report AHC Case: 08-4015

Last Updated: 10/23/08 5:45 PM
Pathologist: Gary D. Marty
Received Date: 10/21/08
Collected Date:
Client Ref No: 6916

Veterinarian: **Diane Morrison**
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Phone: (250) 850-3276
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Submitter: **Tiffany MacWilliam**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted three samples for PCR.

Routine site visit. Three fresh morts sampled with lesions that were likely mechanical damage. Samples sent in for PCR to rule out viral agent. First virology submission for this site.

Vaccinated. Date fish died: Oct 15, 2008.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/23/08 @ 5:45 PM

Specimen	ID	Test	Result
Tissue	org #1	PCR - IHNV	Negative
Tissue	org #2	PCR - IHNV	Negative
Tissue	org #3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/23/08 @ 5:45 PM

Specimen	ID	Test	Result
Tissue	org #1	PCR - VHSV	Negative
Tissue	org #2	PCR - VHSV	Negative
Tissue	org #3	PCR - VHSV	Negative

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

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Final Report AHC Case: 08-4016

Last Updated: 10/23/08 5:45 PM
Pathologist: Gary D. Marty
Received Date: 10/21/08
Collected Date:
Client Ref No: 6921

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
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Submitter: **Brad Boyce**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted ten tissue samples for PCR IHN and VHS.

Ten samples (3 pools) taken during routine site visit.

Vaccinated. Date fish died: Oct 16, 2008.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/23/08 @ 5:45 PM

Specimen	ID	Test	Result
Tissue	org #1	PCR - IHNV	Negative
Tissue	org #2	PCR - IHNV	Negative
Tissue	org #3	PCR - IHNV	Negative
Tissue	org #4	PCR - IHNV	Negative
Tissue	org #5	PCR - IHNV	Negative
Tissue	org #6	PCR - IHNV	Negative
Tissue	org #7	PCR - IHNV	Negative
Tissue	org #8	PCR - IHNV	Negative
Tissue	org #9	PCR - IHNV	Negative
Tissue	org #10	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/23/08 @ 5:45 PM

Specimen	ID	Test	Result
Tissue	org #1	PCR - VHSV	Negative
Tissue	org #2	PCR - VHSV	Negative
Tissue	org #3	PCR - VHSV	Negative

Tissue	org #4	PCR - VHSV	Negative
Tissue	org #5	PCR - VHSV	Negative
Tissue	org #6	PCR - VHSV	Negative
Tissue	org #7	PCR - VHSV	Negative
Tissue	org #8	PCR - VHSV	Negative
Tissue	org #9	PCR - VHSV	Negative
Tissue	org #10	PCR - VHSV	Negative

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

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Final Report AHC Case: 08-4017

Last Updated: 10/23/08 5:46 PM
Pathologist: Gary D. Marty
Received Date: 10/21/08
Collected Date:
Client Ref No: 6917

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
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Submitter: **Tiffany MacWilliam**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted three samples for PCR.

Three fresh morts sampled, with hem of liver and 2/3 with furuncles. A. salmonicida isolated. Tissues submitted for PCR for IHN and VHS to rule out viral agent. First virology sample from this site.

Vaccinated. Date fish died: Oct 15, 2008.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/23/08 @ 5:46 PM

Specimen	ID	Test	Result
Tissue	org #1	PCR - IHNV	Negative
Tissue	org #2	PCR - IHNV	Negative
Tissue	org #3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/23/08 @ 5:46 PM

Specimen	ID	Test	Result
Tissue	org #1	PCR - VHSV	Negative
Tissue	org #2	PCR - VHSV	Negative
Tissue	org #3	PCR - VHSV	Negative

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Final Report AHC Case: 08-4037

Last Updated: 10/29/08 12:08 PM
Pathologist: Gary D. Marty
Received Date: 10/22/08
Collected Date: 10/14/08
Client Ref No: PO# 12305

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Zarah Vansnick**
Phone: (250) 725-1255
Fax: (250) 725-1250
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted head kidney, trunk kidney, liver, and spleen for histology and PCR.

Mortality remains low, however, symptoms of S.R.S. persist. See e-mail for field notes. PO# 12305. Saltwater, vaccinated.

Fortune Channel: I have sent in 2 histocasettes, from fish from Fortune Channel. Tissue preserved Oct.14,2008 (transferred to water the day of shipment). I have been observing S.R.S. like granulomas on livers and spleens. These fish also have swollen kidneys and some have oval shaped lesions on their flanks. I had only observed these symptoms in old morts and very few silvers until more recently. The mortality for this site remains low, however, I am beginning to see more silvers with S.R.S like symptoms.

Fish 1 - Pen 101; 540g, Off feed, Bloody ascites, Granulomas liver, Swollen spleen, Systemic

Fish 2 - Pen 102; 370g, Granulomas liver, Bloody ascites, Hemorrhaged pyloric tissue, Congestion brain

Saranac: I have sent fresh tissue samples from 3 fish to confirm or rule out S.R.S.. Samples collected Oct.20,2008. I have been observing S.R.S. like granulomas on livers and spleens. These fish also have swollen kidneys and some have oval shaped lesions on their flanks. I had only observed these symptoms in old morts and very few silvers until more recently. The mortality for this site remains low, however, I am beginning to see more silvers with S.R.S like symptoms.

Fish 1 - Pen 109; Approx. 600g, Frayed fins, Off feed, Pale Liver

Fish 2 - Pen 107; Approx. 650g, Small lesion dorsal surface, Hemorrhaged vent, Off feed, Granulomas liver, Congestion digestive tract, swollen spleen, Hemorrhaged swim bladder, Fluid filled stomach, Petechial hemorrhaging muscle tissue, Swollen kidney

Fish 3 - Pen 106; Frayed fins, On feed, Petechie liver, Congested ceca and digestive tract

Rant Point: I have sent fresh tissue samples from 3 fish to confirm or rule out S.R.S.. Samples collected Oct.20, 2008. I have been observing S.R.S. like granulomas on livers and spleens. These fish also have swollen kidneys and some have oval shaped lesions on their flanks. I had only observed these symptoms in old morts and very few silvers until more recently. The mortality for this site remains low; however, I am

beginning to see more silvers with S.R.S like symptoms.

Fish 1 - Pen 113; Approx. 600g, Frayed fins, Scale loss, Hemorrhaged vent, Some petechiae belly, Off feed, Bloody ascites

Fish 2 - Pen 111; Approx. 700g, Frayed fins, Skin lesion, Off feed, Enlarged pale liver, Swollen spleen and kidney

Fish 3 - Pen 104; Approx. 795g, Enlarged liver with granulomas, Ascites, Systemic, Petechiae muscle tissue, Swollen spleen and kidney with granulomas

Final Diagnosis

1. Brain: no lesions (slide 2)

2. Other organs: tissue preservation not suitable for histopathology (slides 1 and 2)

Final Comment: PCR results confirm the presence of *Piscirickettsia salmonis* in fish from Saranac and Rant Point. Unfortunately, unusual artifact in the formalin-fixed tissues prevent me from being able to analyze the tissues from Fortune Channel (tissues from Fortune channel for PCR are part of case 2008-4076). This is the first time I have seen this type of what I think is preservation artifact involving nearly all organs. Were these tissues handled in some way that was different from normal? As evidence that the tissues were immersed on 10% formalin, the structure of the brain in slide 2 is fairly well preserved. The lipid-rich nature of the brain might have protected it from whatever cause the unusual artifact in the other tissues.

Fixation protocol review - Tissues should be trimmed to a thickness less than 3 mm, and then immersed in 10% neutral buffered formalin for 24 hours. After 24 hours, they may be transferred to water for shipment (nonhazardous material).

Histopathology

Formalin -fixed tissues were submitted in 2 cassettes for histopathology.

Slides 1 and 2 (Fortune) - brain, heart, liver, spleen, head kidney, trunk kidney, and intestinal ceca

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

Quality control: Liver autolysis: unable to determine (slides 1 and 2). Except for the brain in slide 2, the tissues were not preserved properly for histopathology. Basic tissue structure is present, but nuclear staining is absent.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/24/08 @ 1:21 PM

Specimen	ID	Test	Result
Tissue	A)Saranac Fish#1	PCR - IHNV	Negative
Tissue	B)Saranac Fish#2	PCR - IHNV	Negative
Tissue	C)Saranac Fish#3	PCR - IHNV	Negative
Tissue	D)RantPt. Fish#1	PCR - IHNV	Negative
Tissue	E)RantPt. Fish#2	PCR - IHNV	Negative
Tissue	F)RantPt. Fish#3	PCR - IHNV	Negative

PCR-Piscirickettsia salmo Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/29/08 @ 12:04 PM

Specimen	ID	Test	Result
Tissue	A)Saranac Fish#1	PCR - Piscirickettsia salmonis	Negative

Tissue	B)Saranac Fish#2	PCR - Piscirickettsia salmonis	Positive
Tissue	C)Saranac Fish#3	PCR - Piscirickettsia salmonis	Negative
Tissue	D)RantPt. Fish#1	PCR - Piscirickettsia salmonis	Negative
Tissue	E)RantPt. Fish#2	PCR - Piscirickettsia salmonis	Positive
Tissue	F)RantPt. Fish#3	PCR - Piscirickettsia salmonis	Positive

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/24/08 @ 1:22 PM

Specimen	ID	Test	Result
Tissue	A)Saranac Fish#1	PCR - VHSV	Negative
Tissue	B)Saranac Fish#2	PCR - VHSV	Negative
Tissue	C)Saranac Fish#3	PCR - VHSV	Negative
Tissue	D)RantPt. Fish#1	PCR - VHSV	Negative
Tissue	E)RantPt. Fish#2	PCR - VHSV	Negative
Tissue	F)RantPt. Fish#3	PCR - VHSV	Negative

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

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Final Report AHC Case: 08-4076

Last Updated: 10/30/08 4:32 PM
Pathologist: Gary D. Marty
Received Date: 10/23/08
Collected Date: 10/21/08
Client Ref No: PO #12312

Veterinarian: **Dr. Peter McKenzie**
Clinic: **Mainstream Canada-T**
Phone: (250) 725-1255
Fax: (250) 725-1250

Submitter: **Mainstream Canada**
Phone:
Fax:
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh and formalized fish tissues and one bact. plate. Request Histo, Bact and PCR.

Saltwater. Vaccinated.

Farms: Ross Pass, Fortune Channel.

Submitted: brain, gill, heart, head kidney, trunk kidney, liver, spleen, intestine/ceca.

PO #12312

Ross pass - fresh tissue samples, 4 histocassettes, and 1 culture plate from 4 fish. These fish were dip netted from pen 106. This pen has had fish swimming slowly in corners and most exhibit exophthalmia. These fish completed treatment for mouth rot on October 8, 2008 and mortality is low.

Fish 1 -Approx. 70g; Frayed fins, Pop eye, Off feed, Pale gills, Pale Liver

Fish 2 - Approx.70g; Pop eye, Hemorrhaged vent and belly, Off feed, Bloody ascites

Fish 3 - 65g; Off feed, Pop eye, Small skin lesion

Fish 4 - 70g; Frayed fins, Pop eye, Drop jaw, Pale liver, Swollen spleen

Fortune Channel - fresh tissue samples from 4 fish to confirm or rule out S.R.S.. Samples collected Oct.21,2008. I have been observing S.R.S. like granulomas on livers and spleens. These fish also have swollen kidneys and some have oval shaped lesions on their flanks. I had only observed these symptoms in old morts and very few silvers until more recently. The mortality for this site remains low; however, I am beginning to see more silvers with S.R.S like symptoms.

Final Diagnosis

1a. Gill: lamellar epithelial hyperplasia and fusion, multifocal, with intralesional diatom spines/setae (*Chaetoceros* sp.?) and filamentous bacteria, moderate (slide 1B)

1b. Gill arch: branchitis, multifocal, coalescing, with intralesional multinucleate giant cells and diatom spines/setae (*Chaetoceros* sp.?), moderate (slide 3B), severe (slide 2B)

1c. Gill: lamellar epithelial hyperplasia and fusion, multifocal, with intralesional diatom spines/setae (*Chaetoceros* sp.?), moderate (slides 3B, 4B)

2a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slides 1A, 2A, 3A, 4A)

2b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 1A, 4A), moderate (slide 2A)

3a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1A, 3A)

3b. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, mild (slide 4A), moderate (slide 2A)

4. Mesenteric adipose tissue: saponification of fat (fat necrosis), with scattered macrophages, diffuse, mild (slide 4A; pericardial adipose tissue only, slide 2A), moderate (slide 1A)

5. Mesenteric adipose tissue: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate (slides 3A, 4A)

Final Comment: Microscopic changes in the fish from Ross Pass are consistent with vaccinated fish that are suffering from harmful algae and associated filamentous bacteria in the gills. Lesions are similar to the previous case from this site (2008-3839), except that filamentous bacteria are less of a component in this case (only 1 of 4 fish affected): consistent with the history of completed mouthrot treatment. Comments on specific lesions follow:

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, in marine waters *Tenacibaculum maritimum* (one cause of necrotizing branchitis) is most common. Infections are usually associated with crowding, poor water quality, or stress.

The diatoms *Chaetoceros concavicornis*, *C. convolutus*, and a *Corethron sp*. have been associated with mortality of salmon reared in seawater netpens at numbers as low as 5/mL (Taylor and Harrison 2002). The space between the base of gill filaments often contain moderate numbers of unstained structures that are consistent with the diatom *Chaetoceros*. Individual spines/setae are about 3.5 µm in diameter; I did not see any cell bodies in these sections. In slides 2B and 3B, the spines have penetrated into the loose connective tissue of the arch, resulting in granulomatous inflammation that would have taken a long time to resolve (the lamellae in slide 2B are fairly normal). Mortality due to *Chaetoceros* can occur within a few days of exposure, as a result of physical damage to the gills and the resultant inflammatory response. Affected fish are also more vulnerable to *Vibrio* infections. Larger fish tend to be more susceptible than small fish. Characteristic gill lesions include mucous cell hyperplasia, lamellar epithelial cell hyperplasia and necrosis, and variable numbers of neutrophils. Diatoms and their processes are on the surface of the gill epithelium and sometimes entrapped by multinucleate giant cells (e.g., slide 2B). [Source: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.] Lesions due to *Corethron* sp. are similar (Spear et al. 1989).

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

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, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe). Vacuoles probably are a result of vaccine material lost during tissue processing.

The lipid in the necrotic fat cells has been replaced by wispy basophilic material. Necrotic cells comprise less than 10% of the volume of the mesenteric adipose tissue. About half of the affected cells contain foamy macrophages. Saponification of fat is not a common lesion in Atlantic salmon. It has been associated with vitamin E deficiency, and it can be secondary to nearby inflammation. It might be part of a vaccine reaction. In severe cases, the adipose tissue appears chalky white.

Literature cited:

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

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

Histopathology

Formalin-fixed tissues from 4 fish from Ross Pass were submitted in 4 cassettes for histopathology. Before processing into paraffin, gills were removed from the original (A) cassettes, placed in separate (B) cassettes, and immersed in Protocol B (hydrochloric acid solution) for 2 hours before being rinsed in water and processed routinely into paraffin.

Slide #s 1-4 are labeled in the same order as client #s 1 - 4 (OCT 21/08 Ross Pass).

Organs included on most A slides - heart, liver, spleen, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue, brain, swimbladder.

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

Quality control: Liver autolysis: none (slides 1A, 2A), mild (slide 3A). Decalcification is complete and differential staining is good. Organs have no postfixation dehydration and no acid hematin deposits.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Jaime Osei-Appiah on 10/27/08 @ 8:58 AM

Specimen	ID	Isolate	Result	Level
Isolate	1 TSA Plate-Quadrants 1-4		No Bacteria Isolated	
**: Please note, Plate had no growth upon arrival in Bacteriology				

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/27/08 @ 2:17 PM

Specimen	ID	Test	Result
Tissue	A)Ross Pass #1	PCR - IHNV	Negative
Tissue	B)Ross Pass #2	PCR - IHNV	Negative
Tissue	C)Ross Pass #3	PCR - IHNV	Negative
Tissue	D)Ross Pass #4	PCR - IHNV	Negative
Tissue	E)Fortune Ch #1	PCR - IHNV	Negative
Tissue	F)Fortune Ch #2	PCR - IHNV	Negative
Tissue	G)Fortune Ch #3	PCR - IHNV	Negative
Tissue	H)Fortune Ch #4	PCR - IHNV	Negative

PCR-Piscirickettsia salmo Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/30/08 @ 4:32 PM

Specimen	ID	Test	Result
Tissue	Pools E-H	PCR - Piscirickettsia salmonis	Positive

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/27/08 @ 2:17 PM

Specimen	ID	Test	Result
Tissue	A)Ross Pass #1	PCR - VHSV	Negative
Tissue	B)Ross Pass #2	PCR - VHSV	Negative
Tissue	C)Ross Pass #3	PCR - VHSV	Negative
Tissue	D)Ross Pass #4	PCR - VHSV	Negative
Tissue	E)Fortune Ch #1	PCR - VHSV	Negative
Tissue	F)Fortune Ch #2	PCR - VHSV	Negative
Tissue	G)Fortune Ch #3	PCR - VHSV	Negative
Tissue	H)Fortune Ch #4	PCR - VHSV	Negative

Gary D. Marty
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Final Report AHC Case: 08-4189

Last Updated: 11/04/08 11:14 AM
Pathologist: Gary D. Marty
Received Date: 10/31/08
Collected Date:
Client Ref No: #6950

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 3 fresh fish tissues for PCR for IHN and VHS.

Saltwater entry: 08 S1. Euthanized: no.

Three fresh morts with clinical signs of VHS.

Ref. #6950.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 11/04/08 @ 11:14 AM

Specimen	ID	Test	Result
Tissue	6950-1	PCR - IHNV	Negative
Tissue	6950-2	PCR - IHNV	Negative
Tissue	6950-3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 11/04/08 @ 11:14 AM

Specimen	ID	Test	Result
Tissue	6950-1	PCR - VHSV	Positive
Tissue	6950-2	PCR - VHSV	Positive
Tissue	6950-3	PCR - VHSV	Positive

Case: 08-4189

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

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Final Report AHC Case: 08-4190

Last Updated: 11/04/08 11:15 AM
Pathologist: Gary D. Marty
Received Date: 10/31/08
Collected Date:
Client Ref No: #6929

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 2 fresh fish tissues for PCR for IHN and VHS.

Saltwater entry: 06 SO. Vaccinated: yes. Euthanized: no.

Two fresh morts sampled - septicemic. Fish #2 had muscle hemorrhage.

Ref. #6929.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 11/04/08 @ 11:15 AM

Specimen	ID	Test	Result
Tissue	6929-1	PCR - IHNV	Negative
Tissue	6929-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 11/04/08 @ 11:15 AM

Specimen	ID	Test	Result
Tissue	6929-1	PCR - VHSV	Negative
Tissue	6929-2	PCR - VHSV	Negative

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

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Final Report AHC Case: 08-4191

Last Updated: 11/04/08 11:15 AM
Pathologist: Gary D. Marty
Received Date: 10/31/08
Collected Date:
Client Ref No: #6911

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Marine Harvest**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted one fresh fish tissue for PCR for IHN and VHS.

Saltwater entry: 2006 SO. Euthanized: no. Fish died Oct. 7/08.

One sample from pen 1 for PCR. Low mortality in pen 1.

Ref. #6911.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 11/04/08 @ 11:15 AM

Specimen	ID	Test	Result
Tissue	6911-Pen 1	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 11/04/08 @ 11:15 AM

Specimen	ID	Test	Result
Tissue	6911-Pen 1	PCR - VHSV	Negative

Gary D. Marty
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Facsimile: (604) 556-3010
Toll-Free : 1-800-661-9903

Final Report AHC Case: 08-4374

Last Updated: 11/17/08 4:44 PM
Pathologist: Gary D. Marty
Received Date: 11/13/08
Collected Date:
Client Ref No: 6942

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Tiffany MacWilliam**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted three fresh tissue samples for PCR IHN and VHS.

Mortality on site, low, Hemorrhaging seen in livers of three fish - NVL.

ID: 6942

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 11/17/08 @ 4:44 PM

Specimen	ID	Test	Result
Tissue	6942-1	PCR - IHNV	Negative
Tissue	6942-2	PCR - IHNV	Negative
Tissue	6942-3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 11/17/08 @ 4:44 PM

Specimen	ID	Test	Result
Tissue	6942-1	PCR - VHSV	Negative
Tissue	6942-2	PCR - VHSV	Negative
Tissue	6942-3	PCR - VHSV	Negative

Gary D. Marty

Case: 08-4374

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Final Report AHC Case: 08-4376

Last Updated: 11/17/08 4:45 PM
Pathologist: Gary D. Marty
Received Date: 11/13/08
Collected Date:
Client Ref No: 6968

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Diane Morrison**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for PCR IHN and VHS.

One sample for PCR, only one fish with hemorrhage on p.c. No other abnormalities.

ID: 6968

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 11/17/08 @ 4:45 PM

Specimen	ID	Test	Result
Tissue	6968	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 11/17/08 @ 4:45 PM

Specimen	ID	Test	Result
Tissue	6968	PCR - VHSV	Negative

Gary D. Marty
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Final Report AHC Case: 08-4377

Last Updated: 11/19/08 11:27 AM
Pathologist: Gary D. Marty
Received Date: 11/13/08
Collected Date:
Client Ref No: 6967

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Diane Morrison**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted formalized tissue for histology.

Histo from two broodstock. #284 suspect nephrocalcinosis. No ID enlarged kidney (6967 A).

ID: 6967.

Final Diagnosis

1a. Spleen and intestinal ceca: disseminated granulomas (vaccine reaction?), severe (slide 1 and 2)

1b. Intestinal ceca: submucosal mineralization (metastatic mineralization?), diffuse, moderate (slide 2, Von Kossa stain)

2. Trunk kidney: lymphosarcoma/lymphoma (slides 3, 4, 5)

Final Comment: These fish have evidence of a vaccine reaction, metastatic mineralization, and lymphosarcoma. Specific comments follow:

Slide 1 contains a section of spleen and surrounding mesenteries in which the mesenteries (but not the splenic parenchyma) are expanded by multiple granulomas admixed with abundant, tightly packed fibrocellular fronds. The granulomas are up to 1.2 mm in diameter and composed of a rim of fibroblasts and macrophages that surround a necrotic core; the core sometimes contains mineral. In slide 2, similar granulomas expand multiple foci in the lamina propria of intestinal ceca, surrounding mesenteries, and exocrine pancreas. These granulomas sometimes contain clefts and vacuoles characteristic of a vaccine reaction. Central mineral is especially prominent on the Von Kossa stain. Fine spicules of mineral in the cecal submucosa are evidence of abnormal calcium metabolism. Lack of organisms on the special stains rules out the primary differential: *Renibacterium salmoninarum* infection.

Slides 3, 4, and 5 contain sections of trunk kidney in which the interstitium is greatly expanded by a poorly demarcated 3-cm - diameter mass of neoplastic round cells. Tumour cells have round to angular nuclei with vesiculated chromatin and up to 10 mitotic figures per 40× objective lens field. Chromatin is scant and basophilic. These features are diagnostic of a round cell sarcoma, probably a lymphosarcoma. Lymphosarcomas occur occasionally in cultured salmonids. Although lymphosarcomas in some fish species have been associated with oncogenic viruses, so such virus has ever been isolated from Atlantic salmon. If this is a single case among thousands of harvested fish, it most likely arose spontaneously.

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology (slides 1 and 2). Three (3) additional cassettes were from tissue trimmed at the Animal Health Centre.

Slide 1 (284) - spleen

Slide 2 (284) - intestinal ceca; special stains on sections from the same block include Twort's Gram, ZN acid-fast, and the Von Kossa mineral stain.

Slide 3, 4 and 5 - trunk kidney

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

Quality control: Tissue autolysis: none (slides 1 and 2), mild (slides 3, 4, 5).

Gary D. Marty
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Final Report AHC Case: 08-4391

Last Updated: 11/26/08 9:23 AM
Pathologist: Gary D. Marty
Received Date: 11/14/08
Collected Date:
Client Ref No: PO 12365 Millar

Veterinarian: **Dr. Peter McKenzie**
Clinic: **Mainstream Canada-T**
Phone: (250) 725-1255
Fax: (250) 725-1250

Submitter: **Mainstream Canada**
Phone:
Fax:
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Received fish tissue sample for PCR and 2 culture plates for identification.

in group: 37,000. Dead: 4000. Euthanized: no. DOD: Nov 12, 08. 80g.

Atlantic salmon.

PO #12365 Millar

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Jaime Osei-Appiah on 11/26/08 @ 9:23 AM

Specimen	ID	Isolate	Result	Level
Isolate	Kidney 1	Vibrio splendidus	Positive	
Isolate	Kidney 1	Aliivibrio wodanis	Positive	
**: Due to poor growth antibiotic sensitivitues were unable to be performed.				
Isolate	Kidney 2	Vibrio splendidus	Positive	
Isolate	Kidney 3	Aliivibrio logei	Positive	
Isolate	Kidney 4	Vibrio splendidus	Positive	
Isolate	Skin 1,2,4		No Bacteria Isolated	
Isolate	Skin 3	Psychrobacter sp.	Positive	
**: Due to poor growth antibiotic sensitivities were unable to be performed.				

Fish Resulted by: Erin Zabek Verified by: Erin Zabek on 11/20/08 @ 11:41 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Aliivibrio logei	Kidney 3	s	s	s	s	s	s

Vibrio splendidus	Kidney 1	r	s	s	s	s	s
Vibrio splendidus	Kidney 2	r	s	s	s	s	s
Vibrio splendidus	Kidney 4	r	s	s	s	s	s

**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 11/18/08 @ 11:45 AM

Specimen	ID	Test	Result
Tissue	organs, Pen 106, fish 1-5	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 11/18/08 @ 11:45 AM

Specimen	ID	Test	Result
Tissue	organs, Pen 106, fish 1-5	PCR - VHSV	Negative

Gary D. Marty
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Final Report AHC Case: 08-4481

Last Updated: 12/03/08 11:30 AM
Pathologist: Gary D. Marty
Received Date: 11/24/08
Collected Date:
Client Ref No: CB201108

Veterinarian: **Dr. Peter McKenzie**
Clinic: **Mainstream Canada**
Phone: (250) 286-0022
Fax: (250) 286-0042

Submitter: **Nathan Cassan**
Phone:
Fax:
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 5 fish plates for identification by bacteriology.

Fish are hemorrhaged. Pop-eye exhibited across these 9 fish. 5 plates for ident w/liver and kidney cultured for each. Plates labelled for reference LV- liver; Kd - kidney.

Species: Atlantic salmon. Saltwater. Vaccinated: yes. # in group: 9. Euthanized: no. Fish died Nov 19/08. Farm name: Cliff Bay. Client ref. number: CB201108.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 12/03/08 @ 11:29 AM

Specimen	ID	Isolate	Result	Level
Isolate	Pen4-Fish3-Liver & Kidney	Carnobacterium sp.	Positive	
**: Carnobacterium sp. identified as Carnobacterium divergens				
Isolate	Pen4-Fish3-Liver & Kidney	Vibrio splendidus	Positive	
Isolate	Pen4-Fish3-Liver & Kidney	Aliivibrio wodanis	Positive	
Isolate	Pen4-Fish1-Liver & Kidney	Shewanella sp.	Positive	
Isolate	Pen4-Fish1-Liver & Kidney	Aliivibrio wodanis	Positive	
Isolate	Pen4-Fish2-Liver & Kidney	Aliivibrio wodanis	Positive	
Isolate	Pen4-Fish2-Liver & Kidney	Vibrio splendidus	Positive	
Isolate	Pen4-Fish2-Liver &	Moritella viscosa	Positive	

	Kidney		
Isolate	Pen3-Fish2-Liver & Kidney	Vibrio splendidus	Positive
Isolate	Pen3-Fish2-Liver & Kidney	Aliivibrio wodanis	Positive
Isolate	Pen3-Fish3-Liver & Kidney	Aliivibrio wodanis	Positive
Isolate	Pen3-Fish3-Liver & Kidney	Photobacterium sp.	Positive
Isolate	Pen3-Fish1-Liver & Kidney	Vibrio splendidus	Positive
Isolate	Pen2-Fish3-Liver & Kidney	Vibrio splendidus	Positive
Isolate	Pen2-Fish1-Liver & Kidney	Vibrio splendidus	Positive
Isolate	Pen2-Fish2-Liver & Kidney	Vibrio splendidus	Positive
Isolate	Pen2-Fish2-Liver & Kidney	Aliivibrio logei	Positive

**: Some isolates were unable to have antibiotic sensitivity testing performed due to poor growth.

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 12/03/08 @ 11:30 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Carnobacterium sp.	Pen4-Fish3-Liver & Kidney	s	s	s	r	s	s
Shewanella sp.	Pen4-Fish1-Liver & Kidney	s	s	s	s	s	s
Aliivibrio logei	Pen2-Fish2-Liver & Kidney	s	s	s	s	s	s
Vibrio splendidus	Pen4-Fish3-Liver & Kidney	s	s	s	s	s	s

**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline

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

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Final Report AHC Case: 08-4534

Last Updated: 12/01/08 4:01 PM
Pathologist: Gary D. Marty
Received Date: 11/27/08
Collected Date:
Client Ref No: PO 12312

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Zarah Vansnick**
Phone:
Fax:
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh Atlantic salmon tissues - head kidney, liver and spleen. Request P. salmonis by PCR.

Farm: Ross Pass. Age: 100g. Saltwater. Vaccinated. Prior submission - PO 12312. Fish died Nov. 24/08.

Granuloma liver. Ascites. Swollen spleen. Swollen kidney.

Molecular Diagnostics

PCR-Piscirickettsia salmo Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 4:01 PM

Specimen	ID	Test	Result
Tissue		PCR - Piscirickettsia salmonis	Positive

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

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Final Report AHC Case: 08-4535

Last Updated: 12/01/08 2:27 PM
Pathologist: Gary D. Marty
Received Date: 11/27/08
Collected Date:
Client Ref No: PO 12413

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Zarah Vansnick**
Phone:
Fax:
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted formalized Atlantic salmon tissues for histopathology; brain, heart, head kidney, liver, spleen and intestine.

Farm: Millar Ch.Age: 85g. Saltwater. Vaccinated. Duration of illness: 14 days. Prior submission: PO 12365 Millar. Fish died Nov. 12/08.

Ref. PO 12413.

Final Diagnosis

1. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slides 1A, 2A, 3A, 4A, 5A)

2a. Spleen: peritonitis, granulomatous, regionally diffuse, mild (slides 3A, 5A), with intralesional vacuoles about 50 µm in diameter, mild (slide 1A), moderate (slide 4A)

2b. Intestinal mesenteries: peritonitis, granulomatous, regionally diffuse, mild (slide 1A)

3a. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 1A, 4A), moderate (slides 2A, 3A. 5A)

3b. Trunk kidney: interstitial fibrosis, subacute, focal, moderate (slide 5A)

4. Heart: endocarditis, multifocal, with endothelial cell hypertrophy and a thin layer of lymphocytes and macrophages, mild (slides 2A, 3A, 4A, 5A), moderate (slide 1A)

Final Comment: Most of the lesions in these fish are consistent with the clinical history of mouthrot (described with the submission form for case 2008-4537 (same PO#, 12413) and a vaccine reaction. However, no mouth lesions were included for confirmation. Lack of hepatic vacuoles in any of the fish is evidence that they were not feeding normally for at least 2 days before they died. One fish has interstitial renal fibrosis, which was probably caused by something other than mouthrot or a vaccine. Comments on specific lesions follow:

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

with ulcers (e.g., mouthrot).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe). Vacuoles probably are a result of vaccine material lost during tissue processing.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642).

Renal interstitial fibrosis is evidence of extensive damage and efforts at repair, and it usually occurs following tubular epithelial necrosis. This case (slide 5A) has no necrotic tubules; therefore, if necrosis is the underlying cause, it had resolved by the time the fish died. Causes of renal tubular necrosis include viral hemorrhagic septicemia virus (VHSV), exposure to toxins (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin), or hypoxia followed by re-oxygenation. I do not commonly associated renal tubular necrosis with mouthrot.

The pattern of inflammation in the heart is consistent with systemic immune stimulation; differentials include a bacterial or viral infection. Inflammatory cells lining the endocardial surface in foci of inflammation are rarely more than 2 cell layers thick. This pattern of inflammation has also been described with Heart and Skeletal Muscle Inflammation in Atlantic salmon reared in Europe, but this disease has not been identified in BC salmon.

Histopathology

Formalin-fixed tissues from 5 fish were submitted in 5 cassettes for histopathology. Before processing into paraffin, gills were removed from each cassette and placed into a separate cassette for decalcification.

Slide #s 1A-5A are labeled in the same order as client #s 1 - 5 [Millar Nov 12/08] - Organs included on most slides - heart, liver, spleen, trunk kidney, intestinal ceca, and mesenteric adipose tissue

Slide #s 1G-5G are labeled in the same order as client #s 1 - 5 - gill

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

Quality control: Liver autolysis: none (slides 1A, 2A, 4A, 5A), moderate (slide 3A). Gill autolysis: mild (slides 1G, 2G, 4G, 5G), moderate (slide 3G). Decalcification is complete. Organs have no postfixation dehydration and no acid hematin deposits.

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

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Final Report AHC Case: 08-4537

Last Updated: 12/04/08 1:13 PM
Pathologist: Gary D. Marty
Received Date: 11/27/08
Collected Date:
Client Ref No: PO 12413

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Zarah Vansnick**
Phone:
Fax:
Owner: **Mainstream Canada-T**
Phone:
Fax:(250) 725-1250

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 6 bacteriology plates for culture and sensitivities. Also submitted fresh head kidney, liver and spleen for viral PCR.

Farm: Millar Channel. Species: Atlantic salmon. Age: 85g. Saltwater. Vaccinated. # in group: 500,000. Dead: 160. Duration of illness: 5 days. Prior submission: PO 12365 Millar. Fish died Nov. 24/08.

These fish have been treated with Tribrissen - Aquaflor each pen 10 days post entry. We started to see Mouth Rot on Nov. 20/08. On Nov. 24/08 I observed petechial hemorrhaging on pyloric ceca.

Ref. PO 12413.

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 12/04/08 @ 1:11 PM

Specimen	ID	Isolate	Result	Level
Isolate	Kidney 1,2,4		No Bacteria Isolated	
Isolate	Kidney 3	Vibrio lentus	Positive	
Isolate	Kidney 5,7		No Bacteria Isolated	
Isolate	Kidney 6	Aliivibrio wodanis	Positive	
Isolate	Kidney 8	Vibrio sp.	Positive	
Isolate	Kidney 8	Aliivibrio wodanis	Positive	
Isolate	Brain 1,5,6,7		No Bacteria Isolated	
Isolate	Brain 2	Aliivibrio wodanis	Positive	
Isolate	Brain 3	Vibrio lentus	Positive	
Isolate	Brain 4	Vibrio lentus	Positive	
Isolate	Brain 8	Aliivibrio logei	Positive	

**: Sensitivities not available on this isolate due to poor growth. Bacteria identified by DNA sequencing of 16S rRNA gene.

Fish Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 12/04/08 @ 1:13 PM

Organism	ID	e	ffc	sor	s3	sxt	ot
Vibrio lentus	Kidney 3	s	s	s	s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline							
Aliivibrio wodanis	Kidney 6	s	s	s	s	s	s

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:40 AM

Specimen	ID	Test	Result
Tissue	organ	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:40 AM

Specimen	ID	Test	Result
Tissue	organ	PCR - VHSV	Negative

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

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Final Report AHC Case: 08-4546

Last Updated: 12/01/08 9:41 AM
Pathologist: Gary D. Marty
Received Date: 11/27/08
Collected Date: 11/18/08
Client Ref No: 6987

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Brad Boyce**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted three fresh tissue samples for PCR IHN and VHS.

3 fresh morts sampled - internal hem and muscle hem. Saltwater entry: 07, S1.

Ref: 6987.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:40 AM

Specimen	ID	Test	Result
Tissue	A) 6987-1	PCR - IHNV	Negative
Tissue	B) 6987-2	PCR - IHNV	Negative
Tissue	C) 6987-3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:41 AM

Specimen	ID	Test	Result
Tissue	A) 6987-1	PCR - VHSV	Negative
Tissue	B) 6987-2	PCR - VHSV	Negative
Tissue	C) 6987-3	PCR - VHSV	Negative

Gary D. Marty

Case: 08-4546

END OF REPORT

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Final Report AHC Case: 08-4547

Last Updated: 12/01/08 9:41 AM
Pathologist: Gary D. Marty
Received Date: 11/27/08
Collected Date: 11/26/08
Client Ref No: 6981

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Jerry Burry**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted two fresh tissue samples for PCR IHN and VHS.

Samples #1, 2. Low mortality at site, however one fish with petechial hem of p.c. Saltwater entry: 08, S0. DOD: Nov 12, 08.

Ref: 6981.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:41 AM

Specimen	ID	Test	Result
Tissue	A) 6981-1	PCR - IHNV	Negative
Tissue	B) 6981-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:41 AM

Specimen	ID	Test	Result
Tissue	A) 6981-1	PCR - VHSV	Negative
Tissue	B) 6981-2	PCR - VHSV	Negative

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

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Final Report AHC Case: 08-4548

Last Updated: 12/01/08 9:41 AM
Pathologist: Gary D. Marty
Received Date: 11/27/08
Collected Date: 11/26/08
Client Ref No: 7008

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Tiffany MacWilliam**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted one fresh tissue samples for PCR.

One fish presented with hem in muscle, liver and pyloric caeca. DOD: Nov 25, 08. Saltwater entry: 08, S1.

Ref: 7008.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:41 AM

Specimen	ID	Test	Result
Tissue	organ	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:41 AM

Specimen	ID	Test	Result
Tissue	organ	PCR - VHSV	Negative

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Final Report AHC Case: 08-4549

Last Updated: 12/22/08 10:16 AM
Pathologist: Gary D. Marty
Received Date: 11/27/08
Collected Date: 11/26/08
Client Ref No: 7007

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Tiffany MacWilliam**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue samples for PCR, viral culture.

One fish presented with hem in muscle, liver, pyloric fat. DOD: Nov 25, 08. Saltwater entry: 08, S1.

Ref: 7007.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:41 AM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:42 AM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 12/22/08 @ 10:16 AM

Specimen	ID	Isolate	Result	Level
Tissue			No viruses isolated	

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Final Report AHC Case: 08-4550

Last Updated: 12/01/08 9:42 AM
Pathologist: Gary D. Marty
Received Date: 11/27/08
Collected Date: 11/26/08
Client Ref No: 6995

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Tiffany MacWilliam**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted six fresh tissue samples for PCR, IHN and VHS.

6 samples for PCR 1,2,3,4,5,6 presented with hem in muscle, liver. Some transport damage visible. 5 presented telangiectasis (bumpy spleen). No other abnormality. DOD: Nov 19, 08. Saltwater entry: 07, S1.

Ref: 6995.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:42 AM

Specimen	ID	Test	Result
Tissue	A) 6995-1	PCR - IHNV	Negative
Tissue	B) 6995-2	PCR - IHNV	Negative
Tissue	C) 6995-3	PCR - IHNV	Negative
Tissue	D) 6995-4	PCR - IHNV	Negative
Tissue	E) 6995-5	PCR - IHNV	Negative
Tissue	F) 6995-6	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:42 AM

Specimen	ID	Test	Result
Tissue	A) 6995-1	PCR - VHSV	Negative
Tissue	B) 6995-2	PCR - VHSV	Negative
Tissue	C) 6995-3	PCR - VHSV	Negative
Tissue	D) 6995-4	PCR - VHSV	Negative
Tissue	E) 6995-5	PCR - VHSV	Negative
Tissue	F) 6995-6	PCR - VHSV	Negative

Gary D. Marty
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Case: 08-4550

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Final Report AHC Case: 08-4551

Last Updated: 12/01/08 9:42 AM
Pathologist: Gary D. Marty
Received Date: 11/27/08
Collected Date: 11/26/08
Client Ref No: 7001

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Brad Boyce**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted one fresh tissue sample for PCR.

One sample for PCR from "pen 6", with swollen kidney and hem on margins of kidney. Bacteriology pending. Mortality low at site. DOD: Nov 20, 08. Saltwater entry: 08, S0.

Ref: 7001.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:42 AM

Specimen	ID	Test	Result
Tissue	organ	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:42 AM

Specimen	ID	Test	Result
Tissue	organ	PCR - VHSV	Negative

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

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Final Report AHC Case: 08-4552

Last Updated: 12/01/08 9:42 AM
Pathologist: Gary D. Marty
Received Date: 11/27/08
Collected Date: 11/26/08
Client Ref No: 6999

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
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Submitter: **Brad Boyce**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted one fresh tissue sample for PCR for IHN and VHS.

One sample for PCR labelled "SP5". Routine sampling of fish, some with signs of furunculosis. DOD: Nov 19, 08. Saltwater entry: 07, S1.

Ref: 6999.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:42 AM

Specimen	ID	Test	Result
Tissue	organ	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:42 AM

Specimen	ID	Test	Result
Tissue	organ	PCR - VHSV	Negative

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Final Report AHC Case: 08-4553

Last Updated: 12/01/08 9:43 AM
Pathologist: Gary D. Marty
Received Date: 11/27/08
Collected Date: 11/26/08
Client Ref No: 6994

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Tiffany MacWilliam**
Phone: (250) 850-3276
Fax: (250) 850-3275
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted one fresh tissue sample for PCR for IHN and VHS.

Some fish with mechanical damage. One fish with hem of internal organs. DOD: Nov 19, 08. Saltwater entry: 06, S0.

Ref: 6994.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:43 AM

Specimen	ID	Test	Result
Tissue	organ	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 12/01/08 @ 9:43 AM

Specimen	ID	Test	Result
Tissue	organ	PCR - VHSV	Negative

Gary D. Marty
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Final Report AHC Case: 08-4567

Last Updated: 12/03/08 10:37 AM
Pathologist: Gary D. Marty
Received Date: 11/28/08
Collected Date:
Client Ref No: BI251108

Veterinarian: **Dr. Peter McKenzie**
Clinic: **Mainstream Canada**
Phone: (250) 286-0022
Fax: (250) 286-0042

Submitter: **Nathan Cassan**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 5 fish cassettes (head kidney, liver, intestine/ceca) for histopathology.

Farm: Brent Island. Species: Atlantic salmon. Saltwater. Vaccinated. # in group: 5. Euthanized: yes, "bonked". Fish died Nov. 25/08.

Fish not responding to feed. All 5 fish showed signs of vac scarring & adhesion.

Cassettes labelled 1, 2, 3,4, 5 from Pens 3, 4, 10, 11 and 12 respectively.

Final Diagnosis

1a. Liver: sinusoidal congestion, multifocal, with focal intracytoplasmic spherical eosinophilic to amphophilic inclusions, mild (slide 5)

1b. Liver: yellow-brown pigmented macrophage aggregates (lipofuscin and hemosiderin?), multifocal, mild (slide 5)

1c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 2, 3, 4), moderate (slide 5)

1d. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 1, 2)

1e. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 3, 4)

2. Intestinal ceca: enteritis, submucosal, lymphohistiocytic, focal, moderate (slide 3)

3a. Stomach: peritonitis, granulomatous, regionally diffuse, mild (slides 2, 3)

3b. Intestine: peritonitis, granulomatous, regionally diffuse, severe (slides 4, 5)

3c. Intestinal ceca: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 3, 4, 5)

4. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 3, 4, 5)

Final Comment: Most of the lesions in these fish are consistent with the clinical history of a vaccine reaction. One fish has hepatic sinusoidal congestion that might have been caused by something other than a vaccine. Comments on specific lesions follow:

Sinusoidal congestion in the liver (fish 5) is evidence of circulating vasodilators. I have seen it associated with viral hemorrhagic septicemia virus and *Listonella anguillarum* infection. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology and virology and PCR for VHSV and IHNV (if not already done). The golden to amphophilic cytoplasmic inclusions in hepatocytes vary from 0.5 to 1.5 times 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).

Pigment in the liver (fish 5) probably is lipofuscin, and it might also include hemosiderin. 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; it is more common in older fish. 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 might result from 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.

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

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins or inflammatory mediators) or come from outside the fish (e.g., from the water or the feed). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "silvers" that die in marine net pens, affecting 12% of the 645 Atlantic salmon livers examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2007 was sufficient to identify a trend towards greater prevalence in the fall and winter (14-19%) than in the spring and summer (4.1-10%).

One of the 5 sections of intestinal ceca in slide 3 contains a single 1000 × 150 µm focus of lymphohistiocytic inflammation in the deep submucosa. The inflammation does not involve the mucosa. The submucosa normally contains small numbers of lymphocytes, but this focus seems larger than normal. Lymphohistiocytic inflammation 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, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe).

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642).

Histopathology

Formalin-fixed tissues from 5 fish were submitted in 5 cassettes for histopathology. Slide #s 1-5 are labeled in the same order as client #s Fish 1 - Fish 5 [corresponding to pens 3, 4, 10, 11, and 12, respectively.]

Organs included on most slides - liver, trunk kidney, stomach (sometimes with only the tunica muscularis layer), intestine/intestinal ceca, and mesenteric adipose tissue

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

Quality control: Liver autolysis: none (slides 1, 2, 4, 5), mild (slide 3). Large foci of erythrocytes (e.g., liver in slide 2) have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have no postfixation dehydration.

Gary D. Marty
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Final Report AHC Case: 08-4652

Last Updated: 12/09/08 4:00 PM
Pathologist: Gary D. Marty
Received Date: 12/05/08
Collected Date:
Client Ref No: 7004

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Brad Boyce**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted formalized tissues from 3 fish and fresh tissues for PCR for IHN and VHS.

Three moribunds dipped out of pen 10. Very high number of moribunds in this pen - most with cataracts.

Atlantic salmon. Saltwater entry 07 SO. Vaccinated. Euthanized with TMS overdose.

Final Diagnosis

1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1, 2, 3)

1b. Liver: hepatitis, lymphohistiocytic, focal, mild (slide 3)

2a. Skeletal muscle: *Kudoa thyrsites* pseudocyst, focal, 350 × 50 µm, mild (slide 1); focal, mild (slide 1E1); multifocal (each of 4 foci ~30 µm in diameter), mild (slide 2)

2b: Skeletal muscle: myositis, lymphohistiocytic, multifocal, with intralesional *Kudoa thyrsites* spores, mild (slide 3)

3a. Spleen: splenitis, granulomatous, focal, with a multinucleate giant cell, mild (slide 1)

3b.Spleen: peritonitis, multifocal, lymphocytic, with fibrocellular fronds, mild (slide 2)

4. Heart: epicarditis, lymphohistiocytic, mild (focal, slide 1; diffuse, slide 3)

5. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 1), moderate (slides 2, 3)

6. Intestinal mesenteries: peritonitis, multifocal, lymphocytic, with fibrocellular fronds, mild (slide 1), moderate (slide 3)

7. Eye: phacolysis, acute, moderate (slides 1E1, 1E2, 2E2)

8. Eye, lens: mineralization, focal, mild (slide 2E1)

Final Comment: Microscopic changes in these fish are consistent with the clinical history of moribund fish with cataracts, but they provide no clear clues about the cause of morbidity. Comments on specific findings follow:

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

The focus of lymphohistiocytic inflammation in the liver most likely is part of a reaction of a vaccine. The vaccine might have been injected directly into a vascular organ of the affected fish instead of the peritoneal cavity. A less likely differential is infection with *Renibacterium salmoninarum*.

Kudoa thyrsites is a myxosporean that is most common in skeletal muscle, but sometimes occurs in heart muscle. This example has characteristic stellate spores with four unequal polar capsules converging on one end. That all 3 fish (and striated muscle surrounding 1 of 4 eyes) are positive is consistent with a high prevalence of *K. thyrsites* at this site.

The focus of granulomatous inflammation in the spleen most likely is part of a reaction of a vaccine. The vaccine might have been injected directly into a vascular organ of the affected fish instead of the peritoneal cavity. The primary differential is infection with *Renibacterium salmoninarum*.

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 might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson) or stress (e.g., recent vaccination or movement). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642).

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

Phacolysis refers to the dissolution of the crystalline lens in the eye, which usually occurs spontaneously but is occasionally the result of trauma, infectious agents, or malnutrition. Loss of crystalline structure results in the opaque cataract observed grossly. Phacolysis is sometimes associated with lens rupture. Infiltration of relatively few cells provides evidence that the injury occurred recently (e.g., within the past two weeks). Mineralization (e.g., slide 2E1) is evidence of focal damage.

Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology. Eyes were placed in separate large cassettes and processed into paraffin.

Slides 1 (7004-1), 2 (7004-2) and 3 (7004-3) - brain, heart, spleen, liver, head kidney, trunk kidney, skeletal muscle, intestinal ceca, and mesenteric adipose tissue

Slides 1E-1, 1E-2, 2E-1 and 2E-2 - eye (2 pieces each)

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

Quality control: Liver autolysis: none (slides 1, 2, 3). Large foci of erythrocytes (e.g., spleen in slide 2) have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/08/08 @ 3:20 PM

Specimen	ID	Test	Result
Tissue	7004-1	PCR - IHNV	Negative

Tissue	7004-2	PCR - IHNV	Negative
Tissue	7004-3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/08/08 @ 3:20 PM

Specimen	ID	Test	Result
Tissue	7004-1	PCR - VHSV	Negative
Tissue	7004-2	PCR - VHSV	Negative
Tissue	7004-3	PCR - VHSV	Negative

Gary D. Marty
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Final Report AHC Case: 08-4654

Last Updated: 12/10/08 11:13 AM
Pathologist: Gary D. Marty
Received Date: 12/05/08
Collected Date:
Client Ref No: 7005

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Brad Boyce**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted formalized tissues from 2 fish for histopathology and fresh tissues from 3 fish for PCR for IHN and VHS.

Moribunds dipped out of pen 5 - percussion. Severe adhesions.

Atlantic salmon. Saltwater entry 07 SO. Vaccinated. Euthanized - percussion.

Final Diagnosis

1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1, 2)

1b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate (slide 1)

2. Heart: epicarditis, multifocal, lymphoplasmacytic, mild (slides 1, 2)

3. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, moderate (slides 1, 2)

4. Head kidney: granulomatous inflammation, focal, mild (slide 1)

5. Skeletal muscle: *Kudoa thyrsites* pseudocysts, multifocal, mild (slides 1, 2)

Final Comment: These fish have several lesions consistent with the history of morbidity, but these lesions do not include a specific cause for their demise. Comments on specific lesions follow:

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

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These types of vacuoles were less common before 2007 and might be related to the significant increase in the proportion of plant-based components in commercial feeds that

has occurred since 2006. The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown.

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

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or movement) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642)

Differentials for granulomatous inflammation in the head kidney include a reaction to a vaccine and chronic bacterial disease (e.g., *Yersinia ruckeri* or *Renibacterium salmoninarum* infection).

Kudoa thyrsites is a myxosporean that is most common in skeletal muscle, but sometimes occurs in heart muscle. This example has characteristic stellate spores with four unequal polar capsules converging on one end. The presence of spores in both fish is evidence that farm prevalence is high.

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology (the paper towels surrounding the cassettes were nearly dry upon receipt, but the tissues sectioned and stained normally).

Slide 1 (7005-1) and 2 (7005-2) - heart, spleen, liver, intestine, head kidney, trunk kidney, skin/skeletal muscle, mesenteric adipose tissue

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

Quality control: Liver autolysis: none (slides 1, 2). Large foci of erythrocytes (e.g., spleen in slide 1) have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/08/08 @ 3:21 PM

Specimen	ID	Test	Result
Tissue	7005-1	PCR - IHNV	Negative
Tissue	7005-2	PCR - IHNV	Negative
Tissue	7005-3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/08/08 @ 3:21 PM

Specimen	ID	Test	Result
Tissue	7005-1	PCR - VHSV	Negative
Tissue	7005-2	PCR - VHSV	Negative
Tissue	7005-3	PCR - VHSV	Negative

Gary D. Marty

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Final Report AHC Case: 08-4809

Last Updated: 12/19/08 11:10 AM
Pathologist: Gary D. Marty
Received Date: 12/17/08
Collected Date: 12/11/08
Client Ref No: #7039

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Brad Boyce**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 2 fresh tissues for PCR for IHN and VHS.

2 fish with hem in all organs. Please run both samples for PCR.

Atlantic salmon. Saltwater entry: 2007 S1

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/19/08 @ 11:00 AM

Specimen	ID	Test	Result
Tissue	7039-1	PCR - IHNV	Negative
Tissue	7039-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/19/08 @ 11:10 AM

Specimen	ID	Test	Result
Tissue	7039-1	PCR - VHSV	Negative
Tissue	7039-2	PCR - VHSV	Negative

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

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Final Report AHC Case: 08-4811

Last Updated: 12/19/08 11:00 AM
Pathologist: Gary D. Marty
Received Date: 12/17/08
Collected Date:
Client Ref No: #7024

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Burry, Gerry**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted one fresh tissue for PCR for IHN and VHS

One fish with some hem on liver, p.c and belly wall. Some mechanical damage to other fish in pen. Please run PCR to rule out viral agent.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/19/08 @ 11:00 AM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/19/08 @ 11:00 AM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

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

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Final Report AHC Case: 08-4812

Last Updated: 12/19/08 11:01 AM
Pathologist: Gary D. Marty
Received Date: 12/17/08
Collected Date: 12/11/08
Client Ref No: #7018

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Boyce, Brad**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted a 2-fish pool of fresh tissue for PCR for IHN and VHS.

One fish with clinical signs of ERM and a second fish with tail rot. Atlantic salmon. Saltwater entry 2008 S0.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/19/08 @ 11:00 AM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/19/08 @ 11:01 AM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

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

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Final Report AHC Case: 08-4813

Last Updated: 12/19/08 3:03 PM
Pathologist: Gary D. Marty
Received Date: 12/17/08
Collected Date: 12/11/08
Client Ref No: #7023

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Burry, Gerry**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted one fresh tissue for PCR for IHN and VHS and one formalized sample for histopathology.

Many fish with hem of liver, pyloric caeca 2nd body wall. D.O's have been low at 5.3 mg/L 1

Atlantic salmon. Saltwater entry 2006 S0.

Final Diagnosis

1a. Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate

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

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

Final Comment: In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria (e.g., *Listonella anguillarum*), and infection with VHSV, but often the cause remains unknown. Consider bacteriology (if not already done). Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Sinusoidal congestion has also been described in wild fish (dab) surveyed in the north Atlantic, 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 deposits in congested foci, but nowhere else in the liver, are evidence that the congested foci were acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

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

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

Histopathology

Formalin-fixed tissues were submitted in 1 cassette wrapped in moist paper towel for histopathology.

Slide 1 (7023-1) - brain, spleen, heart, liver, intestine, trunk kidney (2 pieces, one with a small piece of swimbladder)

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

Quality control: Liver autolysis: none. Large foci of erythrocytes (e.g., spleen) have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/19/08 @ 11:01 AM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/19/08 @ 11:01 AM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

Gary D. Marty
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Final Report AHC Case: 08-4814

Last Updated: 12/19/08 4:07 PM
Pathologist: Gary D. Marty
Received Date: 12/17/08
Collected Date: 12/09/08
Client Ref No: #7032

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **MacWilliam, Tiffany**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted one formalized sample for histopathology. PCR test requested (VHSV, IHNV).

Fish presented with shrunken liver. Vaccinated. Saltwater entry 2007 S1.

2nd submission form reads: Fish presented w/ hemorrhaging in p.c. fatty tissues and swimbladder.

Final Diagnosis

1a. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate

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

1c. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, with intracellular lipofuscin and hemosiderin, mild

2. Mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild

Final Comment: This fish has a few lesions common in older farm fish in BC, but none of sufficient severity to assign a cause of death. The sections examined did not have evidence of mesenteric hemorrhage. Comments on specific lesions follow:

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These types of vacuoles were less common before 2007 and might be related to the significant increase in the proportion of plant-based components in commercial feeds that has occurred since 2006. The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion 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 inflammatory mediators) or come from outside the fish (e.g., from the water or the feed). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "silvers" that die in marine net pens, affecting 12% of the 645 Atlantic salmon livers examined in 2007 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2007 was sufficient to identify a trend towards greater prevalence in the fall and winter (14-19%) than in the spring and summer (4.1-10%).

Pigment in the liver probably is lipofuscin, and it might also include hemosiderin. 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; it is more common in older fish. 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 might result from increased turnover of red blood cells.

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

Histopathology

Formalin-fixed tissues were submitted in 1 cassette wrapped in moist paper towel for histopathology.

Slide 1 (7032-1) - heart, spleen, liver, intestine, trunk kidney, mesenteric adipose tissue

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

Quality control: Liver autolysis: mild. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/19/08 @ 11:01 AM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/19/08 @ 11:01 AM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

Gary D. Marty
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Final Report AHC Case: 08-4847

Last Updated: 12/23/08 1:33 PM
Pathologist: Gary D. Marty
Received Date: 12/22/08
Collected Date: 11/20/08
Client Ref No: #6997

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Tiffany Mac William**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted one formalized fish tissue for histology.

Saltwater entry 07, S1.

Black lesions throughout body cavity, enlarged black spleen growing out of body wall.

Atlantic salmon.

Final Diagnosis

1. Visceral mass: malignant melanoma

Final Comment: The slide contains a pigmented neoplastic mass that extends to all margins of the section. The mass is composed of loosely arranged tumour cells that have pleomorphic nuclei and abundant melanin granules. Nuclei are mostly oval, with some as large as 20 µm long. Melanomas are not commonly reported in salmon. I could find reference to only two, and these were in Atlantic salmon from Tasmania (Munday et al. 1998); both of these examples were in the muscle. Based on the clinical history, this one was locally invasive, with moderate metastatic potential. Because melanomas are not a threat to human health, its presences in this fish would not preclude the muscle being marketed as normal product; however, carcass quality might not be ideal.

I have diagnosed one other melanoma in Atlantic salmon; the submitter of that case hypothesized that the prevalence was about one in a million, and that melanomas were most commonly identified as an incidental lesion at harvest.

Literature cited:

Munday, BL, Su, XQ, Harshbarger, JC. 1998. A survey of product defects in Tasmanian Atlantic salmon (*Salmo salar*). Aquaculture 169 (3-4): 297-302.

Histopathology

Formalin-fixed tissues were submitted wrapped in a moist paper towel in 1 cassette for histopathology.

Quality control: Autolysis: moderate. Organs have no postfixation dehydration and no acid hematin deposits.

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

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Final Report AHC Case: 08-4848

Last Updated: 01/02/09 3:59 PM
Pathologist: Gary D. Marty
Received Date: 12/22/08
Collected Date: 12/11/08
Client Ref No: #7045

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Brad Boyce**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted many formalized fish tissue for histology. Please compare to case #7047 (2008-4849).

Saltwater entry 07, S0. Euthanized (TMS) Prior submission - yes 7004 (2008-4652). Netpen/Tank Id: 10

Moribund dipped out of the pen - all fish poor performers off feed. Severe vaccine adhesions varying degrees of lens opacity. #1- Bilateral opaque lens #2 No obvious cataracts, external skin lesions #3 Rings in lens bilateral #4 Abnormal shaped lens, no obvious cataracts #5 Unilateral opaque lens.

Final Diagnosis

1a. Eye: phacolysis, acute, peripheral, mild (slides 1B, 3B, 4B, 5C), moderate (slides 2B, 2C, 5B)

1b. Eye, periorbital adipose tissue: steatitis, lymphohistiocytic, multifocal, mild (slides 4B, 4C)

2a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1A, 2A, 3A, 4A, 5A)

2b. Liver: pericholangitis, lymphohistiocytic, multifocal, mild (slide 1A)

2c. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages (lipofuscin and hemosiderin?), disseminated, mild (slide 3A)

3a: Skeletal muscle: myositis, lymphohistiocytic, focal, with intralesional *Kudoa thyrsites* spores, mild (slides 1A, 2A)

3b. Skeletal muscle: *Kudoa thyrsites* pseudocysts, multifocal, moderate (slide 1C)

4. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild (slide 1A)

5. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 1A, 5A), moderate (slide 4A)

6. Intestinal mesenteries: peritonitis, multifocal, lymphocytic, with fibrocellular fronds, mild (slides 2A, 5A), moderate (slide 4A)

7. Spleen: splenitis, granulomatous, focal, with fibrocellular fronds, mild (slide 4A)

Final Comment: Microscopic changes in these fish are consistent with the clinical history of moribund fish with cataracts, but they provide no clear clues about the specific cause of morbidity. Changes are very similar to case 7047 (2008-4849). Comments on specific findings follow:

Phacolysis refers to the dissolution of the crystalline lens in the eye, which usually occurs spontaneously but is occasionally the result of trauma, infectious agents, or malnutrition. Loss of crystalline structure results in the opaque cataract observed grossly. Phacolysis is sometimes associated with lens rupture. The peripheral nature of the lesion provides evidence that the lesion developed recently (e.g., within the past two weeks for mild lesions). Lenses in these fish have no parasites.

Lymphohistiocytic inflammation in the periorbital adipose tissue is evidence of chronic immune stimulation.

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

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.

Pigment in the liver probably is lipofuscin, and it might also include hemosiderin. 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; it is more common in older fish. 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 might result from increased turnover of red blood cells.

Kudoa thyrsites is a myxosporean that is most common in skeletal muscle, but sometimes occurs in heart muscle. This example has characteristic stellate spores with four unequal polar capsules converging on one end.

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

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or movement) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

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

Histopathology

Formalin-fixed tissues from 5 fish were submitted in 10 cassettes for histopathology. Gills were decalcified for ~2 hours in Protocol B (hydrochloric acid solution) before being rinsed in water and processed routinely into paraffin. Eyes were placed in large cassettes prior to routine processing into paraffin. Blocks containing eyes were subjected to surface decalcification with 8% formic acid before sectioning.

Slide #s - 1A (SP-4 7045), 2A (SP-2 7045), 3A (SP-5 7045), 4A (SP-3 7045) and 5A (1-SP 7045)

Organs included on most slides - heart, liver, spleen, head kidney, trunk kidney, intestine, skin/skeletal muscle

Slide #s - 1B & 1C, 2B & 2C, 3B & 3C, 4B & 4C, 5B & 5C - eye (2 pieces of each)

Slide #s - 1G (SP-4 7045), 2G (SP-2 7045), 3G (SP-5 7045), 4G (SP-3 7045) and 5G (1-SP 7045) - gill

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

Quality control: Liver autolysis: none (slides 1A, 2A, 3A, 4A, 5A). Large foci of erythrocytes (e.g., spleen in slide 1A) have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have no postfixation dehydration.

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

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Final Report AHC Case: 08-4849

Last Updated: 01/02/09 3:55 PM
Pathologist: Gary D. Marty
Received Date: 12/22/08
Collected Date: 12/11/08
Client Ref No: #7047

Veterinarian: **Diane Morrison**
Clinic: **Marine Harvest Canada**
Phone: (250) 850-3276
Fax: (250) 850-3275

Submitter: **Brad Boyce**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

Animal Data
Species: Atlantic Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted many formalized fish tissue (6 + eyes, 3 fish) for histology. Please compare to case #7045 (2008- 4848).

Saltwater entry 07, S0. Euthanized - no; Netpen/Tank Id - Pen 5

Moribund dipped out of the pen - all fish poor performers off feed. Severe vaccine adhesions. Majority unilateral Lens opacity with frank blood in eyes.

Final Diagnosis

1a. Eye: phacolysis, acute, peripheral, mild (slides 1B, 1C, 2C, 3B), moderate (slide 2B)

1b. Eye, periorbital adipose tissue: steatitis, lymphohistiocytic, multifocal, mild (slide 2B)

1c. Eye: intraocular hemorrhage, focal, mild (slide 2B), moderate (slices 2C, 3C)

2. Optic nerve: neuritis, lymphocytic, focal, mild (slide 3C); bifocal, mild (slide 3B)

3a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1A, 2A, 3A)

3b. Liver: pericholangitis, lymphohistiocytic, multifocal, mild (slide 1A)

3c. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate (slide 1A)

3d. Liver: hepatitis, lymphohistiocytic, focal, mild (slide 3A)

4. Skeletal muscle: *Kudoa thyrsites* pseudocysts, focal, mild (slides 1B, 3C), moderate (slides 3A, 3B)

5. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 2A), moderate (slides 1A, 3A)

6. Intestinal mesenteries: peritonitis, multifocal, lymphocytic, with fibrocellular fronds, mild (slide 1A), with mineral, mild (slide 3A)

7. Spleen: splenitis, granulomatous, focal, with fibrocellular fronds and intralesional vacuoles, severe (slide 3A)

8. Head kidney: nephritis, interstitial, granulomatous, multifocal, with multinucleate giant cells, mild (slide 3A)

Final Comment: Microscopic changes in these fish are consistent with the clinical history of moribund fish with cataracts, but they provide no clear clues about the specific cause of morbidity. Changes are very similar to case 7045 (2008-4848). Comments on specific findings follow:

Phacolysis refers to the dissolution of the crystalline lens in the eye, which usually occurs spontaneously but is occasionally the result of trauma, infectious agents, or malnutrition. Loss of crystalline structure results in the opaque cataract observed grossly. Phacolysis is sometimes associated with lens rupture. The peripheral nature of the lesion provides evidence that the lesion developed recently (e.g., within the past two weeks for mild lesions). Lenses in these fish have no parasites.

Lymphohistiocytic inflammation in the periorbital adipose tissue and lymphocytic inflammation in the optic nerve are evidence of chronic immune stimulation.

Intraocular hemorrhage is probably a result of trauma.

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

Lymphohistiocytic inflammation around bile ductules (slide 1A) or within the hepatic parenchyma (slide 3A) is evidence of chronic immune stimulation. Differentials include a focal bacterial infection or reaction to a vaccine.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). In Atlantic salmon livers sampled as part of the Province's Fish Health Auditing and Surveillance Program, prevalence of these vacuoles steadily increased from 42% in 2006 to 50% in 2007 and 55% in 2008; vacuoles tend to be most common in the summer. The increase in vacuole prevalence might be related to the increase in the proportion of plant-based components in commercial feeds that has occurred since 2006. The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown.

Kudoa thyrsites is a myxosporean that is most common in skeletal muscle, but sometimes occurs in heart muscle. This example has characteristic stellate spores with four unequal polar capsules converging on one end.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or movement) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2007 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 34%; n = 642) and Pacific salmon (prevalence = 25%; n = 120).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Vacuoles are probably a result of vaccine material lost during tissue processing.

The small foci of granulomatous inflammation in the head kidney (slide 3A) are probably part of a vaccine reaction. The small size of the foci makes unlikely the primary differential: *Renibacterium salmoninarum* infection.

Histopathology

Formalin-fixed tissues were submitted in 6 cassettes for histopathology. Gills were decalcified for ~2 hours in Protocol B (hydrochloric acid solution) before being rinsed in water and processed routinely into paraffin. Eyes were transferred to large cassettes prior to routine processing into paraffin. Blocks containing eyes were subjected to surface decalcification with 8% formic acid before sectioning.

Slides 1A (BR-3 7047), 2A (BR-2 7047) and 3A (BR-1 7047) - heart, spleen, liver, intestine, head kidney, trunk kidney, skeletal muscle

Slides 1B & 1C, 2B & 2C, 3B & 3C - eye (2 pieces each of each)

Slides 1G (BR-3 7047), 2G (BR-2 7047) and 3G (BR-1 7047) - gill

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

Quality control: Liver autolysis: none (slides 1A, 2A, 3A). Large foci of erythrocytes (e.g., spleen in slide 2A) have precipitates of acid hematin.

Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Gill decalcification is complete. Organs have no postfixation dehydration.

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END OF REPORT