

Final Report AHC Case: 10-103

Last Updated: 01/13/10 4:36 PM

Pathologist: Gary D. Marty

Received Date: 01/11/10

Collected Date:

Client Ref No: 7567

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Brad Boyce- 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 Atlantic salmon tissues for PCR for IHN, VHS and ISA; Also salmon herpesvirus by PCR. (same as case 7566).

Saltwater entry: S0 07. Vaccinated: Yes. Euthanized: No. Prior submission: No.

1 fresh mort - internal hem, exophthalmic, head had skin rubbed off on one side. Small skin lesions. There have been a slight increase in mortis with these signs.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/13/10 @ 9:58 AM

Specimen	ID	Test	Result
Tissue	7567-1	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/13/10 @ 9:58 AM

Specimen	ID	Test	Result
Tissue	7567-1	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/13/10 @ 9:59 AM

Specimen	ID	Test	Result
Tissue	7567-1	PCR - VHSV	Negative

PCR - Herpesvirus salmonis Resulted by: A Scouras Verified by: Dr. J. Robinson on 01/13/10 @ 4:36 PM

Specimen	ID	Test	Result
Tissue	7567-1	PCR - Herpesvirus salmonis	Negative

****:** Test validation in progress.

History of Communication

Date	To	Description
01/14/10 9:22 AM	Morrison, Diane - fax	bc report generated
01/19/10 1:00 PM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-104

Last Updated: 01/14/10 1:59 PM

Pathologist: Gary D. Marty

Received Date: 01/11/10

Collected Date:

Client Ref No: 7566

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Brad Boyce- 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 Atlantic salmon tissues for Histopathology and salmon herpesvirus by PCR.

Saltwater entry: S0 08. Vaccinated: Yes. Insurance legal : No. Euthanized: Yes, percussive. Prior submission: Yes, 7559, 7548.

Moribund fish have skin lesions at various stages.

Fish #1 - skin lesion only.

2-5 - included all organs from Histo.

Fish #3/5 - Had only small lesion and were on feed.

Flesh samples for Histo were trimmed and placed in cassette as per Dr. Gary Marty instructions. (See attached).

Final Diagnosis

1a. Skin: dermatitis, ulcerative, fibrinous, neutrophilic, histiocytic, with plasma-filled dermal scale pockets, focal, severe (slides 1, 5)

1b. Skin: dermatitis, ulcerative, fibrinous, pleocellular, diffuse, severe (slide 3)

1c. Skin: dermatitis and panniculitis, erosive, with fibroplasia and petechiae, diffuse, severe (slide 4)

2a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 2, 3, 4, 5)

2b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slide 3)

2c. Liver: hepatitis, perivascular, lymphocytic, multifocal, mild (slides 4, 5)

2d. Liver: pericholangitis, lymphohistiocytic, focal, mild (slide 5)

2e. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild

Case: 10-104

(slides 3, 4)

3. Head kidney: interstitial golden pigment, focal, mild (slide 3)

4a. Spleen: parenchymal golden pigment (lipofuscin?), scattered, intracellular, mild (slides 3, 4, 5), moderate (slide 2)

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

5. Heart: endocarditis, lymphohistiocytic, with endothelial cell hypertrophy, multifocal, mild (slide 4)

Final Comment: Skin lesions in these fish are the primary cause of morbidity, and the range of diagnoses in these samples is probably representative of the range of lesions occurring in the fish. All the skin lesions could all be a result of the same cause. Ulcers are notorious as significant lesions for which there is often no known cause. The sections in this case contain no obvious organisms, but bacterial culture would probably yield a *Vibrio* or related bacterial species. Whether the cultured organism was a primary or secondary pathogen would be difficult to determine. In general, ulcers tend to be more common among fish under some type of stress (handling, movement, toxin exposure, predators, etc.). Among the clinical differentials, *Herpesvirus salmonis* is unlikely because Atlantic salmon are resistant to infection, and even among susceptible species skin is not a major target organ [reference: Roberts "Fish Pathology, Third Edition," 2001; p. 201]. Most of the other lesions in these fish are probably secondary to the ulcers.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It might be related to increased protein needed as part of an inflammatory response. It is common in juvenile fish with ulcers. Mature females normally have basophilic hepatocytes: needed to produce protein for deposition in their eggs.

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, but then decreased in 2009 back to 43%. The increase in vacuole prevalence might be related changes in the proportion of plant-based components in commercial feeds. At least some types of vacuoles might be normal; their effect on growth and feed conversion is unknown.

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

Lymphohistiocytic inflammation around bile ductules (liver, slide 5) 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 is probably 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 and head kidney most likely is lipofuscin, with same differentials as in the liver. Pigment in the head kidney of fish #2 is limited to several cells in a single 500-µm-diameter focus.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe).

Lymphohistiocytic endocarditis, along with endothelial cell hypertrophy (as in slide 4) is a fairly common combination of lesions related to bacterial and viral infections, but the section contains no obvious organisms. The inflammation might be part of a systemic response to the skin ulcers.

Histopathology

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

Slide 1 (7566-1) - skin and skeletal muscle

Slide 2 (7566-3) - heart, liver, spleen, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide 3 (7566-2) - skin/skeletal muscle, heart, liver, spleen, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide 4 (7566-4) - brain, skin/skeletal muscle, heart, liver, spleen, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide 5 (7566-5) - skin/skeletal muscle, heart, liver, spleen, 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: none (slides 2, 3, 4, 5). Skin autolysis: none (slide 1). Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - Herpesvirus salmoni Resulted by: A Scouras Verified by: Dr. J. Robinson on 01/13/10 @ 4:36 PM

Specimen	ID	Test	Result
Tissue	7566-1	PCR - Herpesvirus salmonis	Negative
Tissue	7566-2	PCR - Herpesvirus salmonis	Negative
** Test validation in progress.			

History of Communication

Date	To	Description
01/14/10 1:59 PM	Morrison, Diane - fax	bc report generated
01/19/10 1:01 PM	Marine Harvest Canada - e-mail	Case Invoiced



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END OF REPORT

Final Report AHC Case: 10-194

Last Updated: 01/19/10 11:20 AM

Pathologist: Gary D. Marty

Received Date: 01/15/10

Collected Date: 12/30/09

Client Ref No: 10-001

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 fresh Atlantic salmon tissue for Histopathology. Request Net pen liver rule out.

Saltwater. Vaccinated: Yes. Insurance: No. Euthanized: Yes, Stun. Prior submission: No. # submitted dead: 4. DOD: Dec. 30/09.

High mortality in population with extensive lesions/winter ulcers displayed in mortality and slow swimmers in corners of pens.

Final Diagnosis

- 1a. Liver: hepatocellular hydropic degeneration, disseminated, acute, mild (slides 1, 2)
- 1b. Liver: hepatocellular single cell necrosis , disseminated, acute, mild (slide 4), moderate (slide 1), severe (slides 2, 3)
- 1c. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slides 1, 4)
- 1d. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slide 2), moderate (slides 1, 2)
- 1e. Liver: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, moderate (slide 1), severe (slide 2)
- 1f. Liver: hepatocellular karyomegaly, diffuse, mild (slides 2, 4)
- 1g. Liver: hepatocellular fatty change (lipidosis), disseminated, moderate (slide 2)
- 1h. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 2, 4)
- 1i. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 4)
2. Mesenteric adipose tissue: peritonitis, granulomatous, multifocal, with occasional fine fibrocellular fronds, mild (slides 2, 4), moderate (slide 1), severe (slide 3)
3. Stomach: intraluminal metazoans with compound eyes (copepods? Slide 2)

3. Intestinal ceca: intraluminal nematode (~90 µm in diameter , with lateral chords and alae, Ascarid?), focal (3 parasite sections are probably of the same individual), mild (slide 3)

Final Comment: These fish have a plethora of liver lesions, many of which are consistent with an early form of the clinical syndrome known as 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, and sinusoidal lipofuscin accumulation. Lack of parenchymal collapse is evidence that the process is relatively acute (going on for several days to a few weeks). Hepatic karyomegaly 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. The presence of copepods in the stomach of fish #2 and a nematode parasite in an intestinal cecum of fish #3 is evidence that these fish were eating environmental organisms rather than formulated feed. By comparison, the stomach in fish #s 1 and 4 contain only formulated feed. Comments on other lesions follow:

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen or fluid). 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, but then decreased back to 43% in 2009. The increase in vacuole prevalence might be related changes in the proportion of plant-based components in commercial feeds. At least some types of vacuoles might be normal; their effect on growth and feed conversion is unknown.

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 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 "fresh silvers" that die in marine net pens, affecting 14% of the 514 Atlantic salmon livers examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

Peritonitis is consistent with a reaction to foreign material ; it is common in fish that have been vaccinated, affecting the spleens of 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe).

Histopathology

Formalin-fixed tissues from 4 fish were submitted in 4 cassettes for histopathology. Slide #s 1-4 are labeled in the same order as client #s 1 - 4.

Organs included on all slides: liver, stomach, intestinal ceca, mesenteric adipose tissue.

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

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

History of Communication

Date	To	Description
01/19/10 11:21 AM	Mainstream Canada - e-mail	bc report generated
01/19/10 11:21 AM	Dr. Peter McKenzie - e-mail	bc report generated
01/22/10 1:12 PM	Mainstream Canada - e-mail	Case Invoiced



Gary D. Marty
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END OF REPORT

Final Report AHC Case: 10-219

Last Updated: 02/11/10 4:24 PM

Pathologist: Gary D. Marty

Received Date: 01/15/10

Collected Date: 01/11/10

Client Ref No: 7572

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 Atlantic tissues for PCR for IHN, VHS, ISA and Salmon herpesvirus; Also cell culture and Histopathology.

Saltwater entry: 08 S0. Vaccinated: Yes. # in group - 6. # dead - 6. Euthanized: Yes, Percussion. Prior submission: Yes, 7548, 7559, 7566. DOD: Jan. 11/10.

Fish 1 had no lesion and fish 2 - 6 had lesions at various stages. Fish 6 had 2 skin lesions. Fish 3 had a lesion that looked partially healed, with scale loss. Flesh samples were trimmed and placed in cassettes as per Dr. Gary Marty instructions. Gills in separate cassettes.

Final Diagnosis

- 1a. Skin: dermatitis and panniculitis, ulcerative, with moderate numbers of superficial bacterial rods (*Vibrio* or related species?), diffuse, severe (slide 6B)
- 1b. Skin: dermatitis, panniculitis, and myositis, ulcerative, pleocellular, with superficial fibrin, petechiae, and a few bacterial rods (*Vibrio* or related species?), diffuse, severe (slides 5C, 6A, 6B)
- 1c. Skin: full-thickness dermal ulceration, with partial re-epithelialization (slides 2A, 4A, 5A, 6B)
- 1d. Skin: dermatitis, ulcerative, fibrinous, neutrophilic, histiocytic, with plasma-filled dermal scale pockets, focal, moderate (slide 6A); multifocal, severe (slide 2A)
- 1e. Skin: dermatitis, panniculitis, and myositis, ulcerative, pleocellular, with superficial fibrin and petechiae, diffuse, moderate (slide 5C), severe (slides 4A, 5C)
- 1f. Skin: dermatitis, ulcerative, with subepidermal granulation tissue, regionally diffuse, severe (slide 2A)
- 1g. Skin: dermatitis, ulcerative, fibrinous, with subepidermal and intradermal granulation tissue, regionally diffuse, severe (slide 2A)
- 1h. Skin: dermatitis and panniculitis, ulcerative, multifocal, with fibroplasia, mild (slide 5C)
- 2a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 4C)

2b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 2C, 3B), moderate (slides 4C, 6D)

2c. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slides 1B, 5A)

2d. Liver: hepatitis, perivascular, lymphocytic, multifocal, mild (slide 2C)

2e. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slides 1B, 2C, 4C, 6D)

3a. Spleen: parenchymal golden pigment (lipofuscin?), scattered, intracellular, mild (slides 1B, 3B, 6D)

3b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 2C, 3B, 5A), moderate (slide 1B)

4. Intestine: peritonitis, chronic, diffuse, with fibrocellular fronds, mild (slide 3B), moderate (slide 5A), severe (slide 1B)

5. Heart: endocarditis, lymphohistiocytic, with endothelial cell hypertrophy, multifocal, mild (slides 1B, 3B, 4C)

6. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (slide 6D), moderate (slide 4C)

Final Comment: Skin lesions in these fish are the primary cause of morbidity, and the variety of diagnoses in these samples is probably representative of the range of lesions occurring in the fish (consistent with gross findings). All the severe skin lesions could be a result of the same underlying cause. The most severe lesions have ulcers that penetrate through the entire dermis (4 of 5 fish affected). All of these lesions have some re-epithelialization over the remaining panniculus: evidence of limited repair. The prognosis for these deep ulcers, however, is poor; even if the underlying problem is resolved, the dermis does not regenerate and these fish will always have scars.

Ulcers are notorious as significant lesions for which there is often no known cause. Unlike the previous submission, however, a few samples in this case contain bacterial rods consistent with a *Vibrio* species. Whether the organisms are a primary or secondary pathogen is unknown, but numbers of organisms are usually low compared with the extent of the lesions.

In general, ulcers tend to be more common among fish under some type of stress (handling, movement, toxin exposure, predators, etc.). Consider also the possibility of trauma contributing to lesion development. Among the clinical differentials, *Herpesvirus salmonis* is unlikely because Atlantic salmon are resistant to infection, and even among susceptible species skin is not a major target organ [reference: Roberts "Fish Pathology, Third Edition," 2001; p. 201]. Most of the other lesions in these fish are probably secondary to the ulcers.

Among the 5 pieces of skin included on slide 3D (fish #3), 4 include scales and 1 has no scales. This is consistent with the gross findings. The piece without scales has no inflammation to link it with the lesions in the other fish (i.e., it might simply be a focus of scale loss independent of what is affecting the other fish). Scales were not included any of the 5 pieces of skin in slide 2A.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It might be related to increased protein needed as part of an inflammatory response. It is common in juvenile fish with ulcers. Mature females normally have basophilic hepatocytes: needed to produce protein for deposition in their eggs.

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). In Atlantic salmon livers sampled as part of the Province's Fish Health Auditing and Surveillance Program, prevalence of these vacuoles steadily increased in Atlantic salmon from 42% in 2006 to 50% in 2007 and 55% in 2008, but then decreased in 2009 back to 43%. The increase in vacuole prevalence might be related changes in the proportion of plant-based components in commercial feeds. At least some types of vacuoles might be normal; their effect on growth and feed conversion is unknown.

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

Pigment in the liver is probably 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, with same differentials as in the liver.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting spleens of 65% of the 509

Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe).

Lymphohistiocytic endocarditis, along with endothelial cell hypertrophy is a fairly common combination of lesions related to bacterial and viral infections, but the heart contains no obvious organisms. The inflammation might be part of a systemic response to the skin ulcers.

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

Histopathology

Formalin-fixed tissues from 6 fish were submitted in 18 cassettes for histopathology.

Slide #s 1A - 6D are labeled in the same order as client #s 1 - 6. All fish have a slide with gill filaments and a slide with organs (heart, liver, spleen, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue; some also include skeletal muscle); all but fish #1 also have a slide with 3 to 5 pieces of skin and skeletal muscle (fish #6 has 2 of these slides). All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Liver autolysis: none (slides 1B, 2C, 3B, 4C, 5A, 6D). Acute telangiectasis in the gill (slides 2B, 6C) is probably secondary to euthanasia by blunt trauma. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 01/19/10 @ 1:53 PM

Specimen	ID	Test	Result
Tissue	7572-1 organs	PCR - IHNV	Negative
Tissue	-2 organs	PCR - IHNV	Negative
Tissue	-3 organs	PCR - IHNV	Negative
Tissue	-4 organs	PCR - IHNV	Negative
Tissue	-5 organs	PCR - IHNV	Negative
Tissue	-6 organs	PCR - IHNV	Negative
Tissue	-2 lesions	PCR - IHNV	Negative
Tissue	-3 lesions	PCR - IHNV	Negative
Tissue	-4 lesions	PCR - IHNV	Negative
Tissue	-5 lesions	PCR - IHNV	Negative
Tissue	-6 lesions	PCR - IHNV	Negative

PCR - ISA Resulted by: A Scouras Verified by: Dr. J. Robinson on 01/19/10 @ 1:53 PM

Specimen	ID	Test	Result
Tissue	7572-1 organs	PCR - ISA	Negative
Tissue	-2 organs	PCR - ISA	Negative
Tissue	-3 organs	PCR - ISA	Negative
Tissue	-4 organs	PCR - ISA	Negative
Tissue	-5 organs	PCR - ISA	Negative
Tissue	-6 organs	PCR - ISA	Negative
Tissue	-2 lesions	PCR - ISA	Negative
Tissue	-3 lesions	PCR - ISA	Negative
Tissue	-4 lesions	PCR - ISA	Negative
Tissue	-5 lesions	PCR - ISA	Negative

Tissue	-6 lesions	PCR - ISA	Negative
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PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 01/19/10 @ 1:53 PM

Specimen	ID	Test	Result
Tissue	7572-1 organs	PCR - VHSV	Negative
Tissue	-2 organs	PCR - VHSV	Negative
Tissue	-3 organs	PCR - VHSV	Negative
Tissue	-4 organs	PCR - VHSV	Negative
Tissue	-5 organs	PCR - VHSV	Negative
Tissue	-6 organs	PCR - VHSV	Negative
Tissue	-2 lesions	PCR - VHSV	Negative
Tissue	-3 lesions	PCR - VHSV	Negative
Tissue	-4 lesions	PCR - VHSV	Negative
Tissue	-5 lesions	PCR - VHSV	Negative
Tissue	-6 lesions	PCR - VHSV	Negative

PCR - Herpesvirus salmoni Resulted by: A Scouras Verified by: Dr. J. Robinson on 01/19/10 @ 4:23 PM

Specimen	ID	Test	Result
Tissue	7572-1 organs	PCR - Herpesvirus salmoni	Negative
Tissue	-2 organs	PCR - Herpesvirus salmoni	Negative
Tissue	-3 organs	PCR - Herpesvirus salmoni	Negative
Tissue	-4 organs	PCR - Herpesvirus salmoni	Negative
Tissue	-5 organs	PCR - Herpesvirus salmoni	Negative
Tissue	-6 organs	PCR - Herpesvirus salmoni	Negative
Tissue	-2 lesions	PCR - Herpesvirus salmoni	Negative
Tissue	-3 lesions	PCR - Herpesvirus salmoni	Negative
Tissue	-4 lesions	PCR - Herpesvirus salmoni	Negative
Tissue	-5 lesions	PCR - Herpesvirus salmoni	Negative
Tissue	-6 lesions	PCR - Herpesvirus salmoni	Negative

**[: Test validation in progress.](#)

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 02/11/10 @ 4:24 PM

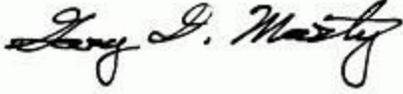
Specimen	ID	Isolate	Result	Level
Tissue	7572-1 organs		No viruses isolated	
Tissue	-2 organs		No viruses isolated	
Tissue	-3 organs		No viruses isolated	
Tissue	-4 organs		No viruses isolated	
Tissue	-5 organs		No viruses isolated	
Tissue	-6 organs		No viruses isolated	
Tissue	-2 lesions		No viruses isolated	
Tissue	-3 lesions		No viruses isolated	
Tissue	-4 lesions		No viruses isolated	
Tissue	-5 lesions		No viruses isolated	
Tissue	-6 lesions		No viruses isolated	

Staff Comments:

Histopathology and PCR results sent to MH staff via Outlook on Tues 2010-01-19 4:35PM by GD Marty.

History of Communication

Date	To	Description
02/11/10 5:45 PM	Morrison, Diane - fax	bc report generated
02/16/10 8:56 AM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-310

Last Updated: 01/27/10 11:31 AM
Pathologist: Gary D. Marty
Received Date: 01/22/10
Collected Date:
Client Ref No: 7572

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 2 Atlantic salmon cultures for Bacteriology.

Saltwater entry: 08S0. Vaccinated: Yes. # in group - 2. Euthanized: Yes, percussion. Prior submission: Yes, #7548, 7559, 7566.

Fish had lesions on side, #4 and #6 (had 2 lesions) Sample #4 and #6B sent for Bacterial Identification.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 01/27/10 @ 11:31 AM

Specimen	ID	Isolate	Result	Level
Isolate	7572 #4	Pseudoalteromonas sp.	Positive	
**: Pseudoalteromonas sp. identified as Pseudoalteromonas denitrificans.				
Isolate	7572 #6B	Vibrio tapetis	Positive	

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 01/27/10 @ 11:31 AM

Organism	ID	e	ffc	s3	sxt	ot
Pseudoalteromonas sp.	7572 #4	s	s	s	s	s
Vibrio tapetis	7572 #6B	s	s	s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethopri, ot = Oxytetracycline						

History of Communication

Date	To	Description
01/27/10 4:22 PM	Morrison, Diane - fax	bc report generated
01/28/10 1:50 PM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-312

Last Updated: 01/26/10 9:42 AM

Pathologist: Gary D. Marty

Received Date: 01/22/10

Collected Date:

Client Ref No: 7579

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 formalized Atlantic tissue for Histopathology.

Saltwater entry: 09 S0. Vaccinated: Yes. Insurance: No. Euthanized: No. Prior submission: No.

Sampled one fresh mort. Kidney was swollen and appeared to have Nephrocalcinosis. Fish were entered into saltwater 3 weeks ago.

Final Diagnosis

1. Trunk kidney: renal tubular intraluminal necrotic cells, interstitial fibrosis, and tubular luminal dilation, multifocal, moderate

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

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

Final Comment: Small numbers of renal tubular intraluminal necrotic cells are fairly common in fish that have significant inflammation somewhere in their body. With this lesion, tubule lumens usually contain only one or two necrotic cells. In many cases (like this one), tubular epithelium surrounding these cells is attenuated (evidence of epithelial cell loss, with stretching of the remaining epithelium to fill the resultant gap). The underlying cause of the necrotic cells is often not determined, but differentials include anything that causes significant inflammation. Fibrosis is evidence of more extensive damage and efforts at repair. Causes in fish 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. The section has no evidence of mineralization.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. 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.

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

Slide 1 (7579-1) - heart, spleen, liver, head kidney/trunk kidney transition, trunk kidney, intestine, mesenteric adipose tissue

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

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

History of Communication

Date	To	Description
01/26/10 9:46 AM	Morrison, Diane - fax	bc report generated
01/28/10 1:51 PM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-314

Last Updated: 01/27/10 12:07 PM

Pathologist: Gary D. Marty

Received Date: 01/22/10

Collected Date:

Client Ref No: 7587

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 Atlantic tissue for Histopathology and PCR for IHN, VHS and ISA.

Saltwater entry: 09 S1. Vaccinated: Yes. Insurance: No. Euthanized: Yes, percussive. Prior submission: No.

Staff noticed "Yellow belly" on apparently healthy fish. Internally liver was hemorrhaged and yellow/orange in colour. Fish had not been eating recently.

Final Diagnosis

1a. Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate

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

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

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

2a. Spleen: splenitis, neutrophilic, multifocal, moderate

2b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

3a. Trunk kidney: nephritis, interstitial, neutrophilic, multifocal, moderate

3b. Trunk kidney: renal tubular intraluminal necrotic cells, with luminal dilation, multifocal, mild

3c. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild

Final Comment: This fish has several lesions that provide clues to morbidity, but a specific cause cannot be identified. Comments on specific lesions follow:

Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined (as in this case). Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia (and PCR results rule it out in this case). 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 are probably 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 liver, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins or 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 "fresh silvers" that die in marine net pens, affecting 14% of the 514 Atlantic salmon livers examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. 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.

Neutrophilic splenitis and interstitial nephritis are evidence of acute inflammation. The multifocal distribution is evidence of a pathogen disseminated through the blood, but the section has no organisms on Twort's Gram stain.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe).

Small numbers of renal tubular intraluminal necrotic cells are fairly common in fish that have significant inflammation somewhere in their body. With this lesion, tubule lumens usually contain only one or two necrotic cells. In many cases, tubular epithelium surrounding these cells is attenuated (evidence of epithelial cell loss, with stretching of the remaining epithelium to fill the resultant gap). In other cases (like this one), the height of tubular lining cells is normal. The underlying cause of the necrotic cells is often not determined, but differentials include anything that causes significant inflammation.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) 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 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469).

Histopathology

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

Slide 1 (7587-1) - heart, spleen, liver, head kidney, trunk kidney, skeletal muscle, intestine.

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). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Ken Sojnyky Verified by: Dr. J. Robinson on 01/26/10 @ 1:24 PM

Specimen	ID	Test	Result
Tissue	7587	PCR - IHNV	Negative

PCR - ISA Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 01/26/10 @ 1:24 PM

Specimen	ID	Test	Result
Tissue	7587	PCR - ISA	Negative

PCR - VHSV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 01/26/10 @ 1:24 PM

Specimen	ID	Test	Result
Tissue	7587	PCR - VHSV	Negative

History of Communication

Date	To	Description
01/27/10 12:07 PM	Morrison, Diane - fax	bc report generated
01/28/10 1:52 PM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-328

Last Updated: 02/16/10 10:34 AM

Pathologist: Gary D. Marty

Received Date: 01/25/10

Collected Date: 01/20/10

Client Ref No: 10-002

Veterinarian: **Dr. Peter McKenzie**

Clinic: **Mainstream Canada**

Phone: (250) 286-0022

Fax: (250) 286-0042

Submitter: **Nathan Cassan - Mainstream Canada**

Phone:

Fax:

Owner: **Mainstream Canada**

Phone:

Fax:(250) 286-0042

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID: Cecil Island

Case History

Submitted formalized Atlantic tissues as well as a culture plate.

Please test according to directions:

Formalized tissues for Histopathology.

Head kidney and spleen for Virology and PCR - VHSV, IHNV - Tissue for Virology/PCR can be pooled.

Brain, trunk kidney plates- For Bacteriology.

Smolt. Saltwater. Vaccinated: Yes. Euthanized: No. Prior submission: No. # dead - 4. DOD: Jan. 20/10.

Lesions/ulcers dominating mortality. Full set of testing requested to cover all possibilities as hemorrhaging present as well. Each fish plated on TSA and TSA w/5%S.B. for Bacti.

Final Diagnosis

- 1a. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slide 2), moderate (slides 1, 4)
- 1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1, 2, 3)
- 2a. Trunk kidney: renal tubular intraluminal necrotic cells, with luminal dilation, multifocal, mild (*Yersinia ruckeri*?, slide 2)
- 2b. Trunk kidney: interstitial cell atrophy, diffuse, moderate (slides 1, 3)
- 2c. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slide 2)
3. Intestinal ceca and mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1), moderate (slide 2)
4. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1)

5. Gill: lamellar epithelial lifting (interstitial/subepithelial edema or postmortem artifact?), multifocal, moderate (slide 3G)

Final Comment: Lesions in these fish are consistent with the clinical history of ulcers, but as commonly occurs with ulcers, the ultimate cause of the ulcers remains unknown. Ulcers on BC farmed Atlantic salmon tend to be covered with filamentous bacteria (*Tenacibaculum maritimum*) or *Vibrio* (or related) species; culture results in this case support the invasion of *Aliivibrio wodanis* through the ulcers. *Aliivibrio wodanis* may be a postmortem contaminant. Although *Aliivibrio wodanis* is commonly cultured from Atlantic salmon with "winter ulcers," Koch's postulates have not been fulfilled. Instead, *Moritella viscosa* (old name = *Vibrio viscosus*) has been identified as the cause of winter ulcers (i.e., Koch's postulates have been fulfilled). [Source T. Lunder, et al. 2000. Phenotypic and genotypic characterization of *Vibrio viscosus* sp. nov. and *Vibrio wodanis* sp. nov. isolated from Atlantic salmon (*Salmo salar*) with 'winter ulcer.' International Journal of Systematic and Evolutionary Microbiology 50:427-450.] Comments on specific lesions follow:

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen or fluid). 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, but then decreased back to 43% in 2009. The increase in vacuole prevalence might be related changes in the proportion of plant-based components in commercial feeds. At least some types of vacuoles might be normal; their effect on growth and feed conversion is unknown

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It might be related to increased protein needed as part of an inflammatory response. It is common in juvenile fish with ulcers.

Small numbers of renal tubular intraluminal necrotic cells are fairly common in fish that have significant inflammation somewhere in their body. With this lesion, tubule lumens usually contain only one or two necrotic cells. In many cases (like this one), tubular epithelium surrounding these cells is attenuated (evidence of epithelial cell loss, with stretching of the remaining epithelium to fill the resultant gap). The underlying cause of the necrotic cells is often not determined, but differentials include anything that causes significant inflammation. In this case, the differential of *Yersinia ruckeri* is supported by bacterial culture results (note, however, that the lesions are not classic for death due to *Yersinia ruckeri* infection).

Interstitial cell atrophy in the kidney might be a result of decreased production or increased release of hematopoietic cells, or a combination of the two. This could be a result of viral, parasitic, or bacterial infection; the sections contain no obvious organisms.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) 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 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 36% ; n = 511).

Peritonitis of the spleen, intestinal ceca, and adjacent mesenteries is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting the mesenteries of 54% of the 514 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (40% were mild, 12% were moderate, and 2.1% were severe).

Separation of gill lamellar epithelium from underlying pillar cells can be a result of edema, and it also is a common postmortem artifact. In this case, accumulation of proteinaceous fluid deep to the separated epithelium supports the diagnosis of edema over artifact. However, the fact that the affected fish died before it was sampled increases the chances that the change is a postmortem artifact (epithelium begins to lift off the lamellae just 5 minutes after fish death). Lamellar edema is commonly associated with exposure to toxins, including formalin and hydrogen peroxide overdose. Lamellar edema is reversible if the inciting cause is removed.

Histopathology

Formalin-fixed tissues from 4 fish were submitted in 4 cassettes for histopathology. Gills were removed from the original cassettes and placed in separate (G) cassettes. The gills were immersed 1.25 h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

Slide #s 1 - 4 are labeled in the same order as client #s 1 - 4

Organs included on most slides 1, 2, 3, and 4 - brain, heart, liver, spleen (not in slides 2, 3), trunk kidney, intestinal ceca, mesenteric adipose tissue.

Slide #s 1G, 2G, 3G, 4G - gill

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

Quality control: Liver autolysis: mild (slides 1, 2, 3, 4). The lack of good preservation in the entire liver in some sections was at least partly a result of too much tissue in the cassettes. Some of this can be avoided by placing the gill in a separate cassette (Animal Health Centre charges are based more on # of fish than # of slides, and 2 cassettes/fish are charged the same as 1 cassette/fish). Organs have no postfixation dehydration and no acid hematin deposits.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 01/28/10 @ 4:21 PM

Specimen	ID	Isolate	Result	Level
Isolate	Fish 1 Brain/Kidney	Aliivibrio wodanis	Positive	
Isolate	Fish 2 Brain/Kidney	Aliivibrio wodanis	Positive	
Isolate	Fish 3 Brain/Kidney	Aliivibrio wodanis	Positive	
**: Unable to perform antibiotic sensitivity testing due to poor growth.				
Isolate	Fish 3 Brain/Kidney	Yersinia ruckeri	Positive	
Isolate	Fish 4 Brain/Kidney	Yersinia ruckeri	Positive	

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 01/28/10 @ 4:22 PM

Organism	ID	e	ffc	s3	sxt	ot
Yersinia ruckeri	Fish 3 Brain/Kidney	r	s	s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethoprim, ot = Oxytetracycline						

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 01/28/10 @ 3:51 PM

Specimen	ID	Test	Result
Tissue	pool of kd,sp fish 1-4	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 01/28/10 @ 3:51 PM

Specimen	ID	Test	Result
Tissue	pool of kd,sp fish 1-4	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 02/16/10 @ 10:34 AM

Specimen	ID	Isolate	Result	Level
Tissue	pool of kd,sp fish 1-4		No viruses isolated	

History of Communication

Date	To	Description
01/29/10 9:55 AM	Mainstream Canada - e-mail	bc report generated
01/29/10 9:55 AM	Dr. Peter McKenzie - e-mail	bc report generated

02/18/10 10:23 AM
02/18/10 10:23 AM
03/03/10 4:23 PM

Mainstream Canada - e-mail
Dr. Peter McKenzie - e-mail
Mainstream Canada - e-mail

bc report generated
bc report generated
Case Invoiced



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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END OF REPORT

Final Report AHC Case: 10-329

Last Updated: 02/16/10 10:35 AM

Pathologist: Gary D. Marty

Received Date: 01/25/10

Collected Date: 01/20/10

Client Ref No: 10-003

Veterinarian: **Dr. Peter McKenzie**

Clinic: **Mainstream Canada**

Phone: (250) 286-0022

Fax: (250) 286-0042

Submitter: **Nathan Cassan - Mainstream Canada**

Phone:

Fax:

Owner: **Mainstream Canada**

Phone:

Fax:(250) 286-0042

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID: Cliff Bay

Case History

Submitted formalized Atlantic tissues as well as a culture plate.

Please test according to directions:

Formalized tissues for Histopathology.

Head kidney and spleen for Virology and PCR - VHSV, IHNV - Tissue for Virology/PCR can be pooled.

Brain, trunk kidney plates- For Bacteriology.

Smolt. Saltwater. Vaccinated: Yes. Euthanized: No. Prior submission: No. # dead - 4. DOD: Jan. 20/10.

Lesions/ulcers dominating mortality with hemorrhaging present in most cases. Full set of testing requested in order to cover all possibilities. Each fish plated on TSA and TSA w/5%S.B. for Bacti.

Final Diagnosis

1a. Gill: lamellar capillary thrombosis, multifocal, acute, mild (slide 4G), moderate (slide 2G), severe (slides 1G, 3G)

1b. Gill: lamellar telangiectasis, focal, mild (slide 2G); multifocal, mild (slide 3G), moderate (slide 1G)

2a. Liver: sinusoidal congestion, with acid hematin granules focal, moderate (slide 2), and intracytoplasmic spherical golden to amphophilic inclusions, acute, focal, moderate (slide 1)

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

3. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slide 4)

4. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild (slide 3)

5. Intestinal ceca and mesenteries: peritonitis, chronic, focal, with fibrocellular fronds and intralesional vacuoles about 50 µm in diameter, mild

(slides 1, 4)

6. Spleen: peritonitis, granulomatous, multifocal, with fibrocellular fronds, mild (slides 3, 4), moderate (slide 2)

Final Comment: In addition to the ulcers described grossly, all fish have some degree of thrombosis of gill lamellar capillaries; in three of the fish, the lesions are of sufficient severity to have contributed to the fish's death. Thrombosis is evidence of increased coagulability, resulting from endothelial damage related to virus, bacterial, or parasitic infection, or exposure to toxins from harmful algal blooms. Comments on specific lesions follow:

Telangiectasis in the gill (lamellar capillary aneurysms or ruptured lamellar capillaries) most commonly results from trauma (e.g., handling).

Focal sinusoidal congestion in the liver is a nonspecific vascular lesion. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. 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 are probably 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 are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

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

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) 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 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 36% ; n = 511).

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 23% of the 512 Atlantic salmon hearts examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

Peritonitis of the spleen, intestinal ceca, and adjacent mesenteries is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting the mesenteries of 54% of the 514 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (40% were mild, 12% were moderate, and 2.1% were severe). Vacuoles are probably a result of vaccine material lost during tissue processing.

Histopathology

Formalin-fixed tissues from 4 fish were submitted in 4 cassettes for histopathology. Gills were removed from the original cassettes and placed in separate (G) cassettes. The gills were immersed 1.25 h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

Slide #s 1 - 4 are labeled in the same order as client #s 1 - 4

Organs included on most slides 1, 2, 3, and 4 - brain, heart, liver, spleen (not in slide 1), trunk kidney, intestinal ceca, mesenteric adipose tissue.

Slide #s 1G, 2G, 3G, 4G - gill

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

Quality control: Liver autolysis: moderate (slides 1, 3), severe (slides 2, 4). Foci of erythrocytes (e.g., liver 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). Gill decalcification is complete and differential staining is good. Organs have no postfixation dehydration.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 01/29/10 @ 1:49 PM

Specimen	ID	Isolate	Result	Level
Brain	Fish 1	Vibrio lentus	Positive	
Kidney	Fish 1		No Bacteria Isolated	
Brain	Fish 2	Photobacterium iliopiscarium	Positive	
Kidney	Fish 2		No Bacteria Isolated	
Brain	Fish 3	Photobacterium iliopiscarium	Positive	
Kidney	Fish 3		No Bacteria Isolated	
Brain	Fish 4	Vibrio lentus	Positive	
Kidney	Fish 4		No Bacteria Isolated	

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 01/29/10 @ 1:49 PM

Organism	ID	e	ffc	s3	sxt	ot
Photobacterium iliopiscarium	Fish 2	s	s	s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethopri ot = Oxytetracycline						
Vibrio lentus	Fish 1	s	s	s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethopri ot = Oxytetracycline						

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 01/28/10 @ 3:51 PM

Specimen	ID	Test	Result
Tissue	pool of kd,sp fish 1-4	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 01/28/10 @ 3:52 PM

Specimen	ID	Test	Result
Tissue	pool of kd,sp fish 1-4	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 02/16/10 @ 10:35 AM

Specimen	ID	Isolate	Result	Level
Tissue	pool of kd,sp fish 1-4		No viruses isolated	

History of Communication

Date	To	Description
01/29/10 3:04 PM	Mainstream Canada - e-mail	bc report generated
01/29/10 3:05 PM	Dr. Peter McKenzie - e-mail	bc report generated

Case: 10-329

02/18/10 10:24 AM
02/18/10 10:24 AM
03/03/10 4:24 PM

Mainstream Canada - e-mail
Dr. Peter McKenzie - e-mail
Mainstream Canada - e-mail

bc report generated
bc report generated
Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-485

Last Updated: 02/12/10 3:22 PM

Pathologist: Gary D. Marty

Received Date: 02/02/10

Collected Date: 01/27/10

Client Ref No: 10-004

Veterinarian: **Dr. Peter McKenzie**

Clinic: **Mainstream Canada**

Phone: (250) 286-0022

Fax: (250) 286-0042

Submitter: **Mainstream Canada**

Phone:

Fax:

Owner: **Mainstream Canada**

Phone:

Fax:(250) 286-0042

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID: Raza Island

Case History

Submitted 12 Atlantic salmon Bacti plates for Identification and Drug sensitivities for Bacteriology.

Age: Smolt. Saltwater. Vaccinated: Yes. Legal: No. Euthanized: Yes, Stun. Prior submission: Yes - 10 - 194. # submitted dead - 10. DOD: Jan. 27/10.

10 fish - Half with both skin lesions and hemorrhaging. Half with hemorrhaging only. (12 plates).

Plated kidney and brain from each on TSA and TSA with 5% sheeps blood for culture and Identification and sensitivities.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 02/10/10 @ 11:33 AM

Specimen	ID	Isolate	Result	Level
Isolate	Fish 1 Kidney	Aliivibrio wodanis	Positive	
Isolate	Fish 1 Brain	Aliivibrio wodanis	Positive	
Isolate	Fish 2 Kidney	Aliivibrio wodanis	Positive	
Isolate	Fish 2 Brain	Aliivibrio wodanis	Positive	
Isolate	Fish 3 Kidney	Photobacterium phosphoreum	Positive	
Isolate	Fish 3 Kidney	Aliivibrio wodanis	Positive	
Isolate	Fish 3 Brain	Aliivibrio wodanis	Positive	
Isolate	Fish 4 Kidney	Aliivibrio wodanis	Positive	
Isolate	Fish 4 Brain		No Bacteria Isolated	
Isolate	Fish 5 Kidney	Aliivibrio wodanis	Positive	
Isolate	Fish 5 Brain	Aliivibrio wodanis	Positive	
Isolate	Fish 6 Kidney	Aliivibrio wodanis	Positive	
Isolate	Fish 6 Brain	Aliivibrio wodanis	Positive	

Isolate	Fish 7 Kidney	Aliivibrio wodanis	Positive
Isolate	Fish 7 Brain	Aliivibrio wodanis	Positive
Isolate	Fish 8 Kidney	Aliivibrio wodanis	Positive
Isolate	Fish 8 Brain	Aliivibrio wodanis	Positive
Isolate	Fish 9 Kidney	Bacteria	Positive
**: Bacteria identified as <i>Sejongia marina</i>			
Isolate	Fish 9 Kidney	Aliivibrio wodanis	Positive
Isolate	Fish 9 Brain	Bacteria	Positive
**: Bacteria identified as <i>Sejongia marina</i>			
Isolate	Fish 9 Brain	Aliivibrio wodanis	Positive
Isolate	Fish 10 Kidney	Aliivibrio wodanis	Positive
Isolate	Fish 10 Brain	Aliivibrio wodanis	Positive

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 02/10/10 @ 11:39 AM

Organism	ID	e	ffc	s3	sxt	ot
Bacteria	Fish 9 Kidney	s	s	r	s	s
**: Bacteria identified as <i>Sejongia marina</i>						
Photobacterium phosphoreum	Fish 3 Kidney	s	s	s	s	s
Aliivibrio wodanis	Fish 1 Kidney	s	s	s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethopri ot = Oxytetracycline						

History of Communication

Date	To	Description
02/10/10 1:18 PM	Mainstream Canada - e-mail	bc report generated
02/10/10 1:18 PM	Dr. Peter McKenzie - e-mail	bc report generated
02/12/10 3:26 PM	Mainstream Canada - e-mail	bc report generated
02/12/10 3:26 PM	Dr. Peter McKenzie - e-mail	bc report generated
02/16/10 9:11 AM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-525

Last Updated: 02/08/10 9:50 AM

Pathologist: Gary D. Marty

Received Date: 02/04/10

Collected Date: 02/02/10

Client Ref No:

Veterinarian: **Dr. Peter McKenzie**

Clinic: **Mainstream Canada**

Phone: (250) 286-0022

Fax: (250) 286-0042

Submitter: **Mainstream Canada**

Phone:

Fax:

Owner: **Mainstream Canada-T**

Phone:

Fax:(250) 725-1250

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID: Dixon Bay

Case History

Submitted fresh Atlantic salmon tissues for PCR.

Saltwater. Vaccinated: Yes. Insurance: No. Euthanized: No. Prior submission: No. DOD: Feb. 2/10.

To confirm/rule out VHS.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/08/10 @ 9:50 AM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/08/10 @ 9:50 AM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Positive

Staff Comments:

The Ct value for the positive VHSV test was 21.7. For real time PCR tests, the Ct value is the number of replications needed for the reaction to reach the threshold for declaring a result positive. Samples beginning with abundant reactive material (nucleic acid) will have a lower Ct value than samples beginning with less reactive material. Criteria for interpreting Ct values at the Animal Health Centre include: < 20.00, very strong; 20.00 to 27.99, strong; 28.00 to 35.00, moderate; > 35.00, weak.

History of Communication

Date	To	Description
02/08/10 10:13 AM	Mainstream Canada-T - fax	bc report generated
02/08/10 10:13 AM	Dr. Peter McKenzie - e-mail	bc report generated
02/11/10 3:14 PM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-635

Last Updated: 02/15/10 10:58 AM

Pathologist: Gary D. Marty

Received Date: 02/11/10

Collected Date: 02/09/10

Client Ref No: PO 14554

Veterinarian: **Dr. Peter McKenzie**

Clinic: **Mainstream Canada-T**

Phone: (250) 725-1255

Fax: (250) 725-1250

Submitter: **Zarah Vansnyck - Mainstream**

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 tissue to rule out VHS by PCR.

Atlantic salmon. Age - 3-4 kg. Saltwater. Vaccinated. Euthanized - no. Fish died Feb. 9/10.

Submitted head kidney and spleen.

Farm name: Millar Ch.

Molecular Diagnostics

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 02/15/10 @ 10:58 AM

Specimen	ID	Test	Result
Tissue	kd,sp	PCR - VHSV	Negative

History of Communication

Date	To	Description
02/17/10 4:16 PM	Mainstream Canada-T - fax	bc report generated
02/17/10 4:16 PM	Dr. Peter McKenzie - e-mail	bc report generated
02/18/10 1:16 PM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-661

Last Updated: 02/17/10 3:20 PM

Pathologist: Gary D. Marty

Received Date: 02/15/10

Collected Date:

Client Ref No: 7605

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 formalized Atlantic Salmon tissue for Histopathology and fresh tissue for PCR for IHN, VHS and ISA.

Saltwater entry: 09 S1. Vaccinated: Yes. Insurance: No. Euthanized: Yes - TMS overdose. Prior submission: No.

Fish are experiencing high mortality due to lesions on sides. Also small circular lesions on head/operculum perhaps damage from lice?

Final Diagnosis

1a. Skin (boneless): dermatitis and panniculitis, ulcerative, granulomatous, with multinucleate giant cells, focal, moderate (slide 2) with very small numbers of bacterial rods, focal, severe (slide 1)

1b. Skin (with bone): dermatitis, ulcerative, granulomatous, neutrophilic, focal, severe (slide 1)

1c. Skin (boneless): panniculitis, lymphohistiocytic, regionally diffuse, moderate (slide 2)

1d. Skin (boneless): immature dermal collagen (healing ulcer?) with scattered multinucleate giant cells, focal, moderate (slide 2)

2. Liver: pericholangitis, lymphohistiocytic, multifocal, mild (slide 1), moderate (slide 2)

3a. Heart: epicarditis, diffuse, lymphohistiocytic, mild (slide 2), moderate (slide 1)

3b. Heart: endocarditis, multifocal, lymphohistiocytic, mild (slide 2), moderate (slide 1)

4. Liver and adjacent mesenteric adipose tissue: peritonitis and steatitis, granulomatous, focal, with adhesion to the liver, moderate (slide 1)

5. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate (slides 1, 2)

Final Comment: Ulcers are the most severe lesions in these fish. Bacterial rods in one lesion (slide 1) are probably a *Vibrio/Alivibrio* species, but number of organisms seems too few for them to be the primary pathogen. Intralesional multinucleate giant cells in other lesions are evidence of persistent antigen, but sections contain no obvious organisms. Neutrophils and fibrin in another lesion are evidence that that lesion was fairly acute (less than 7 days old). Epithelialized immature dermal collagen in slide 2 (one lesion only) might be a focus of healing

(probably > 7 days old). Development of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection). PCR results rule out an active association of the lesions with the tested viruses.

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

Epicarditis and endocarditis are evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Severity greater than mild is uncommon in Atlantic salmon "fresh silvers" that die in marine net pens, affecting only 1.2% of the 512 Atlantic salmon hearts examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program; by comparison, mild epicarditis (21%) and endocarditis (16%) are common.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated; however, it is uncommon to have adhesions between the mesenteries and the liver (as in slide 1).

Histopathology

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

Slide 1 (7605-1) - heart, spleen, liver, trunk kidney, trunk/head kidney transition, skin/bone, skin/skeletal muscle

Slides 2 (7605-2) - heart, spleen, skin, liver, head kidney, trunk kidney, skin, skin/skeletal muscle (2 pieces)

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

Quality control: Liver autolysis: none (slides 1, 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 02/16/10 @ 4:31 PM

Specimen	ID	Test	Result
Tissue	7605-1	PCR - IHNV	Negative
Tissue	7605-2	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/16/10 @ 4:30 PM

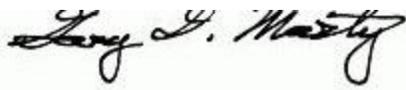
Specimen	ID	Test	Result
Tissue	7605-1	PCR - ISA	Negative
Tissue	7605-2	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/16/10 @ 4:30 PM

Specimen	ID	Test	Result
Tissue	7605-1	PCR - VHSV	Negative
Tissue	7605-2	PCR - VHSV	Negative

History of Communication

Date	To	Description
02/17/10 3:23 PM	Morrison, Diane - fax	bc report generated
02/18/10 1:20 PM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-758

Last Updated: 02/22/10 1:28 PM

Pathologist: Gary D. Marty

Received Date: 02/19/10

Collected Date:

Client Ref No: 7628

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 2 Atlantic salmon formalized tissues for Histopathology.

Saltwater entry: 2008

Histo from 2 fish with skin lesions. SP9 was a fresh mort with lesion starting on skin. SP3 was a moribund with raised scales in numerous locations - no lesion development or scale loss. Histo from SP09 includes 1 cassette with tissues and 3 cassettes of skin lesions - cut mid lesion with face presented . Histo from SP3 - 1 cassettes with gill; 1 cassettes with tissues and 3 cassettes with skin - cut mid section with cut surface presented. Internally fish had ascites and adhesions with no other visible lesions.

Final Diagnosis

- 1a. Skin: dermatitis, ulcerative, superficial, multifocal, moderate, with moderate numbers of bacterial rods (slide 9-3)
- 1b. Skin: denuded epidermis, multifocal, moderate (slides 3-1, 9-4)
- 2a. Liver: peritonitis, fibrinocellular, focal, moderate (slide 3-4)
- 2b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 3-4, 9-2)
- 2c. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slide 9-2), moderate (slide 3-4)
3. Heart: epicarditis, histiocytic, with epicardial cell hypertrophy, diffuse, moderate (slide 3-4)
4. Trunk kidney: renal tubular intraluminal necrotic cells, with luminal dilation, focal, mild (slides 3-4, 9-2)
5. Spleen: peritonitis, granulomatous, with fibrocellular fronds and mesothelial cell hypertrophy, regionally diffuse, moderate (slide 3-4)
6. Skeletal muscle: flocculated sarcoplasm of white myofibres, acute, mild (~5% of myofibres) (slides 3-1, 3-2)

Final Comment: These fish have several lesions that provide evidence of systemic disease, and many of the lesions could be a result of skin

ulcers. Unfortunately, the cause of the ulcers cannot be determined (a common result with ulcer histopathology). Comments on specific lesions follow:

Skin ulcers in slide 9-3 are superficial, with none deeper than the scale pocket. Rod-shaped bacteria (*Vibrio* or *Aliivibrio* spp.) commonly invade the ulcers. Enlargement of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection).

Lack of epidermis on the surface of the skin (slides 3-1 and 9-4) is a common artifact, or it might be a superficial ulcer. In both slides, relatively normal epidermis is adjacent to the denuded area: evidence of artifact. The denuded surface has no exudate or bacteria, also evidence of artifact. Many of the scale pockets in fish 3 are unusually large, but the space is not filled with exudate; these spaces might be artifacts or they might explain the foci of raised scales, described grossly (e.g., as a result of a transudate). If additional samples from these fish are submitted for histopathology, consider sending a digital photograph of the lesion along with a tissue sample.

Hepatic fibrinocellular peritonitis and splenic peritonitis in slide 3-4 are evidence of active inflammation. Inflammation on the liver includes neutrophils and macrophages. Differentials include a bacterial infection or exposure to other antigens. The sections include no obvious organisms ; were any bacteria grown from a kidney swab?

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It might be related to increased protein needed as part of an inflammatory response. It is common in juvenile fish with ulcers. Mature females normally have basophilic hepatocytes: needed to produce protein for deposition in their eggs.

Pigment in the liver is probably 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.

Epicardial inflammation and hypertrophy are evidence of systemic immune stimulation. The cause in fish 3 is probably the same as for the fibrinocellular peritonitis.

Small numbers of renal tubular intraluminal necrotic cells are fairly common in fish that have systemic disease or significant inflammation somewhere in their body. With this lesion, tubule lumens usually contain only one or two necrotic cells. In most cases (but not this one), tubular epithelium surrounding these cells is attenuated (evidence of epithelial cell loss, with stretching of the remaining epithelium to fill the resultant gap). In other cases (like this one), the height of tubular lining cells is normal. The underlying cause of the necrotic cells is often not determined, but differentials include anything that causes significant inflammation.

Flocculated sarcoplasm of skeletal muscle is a degenerative change that can result from trauma, bacterial, fungal, or parasitic infections. It is common in fish that are handled within a few hours of death. These sections have no evidence of infectious organisms.

Histopathology

Formalin-fixed tissues were submitted from two fish in a total of 9 cassettes for histopathology.

Slides 3-1 (7628 SP3 skin lesion), 3-2 (7628 SP3 skin lesion), 3-5 (7628 SP3 skin lesion), 9-1 (7628 SP09 skin lesion), 9-3 (7628 SP09 skin lesion), 9-4 (7628 SP09 skin lesion) - skin/skeletal muscle (2 pieces/slide except for slide 9-3, which has 4 pieces)

Slide 3-3 (7628 SP3 2/16/10) - gill

Slides 3-4 (7628 SP3 2/16/10) and 9-2 (7628 SP09 2/16/10) - heart, spleen, liver, head kidney, trunk kidney, skeletal muscle, and intestine

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

Quality control: Liver autolysis: none (slide 3), moderate (slide 9-2). Large foci of erythrocytes (e.g., spleen in slide 3-4) 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). Organs have no postfixation dehydration.

History of Communication

Date	To	Description
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Case: 10-758

02/22/10 1:28 PM

Morrison, Diane - fax

bc report generated

02/22/10 2:52 PM

Marine Harvest Canada - e-mail

Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-760

Last Updated: 02/24/10 10:53 AM

Pathologist: Gary D. Marty

Received Date: 02/19/10

Collected Date: 02/15/10

Client Ref No: 10-009

Veterinarian: **Dr. Peter McKenzie**

Clinic:

Phone:

Fax:

Submitter: **Mainstream Canada**

Phone:

Fax:

Owner: **Mainstream Canada**

Phone:

Fax:(250) 286-0042

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID: cypress

Case History

Submitted fresh and formalized Atlantic fish tissue for Histopathology, Viral culture and PCR for VHS, IHN and BKD.

Age: ? Sex: Unknown. Saltwater. Vaccinated: Yes. Insurance: No. Euthanized: No. Prior submission: No. # dead - 3. DOD: Feb. 15/10.

Petechial hemorrhaging on the liver and body walls. Swollen kidneys were being observed.

PO#13935.

Final Diagnosis

1. Liver, trunk kidney, head kidney, heart, spleen: disseminated granulomatous inflammation, consistent with *Renibacterium salmoninarum* , multifocal, moderate to severe (slide 16A)
2. Gill: branchitis, granulomatous, with multinucleate giant cells, necrosis, and intralesional short bacterial rods consistent with *Renibacterium salmoninarum* , multifocal, moderate (slide 16B)
3. Perineural connective tissue: vacuolated epithelioid macrophages, with fibrin, multifocal, moderate (slide 11A)
- 4a. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, moderate (slides 11A, 12A, 16A)
- 4b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slides 11A, 12A, 16A)
- 4c. Liver: pericholangitis, lymphohistiocytic, multifocal, mild (slides 11A, 16A)
5. Spleen: parenchymal golden pigment (lipofuscin?), scattered, intracellular, mild (slide 11A)
6. Mesenteric adipose tissue: peritonitis, chronic, multifocal, with fibrocellular fronds, moderate (slide 11A)
- 7a. Heart: myocardial karyomegaly, multifocal, mild (slide 12A)

7b. Heart: epicarditis, multifocal, histiocytic, mild (slide 12A)

8. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slide 16A)

Final Comment: These fish have several lesions that might have contributed to morbidity. The most obvious cause of death is in fish 16, which has disseminated granulomatous inflammation and short bacterial rods consistent with the PCR result of *Renibacterium salmoninarum*. Comments on other specific lesions follow:

Multiple foci of vacuolated epithelioid macrophages, with fibrin, are unusual features of inflammation associated with perineural connective tissue. This type of inflammation is usually a result of persistent antigens, but the section has no obvious infectious organisms.

Pigment in the liver and spleen is probably lipofuscin, and in the liver 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.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It might be related to increased protein needed as part of an inflammatory response. It is common in juvenile fish with ulcers. Mature females normally have basophilic hepatocytes: needed to produce protein for deposition in their eggs.

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.

Peritonitis of the visceral mesenteries is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 54% of the 514 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (40% were mild, 12% were moderate, and 2.1% were severe).

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 9.3% of the 2181 Atlantic salmon hearts examined as part of the province's Fish Health Auditing and Surveillance Program from 2006 through 2009). 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).

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 handling) 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 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 36%; n = 511) and Pacific salmon (prevalence = 42%; n = 71).

Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology. Gills were removed from the original (A) cassettes and placed in separate (B) cassettes.

Slides 11A (Cypress Pen 11 2/15/10) and 12A (Cypress Pen 12 2/15/10) - brain, heart, spleen, liver, trunk kidney, head/trunk kidney transition, intestinal ceca, mesenteric adipose tissue

Slide 16A (Cypress Pen 16 2/15/10) - liver, brain, heart, spleen, head kidney, trunk kidney, intestine, mesenteric adipose tissue

Slides 11B (Cypress Pen 11 2/15/10), 12B (Cypress Pen 12 2/15/10) and 16B (Cypress Pen 16 2/15/10) - gill

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

Quality control: Liver autolysis: none (slide 11A), moderate (slide 16A), severe (slide 12A). Large foci of erythrocytes (e.g., spleen in slide 11A) 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). Organs have no postfixation dehydration.

PCR-Renibacterium salmoni Resulted by: A Scouras Verified by: Dr. J. Robinson on 02/24/10 @ 10:53 AM

Specimen	ID	Test	Result
Tissue	org Pen 12	PCR-Renibacterium salmoninaru	Negative
Tissue	org Pen 16	PCR-Renibacterium salmoninaru	Positive
**: Test validation in progress.			

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 02/23/10 @ 10:47 AM

Specimen	ID	Test	Result
Tissue	org Pen 12	PCR - IHNV	Negative
Tissue	org Pen 16	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 02/23/10 @ 10:47 AM

Specimen	ID	Test	Result
Tissue	org Pen 12	PCR - VHSV	Negative
Tissue	org Pen 16	PCR - VHSV	Negative

History of Communication

Date	To	Description
02/24/10 10:55 AM	Mainstream Canada - e-mail	bc report generated
02/24/10 10:56 AM	Dr. Peter McKenzie - e-mail	bc report generated
02/25/10 1:58 PM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-799

Last Updated: 03/23/10 3:17 PM

Pathologist: Gary D. Marty

Received Date: 02/23/10

Collected Date:

Client Ref No: 7634

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Brad Boyce - 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 Atlantic Salmon tissues for Histopathology, Viral culture and PCR for IHN, VHS and ISA.

Saltwater entry: 09 S0. Vaccinated: Yes. Insurance: No. Euthanized: No. Prior: No.

Fresh mort - Exophthalmic, liver hemmorrhaging.

Final Diagnosis

1. Skeletal muscle and adjacent adipose tissue (skin or parietal peritoneum along body wall): steatitis, neutrophilic, fibrinous, with congestion and hemorrhage, diffuse, acute, severe
2. Liver: sinusoidal congestion, with cellular thrombi, acid hematin granules, and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, severe
- 3a. Trunk kidney: renal tubular luminal dilation, with a few intraluminal necrotic cells, multifocal, moderate
- 3b. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild
4. Head kidney: nephritis, interstitial, granulomatous, focal, mild

Final Comment: This fish probably died of complications related to the focus of neutrophilic steatitis adjacent to the sample of skeletal muscle (body wall?). Fibrin, congestion, and hemorrhage are evidence that the lesion was acute (perhaps no more than 48 hours old). Although the section contains no obvious organisms, bacteria might have been involved. Changes in other organs might be a result of inflammation related to this lesion.

Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion. Scattered cellular thrombi in some foci of congestion are consistent with vascular damage. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with

VHSV; the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology, if not already done. 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 are probably 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 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.

Small numbers of renal tubular intraluminal necrotic cells are fairly common in fish that have systemic disease or significant inflammation somewhere in their body. With this lesion, tubule lumens usually contain only one or two necrotic cells. In most cases (like this one), tubular epithelium surrounding these cells is attenuated (evidence of epithelial cell loss, with stretching of the remaining epithelium to fill the resultant gap). The underlying cause of the necrotic cells is often not determined, but differentials include anything that causes significant inflammation.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) 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 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 36% ; n = 511).

The focus of granulomatous inflammation in the head kidney is evidence of chronic immune stimulation. Differentials include vaccine material and *Renibacterium salmoninarum*. The single lesion in this fish did not contribute to its death.

Histopathology

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

Slide 1 (7634-1) - heart, spleen, liver, head kidney, trunk kidney, skeletal muscle and adjacent adipose tissue

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). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/24/10 @ 3:18 PM

Specimen	ID	Test	Result
Tissue	org 7634	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/24/10 @ 3:18 PM

Specimen	ID	Test	Result
Tissue	org 7634	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/24/10 @ 3:18 PM

Specimen	ID	Test	Result
Tissue	org 7634	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Cheryl Cecconi Verified by: Dr. J. Robinson on 03/23/10 @ 3:17 PM

Specimen	ID	Isolate	Result	Level
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Case: 10-799

Staff Comments:

Interim report (PDF version) with histopathology and PCR results sent to MH fish health staff via Outlook attachment by GD Marty, Wed 2010-02-24 4:23 PM.

History of Communication

Date	To	Description
03/23/10 4:01 PM	Morrison, Diane - fax	bc report generated
03/24/10 9:45 AM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-800

Last Updated: 02/24/10 3:18 PM

Pathologist: Gary D. Marty

Received Date: 02/23/10

Collected Date:

Client Ref No: 7621

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Brad Boyce - 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 Atlantic Salmon tissues for PCR for IHN, VHS and ISA.

Saltwater entry: 08 S0. Vaccinated: Yes. Insurance: No. Euthanized: No. Prior: No.

Sampled 2 fresh morts, routine viral screen.

Molecular Diagnostics

PCR - IHN Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/24/10 @ 3:18 PM

Specimen	ID	Test	Result
Tissue	org 7621-1	PCR - IHN	Negative
Tissue	org 7621-2	PCR - IHN	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/24/10 @ 3:18 PM

Specimen	ID	Test	Result
Tissue	org 7621-1	PCR - ISA	Negative
Tissue	org 7621-2	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/24/10 @ 3:18 PM

Specimen	ID	Test	Result
Tissue	org 7621-1	PCR - VHSV	Negative
Tissue	org 7621-2	PCR - VHSV	Negative

History of Communication

Case: 10-800

Date	To	Description
02/24/10 4:17 PM	Morrison, Diane - fax	bc report generated
02/25/10 2:01 PM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-870

Last Updated: 03/05/10 11:27 AM

Pathologist: Gary D. Marty

Received Date: 03/01/10

Collected Date: 02/26/10

Client Ref No: #7628

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 Atlantic salmon blood agar plates for ID and antibiotic sensitivities.

Saltwater entry. Insurance/legal: no. Prior submission case #758.

Three blood agar plates with isolates taken from the skin and muscle of affected fish sampled in MH case #7628 (AHC #758).

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Jaime Osei-Appiah on 03/05/10 @ 11:27 AM

Specimen	ID	Isolate	Result	Level
Isolate	7628 skin	Flavobacterium frigidarium	Positive	
Isolate	7628 S	Psychrobacter sp.	Positive	
Isolate	7628 muscle	Flavobacterium frigidarium	Positive	

Fish Resulted by: Erin Zabek Verified by: Jaime Osei-Appiah on 03/05/10 @ 11:27 AM

Organism	ID	e	ffc	s3	sxt	ot
Flavobacterium frigidarium	7628 skin	s	s	s	s	s
Psychrobacter sp.	7628 S	s	s	s	s	s

** Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethopri
ot = Oxytetracycline

History of Communication

Date	To	Description
03/05/10 12:57 PM	Morrison, Diane - fax	bc report generated
03/09/10 2:03 PM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-912

Last Updated: 03/05/10 11:40 AM

Pathologist: Gary D. Marty

Received Date: 03/03/10

Collected Date:

Client Ref No: 7645

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Brad Boyce - 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 Atlantic salmon tissues for Histopathology and PCR for IHN, VHS and ISA.

Saltwater entry: 09 S1. Vaccinated: Yes. Insurance: No. Euthanized: Yes, TMS. Prior submission: No.

Viral 1 - 8 and histo 1 - 5, 21 - 25 were from random "healthy fish". Fish overdosed with TMS and bled out by cutting tail. No gross lesions except for viral #9 was from 2 fresh mortis.

Final Diagnosis

Most significant lesions in these fish:

1. Heart: epicarditis and endocarditis, lymphohistiocytic, multifocal, mild (slides 4A, 5A, 22A, 23A, 24A), moderate (slides 2A, 21A)
- 2a. Skeletal muscle: *Kudoa thyrsites* pseudocyst(s), focal, mild (slides 1A, 3A), bifocal, mild (slides 4A, 23A), multifocal, moderate (slide 21A)
- 2b. Skeletal muscle: myositis, focal, mild (slide 21A); multifocal, moderate (slide 23A)
3. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate (slide 1A), severe (slide 22A)
- 4a. Gill: lamellar hyperplasia and fusion, focal, moderate (slide 1B)
- 4b. Gill: branchitis, granulomatous, multifocal, mild (slide 25B)

Final Comment: None of the many lesions in these fish are of sufficient severity to spark concern if imminent death, but the degree of inflammation is more than expected among healthy fish. Most notable are mild to moderate inflammation in the heart (100% prevalence), mild infiltrates of mononuclear cells in the brain (meningitis, 70% affected), and *Kudoa thyrsites* infestation in the skeletal muscle (50% affected). Comments on specific lesions follow:

Lymphohistiocytic epicarditis and endocarditis are evidence of chronic immune stimulation. Differentials include a low-grade bacterial infection and a reaction to a vaccine. The section from the affected fish has no obvious organisms.

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. Extensive inflammation (myositis) is common in severe infections (source Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada). DNA-based QPCR provides the greatest sensitivity for detecting infection, but histopathology is a better predictor of flesh quality (Funk et al. 2007).

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe). Vacuoles are probably a result of vaccine material lost during tissue processing.

Gill lamellar hyperplasia with fusion may be a result of physical damage from exposure to an irritant (e.g., fine feed particles or toxins in the water, including ammonia); differentials include parasites or diatoms, but this lesion contains no organisms (they might have been lost during processing). The lesions in slide 1B are limited to 4-mm long segments of two adjacent filaments.

Granulomatous inflammation in the gill is probably related to vaccination. The lesion in slide 25B affects a small focus, about mid-filament, on 4 adjacent filaments. One focus contains multinucleate giant cells consistent with persistent foreign material; the lesion has no obvious infectious organisms.

Literature cited:

Funk V.A., Raap, M., Sojonky, K., Jones, S., Robinson, J., Falkenberg, C., Miller, K.M. Development and validation of an RNA- and DNA-based quantitative PCR assay for determination of *Kudoa thyrsites* infection levels in Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms 75(3): 239-249.

Histopathology

Formalin-fixed tissues from 10 fish were submitted in 20 cassettes for histopathology. The gills were immersed 2h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

Slide #s 1A/1B - 5A/5B are labeled in the same order as client #s 1 - 5 and Slide #s 21A/21B - 25A/25B are labeled in the same order as client #s 21 - 25

Organs included on most A slides - brain , skin, heart, liver, spleen, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue, skeletal muscle

Organs included on most B slides - gill

Results are detailed by organ on the spreadsheet (2010-0912.xls). This spreadsheet is in the same format as the one used for the BC Fish Health Auditing and Surveillance Program; therefore, many of the scored items do not occur in this group of fish. The main difference is that skeletal muscle lesions are scored here, but skeletal muscle is not part of the government auditing program.

Quality control: Details are included on the spreadsheet (2010-0912.xls). Overall, tissue quality is excellent, particularly the gill. Mild artifact is normal for paraffin-embedded sections. Tissues have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/05/10 @ 10:33 AM

Specimen	ID	Test	Result
Tissue	7645-1	PCR - IHNV	Negative
Tissue	7645-2	PCR - IHNV	Negative
Tissue	7645-3	PCR - IHNV	Negative
Tissue	7645-4	PCR - IHNV	Negative
Tissue	7645-5	PCR - IHNV	Negative

Tissue	7645-6	PCR - IHNV	Negative
Tissue	7645-7	PCR - IHNV	Negative
Tissue	7645-8	PCR - IHNV	Negative
Tissue	7645-9	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/05/10 @ 10:33 AM

Specimen	ID	Test	Result
Tissue	7645-1	PCR - ISA	Negative
Tissue	7645-2	PCR - ISA	Negative
Tissue	7645-3	PCR - ISA	Negative
Tissue	7645-4	PCR - ISA	Negative
Tissue	7645-5	PCR - ISA	Negative
Tissue	7645-6	PCR - ISA	Negative
Tissue	7645-7	PCR - ISA	Negative
Tissue	7645-8	PCR - ISA	Negative
Tissue	7645-9	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/05/10 @ 10:33 AM

Specimen	ID	Test	Result
Tissue	7645-1	PCR - VHSV	Negative
Tissue	7645-2	PCR - VHSV	Negative
Tissue	7645-3	PCR - VHSV	Negative
Tissue	7645-4	PCR - VHSV	Negative
Tissue	7645-5	PCR - VHSV	Negative
Tissue	7645-6	PCR - VHSV	Negative
Tissue	7645-7	PCR - VHSV	Negative
Tissue	7645-8	PCR - VHSV	Negative
Tissue	7645-9	PCR - VHSV	Negative

History of Communication

Date	To	Description
03/03/10 1:12 PM	-	bc report generated
03/05/10 11:40 AM	Morrison, Diane - fax	bc report generated
03/09/10 2:26 PM	Marine Harvest Canada - e-mail	Case Invoiced



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END OF REPORT

Final Report AHC Case: 10-962

Last Updated: 03/16/10 1:40 PM

Pathologist: Gary D. Marty

Received Date: 03/05/10

Collected Date: 03/01/10

Client Ref No: 10-011

Veterinarian: **Dr. Peter McKenzie**

Clinic:

Phone:

Fax:

Submitter: **Kelly Abel - Mainstream Canada**

Phone:

Fax:

Owner: **K.A-Mainstream Canada**

Phone:

Fax:(250) 286-0042

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID: Cypress

Case History

Submitted formalized Atlantic salmon tissues for Histopathology and bacti plates for Bacteriology.

Saltwater. Vaccinated: Yes. Insurance: No. Euthanized: No. Prior submission: Yes, 10-760. DOD: March. 1/10.

Skin lesions being observed on external body

Final Diagnosis

1a. Liver: hepatocellular single cell necrosis , disseminated, acute, mild (slide 2A), moderate (slide 1A)

1b. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slide 2A), moderate (slide 1A)

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

1d. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 2A)

2a. Kidney: interstitial edema and hematopoietic cell atrophy, diffuse, mild (slide 2A), moderate (slide 1A)

2b. Trunk kidney: renal tubular dilation, diffuse, moderate (slide 1A)

2c. Trunk kidney: renal tubular casts of protein and yellow-brown pigment, multifocal, mild (slide 1A)

3. Gill: lamellar fusion (synechiae), multifocal, mild (slides 1B, 2B)

4. Spleen: parenchymal golden pigment (lipofuscin?), scattered, intracellular, mild (slide 2A)

Final Comment: These fish have several lesions consistent with ulcers, but none are specific enough to identify the cause of the ulcers. Ulcers in marine salmonids are commonly invaded by filamentous or rod-shaped bacteria. 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 the most likely filamentous bacteria, and *Vibrio* or related species (*Aliivibrio*) are the most likely rod-shaped

bacteria.

Aliivibrio wodanis is commonly cultured from Atlantic salmon with "winter ulcers," but Koch's postulates have not been fulfilled (i.e., it might be a secondary invader). Instead, *Moritella viscosa* (old name = *Vibrio viscosus*) has been identified as the cause of winter ulcers (i.e., Koch's postulates **have** been fulfilled). [Source T. Lunder, et al. 2000. Phenotypic and genotypic characterization of *Vibrio viscosus* sp. nov. and *Vibrio wodanis* sp. nov. isolated from Atlantic salmon (*Salmo salar*) with 'winter ulcer.' International Journal of Systematic and Evolutionary Microbiology 50:427-450.]

Comments on specific microscopic lesions follow:

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).

Pigment in the liver and spleen is probably lipofuscin, and in the liver 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.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It might be related to increased protein needed as part of an inflammatory response. It is common in juvenile fish with ulcers. Mature females normally have basophilic hepatocytes: needed to produce protein for deposition in their eggs.

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 "fresh silvers" that die in marine net pens, affecting 14% of the 514 Atlantic salmon livers examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

Interstitial renal edema is evidence of dysfunctional circulation, and interstitial cell atrophy is evidence of decreased immune function; both are often a result of a viral infection (e.g., VHSV). With edema, the space between renal tubules is increased without an increase in the number of hematopoietic cells. With interstitial cell atrophy, there is a decrease in the number of hematopoietic cells. The primary differential is postmortem autolysis, in which a space is formed when the base of the tubular epithelial cells pulls away from the tubular basement membrane.

Renal tubular dilation involving both the lumen and the total diameter of the tubular is often the result of increased intraluminal pressure; this might be a result of downstream blockage.

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.

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).

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology. Gills were removed from the original (A) cassettes and placed in separate (B) cassettes. Gills were then immersed for 2 h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

Slides 1A (Cypress Pen 15 March 1 2010) and 2A (Cypress Pen 16 March 1 2010) - brain, heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slides 1B (Cypress Pen 15 March 1 2010) and 2B (Cypress Pen 16 March 1 2010) - gill

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

Quality control: Liver autolysis: mild (slide 1A), moderate (slide 2A). Large foci of erythrocytes (e.g., spleen in slide 2A) have precipitates of acid

Case: 10-962

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). Gill decalcification is complete and differential staining is good. Organs have no postfixation dehydration.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 03/16/10 @ 10:38 AM

Specimen	ID	Isolate	Result	Level
Isolate	Pen 15 Brain	Aliivibrio wodanis	Positive	
Isolate	Pen 15 Kidney	Aliivibrio wodanis	Positive	
Isolate	Pen 16 Brain	Aliivibrio wodanis	Positive	
Isolate	Pen 16 Kidney	Aliivibrio wodanis	Positive	
**: Unable to perform ant biotic sensitivities due to poor growth				

History of Communication

Date	To	Description
03/09/10 12:00 PM	K.A-Mainstream Canada - e-mail	bc report generated
03/09/10 12:01 PM	Dr. Peter McKenzie - e-mail	bc report generated
03/16/10 1:40 PM	K.A-Mainstream Canada - e-mail	bc report generated
03/16/10 1:40 PM	Dr. Peter McKenzie - e-mail	bc report generated
03/17/10 10:31 AM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-963

Last Updated: 03/16/10 10:47 AM

Pathologist: Gary D. Marty

Received Date: 03/05/10

Collected Date:

Client Ref No: 10-010

Veterinarian: **Dr. Peter McKenzie**

Clinic:

Phone:

Fax:

Submitter: **Nathan Cassan - Mainstream Canada**

Phone:

Fax:

Owner: **Mainstream Canada - NC**

Phone:

Fax:(250) 286-0042

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

Case History

Submitted 6 Atlantic salmon bacti plates for Bacteriology.

Saltwater. Vaccinated: Yes. Insurance: No.Prior submission: No.

10 subcultures over 6 plates for Bacterial Identification and sensitivities as labelled.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 03/16/10 @ 10:43 AM

Specimen	ID	Isolate	Result	Level
Isolate	ML1	Vibrio splendidus	Positive	
Isolate	ML2	Vibrio splendidus	Positive	
Isolate	ML3	Vibrio splendidus	Positive	
Isolate	ML4	Vibrio splendidus	Positive	
Isolate	ML5	Vibrio splendidus	Positive	
Isolate	ML6	Vibrio splendidus	Positive	
Isolate	ML7	Vibrio splendidus	Positive	
Isolate	ML8	Vibrio splendidus	Positive	
Isolate	ML8	Pseudoalteromonas sp.	Positive	
**: Psuedoalteromonas sp. identified as Pseudoalteromonas veronii				
Isolate	R48	Pseudoalteromonas sp.	Positive	
Isolate	F15	Psychrobacter sp.	Positive	
Isolate	F15	Pseudoalteromonas sp.	Positive	

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 03/16/10 @ 10:46 AM

Organism	ID	e	ffc	s3	sxt	ot
Pseudoalteromonas sp.	ML8	r	r	s	r	s
**: Pseudoalteromonas sp. identified as Pseudoalteromonas veronii						
Pseudoalteromonas sp.	R48	s	s	s	s	s
Psychrobacter sp.	F15	s	s	s	s	s
Vibrio splendidus	ML1	s	s	s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethopri ot = Oxytetracycline						

Staff Comments:

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. 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.

History of Communication

Date	To	Description
03/16/10 1:13 PM	Mainstream Canada - NC - e-mail	bc report generated
03/16/10 1:13 PM	Dr. Peter McKenzie - e-mail	bc report generated
03/17/10 10:32 AM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-987

Last Updated: 03/10/10 3:25 PM

Pathologist: Gary D. Marty

Received Date: 03/09/10

Collected Date: 02/17/10

Client Ref No: 10-20

Veterinarian: **Barry Milligan**

Clinic:

Phone:

Fax:

Submitter: **Tessa Wyrozub - Grieg Seafood BC Ltd.**

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 Atlantic salmon tissue for Histopathology.

Grow-out. Saltwater. Vaccinated - Yes. # submitted dead - 1. DOD: Feb. 17/10.

Final Diagnosis

1a. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slide 1)

1b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate (slide 1)

1c. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slide 1)

1d. Liver: hepatocellular intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, small numbers (slide 1)

Final Comment: These organs have several lesions that might provide clues to morbidity, but none are of sufficient severity to have killed the fish. Comments on specific lesions follow:

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It might be related to increased protein needed as part of an inflammatory response. It is common in juvenile fish with ulcers. Mature females normally have basophilic hepatocytes: needed to produce protein for deposition in their eggs.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen or fluid). These vacuoles are fairly common in Pacific salmon livers sampled as part of the BC Fish Health Auditing and Surveillance Program, and their prevalence changed little from 76% in 2006 to 84% in 2007 and 86% in 2008, but prevalence dropped to 57% in 2009. At least some types of vacuoles might be normal; their effect on growth and feed conversion is unknown.

Pigment in the liver is probably 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

Case: 10-987

decreased growth and survival in several studies. Hemosiderin accumulation in the liver might result from increased turnover of red blood cells.

Golden to amphophilic cytoplasmic inclusions in hepatocytes vary from ½ to 2x the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology. The gill was removed from the original cassette and placed in a separate cassette. The gill was then immersed for 2 h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

Slide 1 - brain, heart, spleen, liver, trunk kidney (2 pieces)

Slide 2 - gill

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

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

History of Communication

Date	To	Description
03/10/10 3:25 PM	Milligan, Barry - e-mail	bc report generated
03/11/10 10:00 AM	Grieg Seafoods BC Ltd. - e-mail	Case Invoiced



Gary D. Marty
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END OF REPORT

Final Report AHC Case: 10-1034

Last Updated: 03/19/10 2:29 PM

Pathologist: Gary D. Marty

Received Date: 03/11/10

Collected Date: 03/09/10

Client Ref No: PO 14632

Veterinarian: **Dr. Peter McKenzie**

Clinic:

Phone:

Fax:

Submitter: **Zarah Vansnick - 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 Atlantic salmon tissues and one slide for Histo, Bacti and PCR.

Age:350g. Saltwater. Vaccinated: Yes. Prior submission: Yes, #10-670. DOD: March. 9/10.

VHS like symptoms. Some fish with Exophtatmia (popeye). Some fish twirling in pens. Some fish excessive mucus in gills.

Lab note: Bacteriology recieved 6 agar plates for identification

Final Diagnosis

Most significant lesions in these fish:

1. Heart: myocardial, necrosis, multifocal, acute, moderate (slide 10), severe (slide 9)
2. Trunk kidney: renal tubular intraluminal necrotic cells, multifocal, mild (slides 2, 3, 4, 5, 7, 8), moderate (slides 6, 8, 9)
3. Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate (slide 1)
4. Collagenous connective tissue (swimbladder wall?): cellulitis (aerocystitis?), fibrinous, diffuse, severe (slide 2)

Final Comment: These fish have several lesions that could be attributed to infection with VHSV. Some of the lesions could also be caused or exacerbated by bacteria, but sections contain no obvious organisms (i.e., the cultured organisms probably were not primary pathogens).

Aliivibrio logei is a halophilic *Vibrio* species that was first described as *Photobacterium logei*, changed to *Vibrio logei*, and changed again in 2007 to *Aliivibrio logei*. Some strains are bioluminescent (Fidopiastis et al. 1998). Although they are occasionally isolated from sick fish, they are considered part of the normal microbiota of fish and sea water, contaminating the samples rather than acting as opportunistic pathogens (Benediktsdottir et al. 1998).

Comments on specific lesions follow:

The heart in slide #9 has several foci of acute myocardial necrosis that comprise about 15% of the total volume of the heart; myocardial necrosis in fish #10 is less severe. Most foci of necrosis involve the spongy layer of the heart (i.e., the part of the heart that normally is bathed by low-oxygen blood). I don't recall having ever encountered an Atlantic salmon with myocardial necrosis from a VHSV- positive farm. Myocardial necrosis might be a rare manifestation of VHS, but the scientific literature has no reports of histopathology of farmed Atlantic salmon exposed to the Pacific strain of VHSV (type Ia) under controlled laboratory conditions.

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). Moderate to severe cases in BC are often associated with the North American strain of VHSV (Type IVa). Mild cases are fairly common in debilitated fish, regardless of cause. With many mild cases, tubular lumens contain only one or two necrotic cells. In affected kidneys, the height of tubular lining cells is normal. In moderate or severe cases, all epithelial cells within a tubule are necrotic. Renal tubular epithelial necrosis was fairly common among Atlantic salmon sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 12% ; n = 511); the cause was not determined in many cases. Differentials other than VHSV include exposure to toxins (e.g., bacterial toxins, algal toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin).

Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; PCR results point to VHSV as the cause in this case. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

Fibrinous inflammation surrounding both sides of a ring of dense irregular collagenous connective tissue (swimbladder wall? Slide 2) is evidence of acute inflammation. The section has no obvious organisms, but it might be a result of acute infection with VHSV.

Literature cited:

Benediktsdottir, E., Helgason, S., Sigurjonsdottir, H. 1998. *Vibrio spp.* isolated from salmonids with shallow skin lesions and reared at low temperature. *Journal of Fish Diseases* [J. Fish Dis.] 21(1):19-28.

Fidopiastis, P.M., Von Boletzky, S., Ruby, E.G. 1998. A new niche for *Vibrio logei*, the predominant light organ symbiont of squids in the genus *Sepiola*. *Journal of Bacteriology* [J. Bacteriol.] 180(1):59-64.

Histopathology

Formalin-fixed tissues from 10 fish were submitted in 10 cassettes for histopathology. Slide #s 1-10 are labeled in the same order as client #s Barkley Fish 1 - Barkley Fish 10. Results are detailed by organ on the spreadsheet (2010- 1034.xls). This spreadsheet is in the same format as the one used for the BC Fish Health Auditing and Surveillance Program; therefore, many of the scored items do not occur in this group of fish.

Quality control: Details are included on the spreadsheet (2010-1034.xls). Tissue autolysis varies from mild to severe (i.e., within the range of autolysis common in tissues that are part of the BC Fish Health Auditing and Surveillance Program). Mild artifact is normal for paraffin-embedded sections. 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. Tissues have no postfixation dehydration.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 03/19/10 @ 11:46 AM

Specimen	ID	Isolate	Result	Level
Isolate	Kidney 1		No Bacteria Isolated	
Isolate	Kidney 2	Aliivibrio logei	Positive	
Isolate	Kidney 2	Aliivibrio fischeri	Positive	
Isolate	Kidney 3	Aliivibrio logei	Positive	
Isolate	Kidney 4		No Bacteria Isolated	

Isolate	Kidney 5		No Bacteria Isolated
Isolate	Brain 1		No Bacteria Isolated
Isolate	Brain 2	Aliivibrio logei	Positive
Isolate	Brain 2	Photobacterium iliopiscarium	Positive
Isolate	Brain 3		No Bacteria Isolated
Isolate	Brain 4		No Bacteria Isolated
Isolate	Brain 5		No Bacteria Isolated

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 03/19/10 @ 11:48 AM

Organism	ID	e	ffc	s3	sxt	ot
Photobacterium iliopiscarium	Brain 2	s	s	s	s	s
Aliivibrio fischeri	Kidney 2	s	s	s	s	s
Aliivibrio logei	Kidney 2	s	s	s	s	s

** Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethopri
ot = Oxytetracycline

Molecular Diagnostics

PCR - VHSV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 03/15/10 @ 11:40 AM

Specimen	ID	Test	Result
Tissue	org pool 1 (fish 1-5)	PCR - VHSV	Positive
Tissue	org pool 2 (fish 6-10)	PCR - VHSV	Positive

History of Communication

Date	To	Description
03/15/10 2:29 PM	Mainstream Canada-T - fax	bc report generated
03/15/10 2:29 PM	Dr. Peter McKenzie - e-mail	bc report generated
03/16/10 11:35 AM	Mainstream Canada-T - fax	bc report generated
03/16/10 11:35 AM	Dr. Peter McKenzie - e-mail	bc report generated
03/19/10 2:30 PM	Mainstream Canada-T - fax	bc report generated
03/19/10 2:30 PM	Dr. Peter McKenzie - e-mail	bc report generated
03/22/10 2:22 PM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-1046

Last Updated: 03/25/10 11:25 AM

Pathologist: Gary D. Marty

Received Date: 03/12/10

Collected Date:

Client Ref No: PO 14635

Veterinarian: **Dr. Peter McKenzie**

Clinic:

Phone:

Fax:

Submitter: **Zarah Vansnick - Mainstream Canada**

Phone:

Fax:

Owner: **Mainstream Canada-T**

Phone:

Fax:(250) 725-1250

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID: Mac Lake

Case History

Submitted fresh Atlantic salmon tissue and plate for PCR and Bacteriology.

Saltwater. Age: 300g. Vaccinated: Yes.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 03/24/10 @ 3:21 PM

Specimen	ID	Isolate	Result	Level
Isolate	1	Aliivibrio wodanis	Positive	
Isolate	1	Shewanella sp.	Positive	
**: Shewanella sp. identified as S.baltica by PCR				
Isolate	2		No Bacteria Isolated	
Isolate	3	Aliivibrio wodanis	Positive	
**: Unable to perform antibiotic sensitivities due to poor growth				
Isolate	3	Shewanella sp.	Positive	
**: Shewanella sp. identified as S.baltica by PCR				
Isolate	4		No Bacteria Isolated	

Fish Resulted by: Jaime Osei-Appiah Verified by: Jaime Osei-Appiah on 03/25/10 @ 11:25 AM

Organism	ID	e	ffc	s3	sxt	ot
Shewanella sp.	1	r	s	r	r	r
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethoprim, ot = Oxytetracycline						

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/15/10 @ 4:12 PM

Specimen	ID	Test	Result
Tissue	organs	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/15/10 @ 4:12 PM

Specimen	ID	Test	Result
Tissue	organs	PCR - VHSV	Positive

History of Communication

Date	To	Description
03/16/10 1:01 PM	Mainstream Canada-T - fax	bc report generated
03/16/10 1:02 PM	Dr. Peter McKenzie - e-mail	bc report generated
03/25/10 11:31 AM	Mainstream Canada-T - fax	bc report generated
03/25/10 11:31 AM	Dr. Peter McKenzie - e-mail	bc report generated
03/29/10 3:26 PM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-1086

Last Updated: 03/17/10 11:14 AM

Pathologist: Gary D. Marty

Received Date: 03/15/10

Collected Date:

Client Ref No: 10-2877

Veterinarian:

Clinic:

Phone:

Fax:

Submitter: **Tim Hewison - Microtek Int.**

Phone:

Fax:

Owner: **Microtek International In**

Phone:

Fax:(250) 652-4802

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age: Adult

Premise ID:

Case History

Submitted one Atlantic smolt kidney tissue for Histopathology.

The sample was preserved in formalin then transferred to tap water for transport.

The sample is labelled as 10- 2877.

Final Diagnosis

1. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, with intralesional necrosis and small bacterial rods consistent with *Renibacterium salmoninarum* infection, severe

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

3. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild

Final Comment: This fish probably died from complications related to disseminated *Renibacterium salmoninarum* infection (bacterial kidney disease). Interstitial cell hyperplasia in the kidney results from increased demand for erythrocytes or white blood cells somewhere in the body (renal interstitial cells are the blood-forming or hematopoietic cells in the kidney); in this case, the demand probably was for inflammatory cells in response to bacterial kidney disease.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination, handling, or disease). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 36%; n = 511).

Histopathology

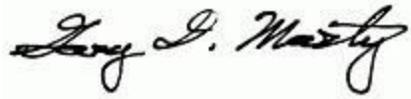
Formalin-fixed tissues were submitted in 1 cassette for histopathology.

Case: 10-1086

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

History of Communication

Date	To	Description
03/17/10 11:15 AM	Microtek International In - e-mail	bc report generated
03/19/10 2:12 PM	Microtek International In - e-mail	Case Invoiced



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END OF REPORT

Final Report AHC Case: 10-1146

Last Updated: 03/22/10 1:28 PM

Pathologist: Gary D. Marty

Received Date: 03/18/10

Collected Date:

Client Ref No: 7653

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Brad Boyce - 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 Atlantic tissues for Histopathology and PCR for IHN, VHS and ISA.

Saltwater entry 09 S0. Vaccinated: Yes. Insurance: No. Euthanized: Yes, TMS. Prior submission: No.

Fish #1/2 - Moribunds - TMS overdose and bled by cutting tail. Histo fish #1 - NVL. #2 - Skin lesion near tail and septicemic. #3 - NLV was a fresh mort.

Final Diagnosis

1. Skin/skeletal muscle: dermatitis, ulcerative, with deep myonecrosis and hemorrhage, with abundant filamentous bacterial, multifocal, coalescing, severe (slide 2A)
2. Head kidney: nephritis, interstitial, granulomatous, multifocal, mild (slides 1A, 3A)
3. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, moderate (slide 1A).
- 4a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1A, 2A)
- 4b. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 3A)
5. Brain: meningitis, granulomatous, multifocal, mild (slide 2A)
6. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, mild (slide 2A), moderate (slide 3A), severe (slide 1A)
7. Intestinal ceca and mesenteries: peritonitis, chronic, focal, with fibrocellular fronds and intralesional vacuoles about 50 µm in diameter, moderate (slide 2A)

Final Comment: These fish have several lesions that might provide clues to morbidity, but only the ulcer in fish 2 is of sufficient severity to have

killed the affected fish. Filamentous bacteria commonly invade skin ulcers. 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 (not available at the Animal Health Centre) or PCR. In saltwater, *Tenacibaculum maritimum* is likely. Comments on other lesions follow:

The most common organism associated with disseminated granulomas in farmed BC salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. Differentials include a reaction to a vaccine and chronic *Yersinia ruckeri* infection.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination, handling, or other disease). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 36% ; n = 511).

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. 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 "fresh silvers" that die in marine net pens, affecting 14% of the 514 Atlantic salmon livers examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

Cerebral meninges in slide 2A are thickened slightly by foci of activated macrophages. The section contains no obvious organisms, but differentials include infection with *Renibacterium salmoninarum*, *Yersinia ruckeri*, and an undescribed presporogonic myxosporean.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting the spleens of 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe). Vacuoles are probably a result of vaccine material lost during tissue processing.

Histopathology

Formalin-fixed tissues were submitted in 6 cassettes for histopathology. The gills (G) cassettes were immersed 3 h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

Slides 1A (7653-1) and 3A (7653-3) - brain, heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide 2A (7653-2) - brain, heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, skin/skeletal muscle, mesenteric adipose tissue

Slides 1G (7653-1), 2G (7653-2) and 3G (7653-3) - gill

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

Quality control: Liver autolysis: none (slides 1A, 2A, 3A). Gill decalcification is complete, and differential staining is good. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 03/22/10 @ 10:01 AM

Specimen	ID	Test	Result
Tissue	7653-1	PCR - IHNV	Negative
Tissue	7653-2	PCR - IHNV	Negative
Tissue	7653-3	PCR - IHNV	Negative
Tissue	7653-4	PCR - IHNV	Negative
Tissue	7653-5	PCR - IHNV	Negative

PCR - ISA Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 03/22/10 @ 10:01 AM

Case: 10-1146

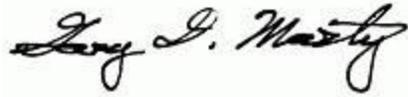
Specimen	ID	Test	Result
Tissue	7653-1	PCR - ISA	Negative
Tissue	7653-2	PCR - ISA	Negative
Tissue	7653-3	PCR - ISA	Negative
Tissue	7653-4	PCR - ISA	Negative
Tissue	7653-5	PCR - ISA	Negative

PCR - VHSV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 03/22/10 @ 10:01 AM

Specimen	ID	Test	Result
Tissue	7653-1	PCR - VHSV	Negative
Tissue	7653-2	PCR - VHSV	Negative
Tissue	7653-3	PCR - VHSV	Negative
Tissue	7653-4	PCR - VHSV	Negative
Tissue	7653-5	PCR - VHSV	Negative

History of Communication

Date	To	Description
03/22/10 1:29 PM	Morrison, Diane - fax	bc report generated
03/24/10 10:05 AM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-1156

Last Updated: 03/24/10 3:25 PM

Pathologist: Gary D. Marty

Received Date: 03/18/10

Collected Date: 03/16/10

Client Ref No: P.O. 14661

Veterinarian: **Dr. Peter McKenzie**

Clinic:

Phone:

Fax:

Submitter: **Zarah Vansnick - Mainstream Canada**

Phone:

Fax:

Owner: **Mainstream Canada-T**

Phone:

Fax:(250) 725-1250

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID: Binns Is.

Case History

Submitted fresh and formalized Atlantic salmon tissues for PCR and Histopathology; also plate for sensitivities by Bacteriology.

Saltwater. Age: 80g. Vaccinated: Yes. Duration of illness: 5 days. Prior submission: No. DOD: March. 16/10.

Gaping mouths - Flared opercula - some necrosis and mucus in gills.

Final Diagnosis

1. Gill: lamellar epithelial hyperplasia and fusion, multifocal, coalescing, with intralesional diatom spines/setae (*Chaetoceros sp.*), subacute, severe (slides 1G, 2G, 3G, 4G, 5G, 6G, 7G, 8G, 9G) and scattered bacterial rods (slides 2G, 3G, 4G, 6G, 7G, 8G, 9G) or filamentous bacteria (*Tenacibaculum maritimum?* Slide 5G), plus fibrin, hemorrhage, and necrosis (slides 3G, 4G, 5G)
- 2a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1A, 2A, 3A, 7A, 8A, 9A)
- 2b. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 4A), moderate (slide 2A)
- 2c. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate (slide 6A)
3. Spleen: peritonitis, granulomatous, multifocal, mild (slides 4A, 6A, 8A), moderate (slides 2A, 3A)
4. Intestinal ceca and mesenteries: peritonitis, chronic, focal, with fibrocellular fronds mild (slides 2A, 4A). Moderate (slide 7A)
5. Head kidney: vascular intracellular protein droplets (erythrophagocytosis?), diffuse, mild (slides 1A, 8A), moderate (slides 2A, 3A, 4A, 5A, 6A, 7A, 9A)
6. Trunk kidney: renal tubular mineralization, multifocal, mild (slide 5A), with dilated tubules and tubular epithelial hyperplasia, focal, moderate (slide 6A)

Final Comment: Clinical signs in these fish were a result of accumulation of diatoms in the gills. The nature of the reaction is consistent with most of the exposure occurring within 2-3 days of when the fish were sampled. 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, 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 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.] Bacteria that are probably secondary invaders include rods (e.g., a *Vibrio* or related species) and filaments (*Tenacibaculum maritimum*).

Other changes are probably secondary to the gill lesions or vaccines:

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. 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.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen or fluid). 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, but then decreased back to 43% in 2009. The change in vacuole prevalence might be related changes in the proportion of plant-based components in commercial feeds. At least some types of vacuoles might be normal; their effect on growth and feed conversion is unknown.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting spleens of 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe).

Accumulation of relatively large protein droplets with cells lining capillaries in the head kidney is an unusual finding. The size and staining pattern of the droplets is consistent with erythrocyte cytoplasm. This type of reaction in higher vertebrates is sometimes associated with damaged erythrocytes. Many of the spleens have similar eosinophilic droplets, but they are not diagnoses separately.

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.

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."

Histopathology

Formalin-fixed tissues from 9 fish were submitted in 9 cassettes for histopathology. For all cassettes, gills were removed from the original (A) cassettes and placed in separate (G) cassettes. The gills were immersed 3 h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

Slide #s 1A/1G-9A/9G are labeled in the same order as client #s Fish 1 Binns Mar 16/10 through Fish 9 Binns Mar 16/10

Organs included on most slides - gill, skin, heart, liver, spleen, head kidney (most slides), trunk kidney (some slides), intestinal ceca, mesenteric adipose tissue, brain

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

Quality control: Liver autolysis: none (slides 1A, 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A). Gill decalcification is complete, and differential staining is good. Organs have no postfixation dehydration and no acid hematin deposits.

Bacteriology

Specimen	ID	Isolate	Result	Level
Isolate	1	Aliivibrio wodanis	Positive	
Isolate	2	Aliivibrio wodanis	Positive	
Isolate	3	Aliivibrio wodanis	Positive	
Isolate	4	Aliivibrio wodanis	Positive	
Isolate	6		No Bacteria Isolated	
Isolate	7		No Bacteria Isolated	
Isolate	8		No Bacteria Isolated	
Isolate	9	Vibrio splendidus	Positive	

Fish Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 03/24/10 @ 3:25 PM

Organism	ID	e	ffc	s3	sxt	ot
Vibrio splendidus	9	s	s	s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethopri ot = Oxytetracycline						
Aliivibrio wodanis	1	s	s	s	s	s

Molecular Diagnostics

PCR - IHN Resulted by: Ken Sojonyk Verified by: Dr. J. Robinson on 03/22/10 @ 10:01 AM

Specimen	ID	Test	Result
Tissue	Pen 101	PCR - IHN	Negative
Tissue	Pen 105	PCR - IHN	Negative

PCR - VHSV Resulted by: Ken Sojonyk Verified by: Dr. J. Robinson on 03/22/10 @ 10:01 AM

Specimen	ID	Test	Result
Tissue	Pen 101	PCR - VHSV	Negative
Tissue	Pen 105	PCR - VHSV	Negative

History of Communication

Date	To	Description
03/22/10 3:48 PM	Mainstream Canada-T - fax	bc report generated
03/22/10 3:48 PM	Dr. Peter McKenzie - e-mail	bc report generated
03/25/10 11:26 AM	Mainstream Canada-T - fax	bc report generated
03/25/10 11:27 AM	Dr. Peter McKenzie - e-mail	bc report generated
03/25/10 3:38 PM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-1255

Last Updated: 03/26/10 2:02 PM

Pathologist: Gary D. Marty

Received Date: 03/25/10

Collected Date:

Client Ref No: 7673

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Brad Boyce - Marine Harvest Can**

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 Atlantic salmon tissue for Histopathology.

Saltwater entry: 09 S0. Euthanized: No. Prior submission: No. Vaccinated: No. Insurance: No.

Fresh mort with swollen granular kidney. No visible lesions in other organs except some vaccine droplets in muscle around injection site.

Final Diagnosis

1a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, with intralesional vacuoles up to 150 µm in diameter, severe

1b. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, moderate

2a. Liver: peritonitis, fibrinous, granulomatous, with subcapsular sinusoidal congestion, focal (along one margin of the liver), subacute, moderate

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

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

Final Comment: This fish might have died from complications related to fibrinous peritonitis, and granulomatous interstitial nephritis. Granulomatous inflammation is evidence of persistent antigen, and the vacuoles are foci where material was lost during processing. This type of reaction is consistent with a vaccine reaction. Lack of organisms on the Gram stain rules out the primary differential: *Renibacterium salmoninarum* infection.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination, handling, or other disease). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 36% ; n = 511).

The focus of fibrinous peritonitis on one margin of the liver includes a 20-µm-thick band of fibrin adhered to the hepatic capsule. The surface of the band is covered by a 60-µm- thick band of granulomatous inflammation. Sinusoids deep to the inflammation are congested. The cause of this lesion is probably the same as for the granulomatous inflammation in the kidney (i.e., probably a vaccine reaction). The lesion has no organisms on Twort's Gram stain.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. 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 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe).

Histopathology

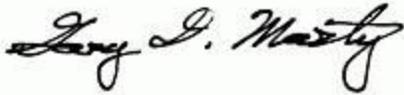
Formalin-fixed tissues were submitted in 1 cassette for histopathology.

Slide 1 (7673-1) - heart, spleen, liver, trunk kidney; sections were stained with H&E and Twort's Gram. 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.

History of Communication

Date	To	Description
03/26/10 2:03 PM	Morrison, Diane - fax	bc report generated
03/29/10 4:05 PM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-1324

Last Updated: 03/31/10 1:41 PM

Pathologist: Gary D. Marty

Received Date: 03/30/10

Collected Date:

Client Ref No: 10-016

Veterinarian: **Dr. Peter McKenzie**

Clinic:

Phone:

Fax:

Submitter: **N.C- Mainstream Canada**

Phone:

Fax:

Owner: **Mainstream Canada - NC**

Phone:

Fax:(250) 286-0042

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

Case History

Submitted formalized Atlantic salmon tissue for Histopathology. (Show cassettes to Dr. Marty for trimming as labelled on jar).

Age:? Saltwater: Yes. Vaccinated: Yes. Insurance: No. Prior submission: No. DOD: Smoked consumer product.

Strange exhibition of crystal like spotting throughout cold smoked fillet, cut 1cm thick as directed. 2 cassettes each from affected (labelled A1 & A2) and Non affected (labelled N1 & N2) areas formalized for Histo.

Final Diagnosis

1. Skeletal muscle (near smoked surface): intracellular crystals , multifocal (up to 0.5 mm in diameter), moderate (slides A1, A2)

Final Comment: The pale white foci on the surface of the smoked product (described grossly) are consistent with insoluble white crystals in sections of the affected samples; in contrast, the nonaffected samples have no crystals. The crystals extend from myofibre cell membrane (e.g., slide A1 DL1, stage coordinates on my microscope = 17 x 115) into the cytoplasm. Larger crystals coalesce and efface several adjacent cells. Because the crystals are limited to the smoked surface of the samples, they probably developed during the smoking process (i.e., they are probably not a result of pre-existing lesions in the fish). The affected samples have less adipose tissue than the nonaffected samples, and the amount of adipose tissue in the nonaffected samples is similar to most Atlantic salmon skeletal muscle samples that I examine. It might be worthwhile to examine whether differences in fat content occur within the same fish, or if differences in fat content represent differences between fish (e.g., genetic or feeding differences). Sections have no evidence of *Kudoa thyrsites* or any other infectious organisms or lesions.

Histopathology

Four cassettes nearly filled with samples of smoked skeletal muscle from Atlantic salmon were submitted for histopathology. The pieces were trimmed to a smaller size for processing. For each piece, the first cut was parallel with the smoked edge of the sample, and the part furthest from the smoked edge was archived (but not processed). The remaining piece was cut in half transversely; both resultant pieces were processed routinely into paraffin. In this way, the entire smoked edge of each sample was included on the slides for examination. Three step-sections were cut through each block at approximately 250-µm intervals (= deeper levels or DL). Slides are numbered the same as the 4 submitted cassettes: affected (A1 & A2) and nonaffected (N1 & N2). Photomicrographs of representative affected and nonaffected skeletal muscle were e-mailed separately to Nathan Cassan and Peter McKenzie (the Animal Health Centre's VADDS database cannot handle high

resolution micrographs).

Quality Control: Preservation of microscopic structure is good. The surface of the section has evidence of dehydration (e.g., nuclei stain dull blue, and cytoplasm stains poorly or not at all), and blood vessels throughout the samples are coagulated, but these changes are probably normal for smoked product. Tissues have no acid hematin deposits.

History of Communication

Date	To	Description
03/31/10 1:42 PM	Mainstream Canada - NC - e-mail	bc report generated
03/31/10 1:42 PM	Dr. Peter McKenzie - e-mail	bc report generated
03/31/10 3:49 PM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-1336

Last Updated: 04/06/10 1:39 PM

Pathologist: Gary D. Marty

Received Date: 03/31/10

Collected Date: 03/24/10

Client Ref No: 7688

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Tiffany MacWilliam - 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 Atlantic salmon tissues for Histopathology.

Saltwater entry: 2008 S0. Vaccinated: Yes. Euthanized: Percussion stun. Prior submission: No. # submitted dead - 2. DOD: Mar. 24/10.

Fish in pen developing external lesion. Fish 1 - Cassette 1 had small oval lesion on body (assuming sealice) and shrunken liver. Fish 2 - Had small circular lesions on operculum, fins and 1 on body: Cassette #2 - contains lesion from operculum; Cassette #3 - contains lesion from body. Please check placement of lesions as per email.

Final Diagnosis

1. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, moderate (slide 1)
2. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 1)
3. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 1)
4. Skin (operculum): dermatitis, ulcerative, focal, with scale pocket lined by macrophages filled with filamentous bacteria, moderate (slide 2)
5. Skin (adjacent to skeletal muscle): dermatitis, ulcerative, focal, lymphohistiocytic with small numbers of macrophages containing filamentous bacteria, mild (slide 3)

Final Comment: The small circular lesions on fish #2 are ulcers infected by filamentous bacteria. Filamentous bacteria commonly invade skin ulcers. 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 (not available at the Animal Health Centre) or PCR. In saltwater, *Tenacibaculum maritimum* is likely.

The liver in fish #1 has several minor changes, but none are of sufficient severity to have killed the fish. Comments on specific changes follow:

Pigment in the liver is probably 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.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It might be related to increased protein needed as part of an inflammatory response. It is common in juvenile fish with ulcers. Mature females normally have basophilic hepatocytes: needed to produce protein for deposition in their eggs.

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

Histopathology

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

Slide 1 (7688-1) - liver, spleen, skin/skeletal muscle

Slide 2 (7688-2) - operculum

Slide 3 (7688-3) - skin/skeletal muscle

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

Quality control: Liver autolysis: none (slide 1). Hepatocytes adjacent to large foci of erythrocytes (slide 1) have a few 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. Organs have no postfixation dehydration.

History of Communication

Date	To	Description
04/06/10 1:40 PM	Morrison, Diane - fax	bc report generated
04/12/10 2:23 PM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-1367

Last Updated: 04/08/10 11:30 AM

Pathologist: Gary D. Marty

Received Date: 04/06/10

Collected Date: 03/30/10

Client Ref No: 7698

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Gerry Burry - Marine Harvest Can**

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 Atlantic salmon tissue for PCR for IHN, ISA and VHS.

Saltwater entry:2009 S0. Vaccinated: Yes. Insurance: No. Prior submission: No. DOD: Mar. 30/10

1 sample for PCR. Fish collected during routine mort dive.

Molecular Diagnostics

PCR - IHN Resulted by: A Scouras Verified by: Dr. J. Robinson on 04/08/10 @ 11:30 AM

Specimen	ID	Test	Result
Tissue	organs	PCR - IHN	Negative

PCR - ISA Resulted by: A Scouras Verified by: Dr. J. Robinson on 04/08/10 @ 11:30 AM

Specimen	ID	Test	Result
Tissue	organs	PCR - ISA	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 04/08/10 @ 11:30 AM

Specimen	ID	Test	Result
Tissue	organs	PCR - VHSV	Negative

History of Communication

Date	To	Description
04/08/10 1:10 PM	Morrison, Diane - fax	bc report generated
04/12/10 2:31 PM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-1368

Last Updated: 05/05/10 2:07 PM

Pathologist: Gary D. Marty

Received Date: 04/06/10

Collected Date: 04/01/10

Client Ref No: 7701

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Gerry Burry - Marine Harvest Can**

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 Atlantic salmon tissue for Histopathology and PCR for IHN, ISA and VHS and tissue culture.

Saltwater entry:2010 S1. DOD:Apr. 1/10.

Fresh dead fish were collected from recent mortalities. All fish had varying degrees of hemorrhaging on liver, pyloric caeca and swim bladder and hemorrhage in musculature. Bacteriology from two days earlier on Mar. 30/10 have revealed no bacterial growth. Bacteriology from this group of fish is pending. Please run PCR and Histo as soon as possible.

Final Diagnosis

1a. Trunk kidney: intratubular hemorrhage and fibrin, with hemorrhage within the urinary space, multifocal, moderate (slides 1, 2)

1b. Trunk kidney: renal tubular mineralization, multifocal, with hemorrhage, dilated tubules and tubular epithelial hyperplasia (nephrocalcinosis), mild (slide 2), moderate (slide 1)

2. Heart, compact cortical layer: congestion and hemorrhage, multifocal, moderate (slide 1)

3a. Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate (slide 2)

3b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate (slide 1)

4. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1)

Final Comment: Congestion and hemorrhage in multiple organs is consistent with gross findings. Differentials include infectious disease and electrocution. Comments on specific lesions follow:

Renal intratubular hemorrhage and fibrin is evidence of damaged glomeruli or other renal vessels. In this case, the hemorrhage is often within tubules that are lined by deeply basophilic epithelial cells (i.e., regenerative epithelial cells). Hemorrhage in the urinary space is evidence that

glomeruli are the source of the hemorrhage. This is an unusual lesion that I have seen only 3 other times, all with Marine Harvest cases and all about the same time of year: 2008-1098(MH6605), 2008-1462(MH6615), 2009- 1433(MH7214). The fish have no evidence of infectious disease other than hemorrhage; is there any chance that these fish were electrocuted?

Renal mineralization is common in cultured fish species; when moderate to severe, the condition is termed nephrocalcinosis. The association with hemorrhage in this case is unusual. Renal mineralization 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.

Congestion and hemorrhage in the stratum compactum of the heart (i.e., the peripheral layer of dense cardiac muscle) is a distinctive lesion that I started seeing in 2008 in clinical submissions from both Atlantic and Pacific salmon. I noted this change only once among samples examined from 2006 - 2008 as part of the BC Audit and Surveillance Program, but in 2009 I had several cases. Clinical cases included all Marine Harvest cases that also had renal intratubular hemorrhage (i.e., those cases listed above). Therefore, differentials are the same as for the renal hemorrhage.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen or fluid). 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, but then decreased back to 43% in 2009. The change in vacuole prevalence might be related changes in the proportion of plant-based components in commercial feeds. At least some types of vacuoles might be normal; their effect on growth and feed conversion is unknown.

Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. 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, IHNV, and ISAV (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe).

Histopathology

Formalin-fixed tissues from 2 fish were submitted in 2 cassettes for histopathology. Slide #s 1 - 2 are labeled in the same order as client #s 7701-1 - 7701-2. Organs included on both slides - heart, liver, spleen, trunk kidney (several pieces), intestinal ceca, mesenteric adipose tissue. All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Liver autolysis: mild (slide 2), severe (slide 1). Organs have no postfixation dehydration and no acid hematin deposits (other than the congested foci in the slide - 2 liver).

Measures of physiologic condition:

Hepatocellular glycogen: none (both slides)

Mesenteric adipose tissue: abundant (both slides)

These measures of physiologic condition are consistent with healthy fish that were not eating full rations in the two days before they died.

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 04/08/10 @ 11:30 AM

Specimen	ID	Test	Result
Tissue	7701-1	PCR - IHNV	Negative
Tissue	7701-2	PCR - IHNV	Negative

PCR - ISA Resulted by: A Scouras Verified by: Dr. J. Robinson on 04/08/10 @ 11:30 AM

Specimen	ID	Test	Result
Tissue	7701-1	PCR - ISA	Negative
Tissue	7701-2	PCR - ISA	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 04/08/10 @ 11:30 AM

Specimen	ID	Test	Result
Tissue	7701-1	PCR - VHSV	Negative
Tissue	7701-2	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Hughes, Giselle Verified by: Dr. J. Robinson on 05/05/10 @ 2:07 PM

Specimen	ID	Isolate	Result	Level
Tissue	7701-1		No viruses isolated	
Tissue	7701-2		No viruses isolated	

Staff Comments:

Interim report (PDF version) with histopathology and PCR results sent to MH fish health staff via Outlook attachment by GD Marty, Thu 2010-04-08 1:17 PM.

History of Communication

Date	To	Description
05/06/10 4:29 PM	Morrison, Diane - fax	bc report generated
05/10/10 10:58 AM	Marine Harvest Canada - e-mail	Case Invoiced



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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END OF REPORT

Final Report AHC Case: 10-1417

Last Updated: 04/13/10 1:30 PM

Pathologist: Gary D. Marty

Received Date: 04/08/10

Collected Date: 03/31/10

Client Ref No: PO.14750

Veterinarian: **Dr. Peter McKenzie**

Clinic:

Phone:

Fax:

Submitter: **Zarah Vansnick - Mainstream Canada**

Phone:

Fax:

Owner: **Mainstream Canada-T**

Phone:

Fax:(250) 725-1250

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID: 10-016

Case History

Submitted fresh and formalized Atlantic salmon tissues for Histopathology and PCR for viral BKD.

Age: 100g. Saltwater. Vaccinated: No. Prior submission: No. DOD: Mar. 31/10.

Mortality post entry into salt water. Transport tank had dissolved oxygen issues. Saltwater free of plankton. Fish have swollen gills - discolouration dorsal surface. Acites - mucus in stomachs.

Final Diagnosis

1a. Liver: hepatocellular hydropic degeneration, disseminated, acute, mild (slide 3)

1b. Liver: biliary preductular cell hyperplasia, diffuse, mild (slides 3, 6, 8, 10)

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

2b. Trunk kidney: renal tubular mineralization, multifocal, with dilated tubules and tubular epithelial hyperplasia (nephrocalcinosis), moderate (slide 2)

2c. Trunk and head kidney: interstitial macrophages distended with homogenous protein (erythrophagocytosis?), disseminated, moderate (slide 4)

2d. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slides 6, 9)

3. Spleen: peritonitis, granulomatous, focal, with fibrocellular fronds, mild (slides 2, 5, 6, 8, 9), moderate (slides 1, 3, 10), severe (slide 7)

4. Intestinal ceca and mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1, 5, 7, 8)

5. Heart: endocardial histiocytosis, focal, mild (slide 4)

6. Brain: meningeal and neuropil hemorrhage, focal, moderate (slide 5)

7. Gill: lamellar epithelial hyperplasia, diffuse, moderate (slides 6G, 7G, 10G)

Final Comment: These fish have several lesions, but few are of sufficient severity of have killed the fish. Potentially lethal lesions are in fish #3 (renal tubular necrosis) and fish #5 (cerebral hemorrhage). Gill lamellae in several fish are lined by prominent chloride cells that might be in numbers greater than normal. Although fish have no microscopic evidence of BKD, three fish positive by PCR for *Renibacterium salmoninarum* provide evidence that this population has been exposed and some individuals will probably eventually die of the disease. Comments on specific lesions follow:

Hydropic degeneration among small numbers of hepatocytes provides evidence that the affected livers were being exposed to toxins (e.g., algal toxins or other natural toxins). Potential sources of the inciting toxins include the water or feed, a bacterial infection, or circulating oxygen radicals following a period of hypoxia. 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. Reference: Wolf, J.C., and M.J. Wolfe. 2005. A brief overview of nonneoplastic hepatic toxicity in fish. *Toxicologic Pathology*. 33(1):75-85.

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 "fresh silvers" that die in marine net pens, affecting 14% of the 514 Atlantic salmon livers examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

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). Mild cases are fairly common in debilitated fish. Renal tubular epithelial necrosis was fairly common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 12% ; n = 511); 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, if not already done.

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.

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

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination, handling, or other disease). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 36% ; n = 511).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting the spleen of 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe).

Macrophages line the endocardial surface in a small focus of the ventricle (fish #4). Differentials include reaction to a vaccine and a low grade bacterial infection.

The most common cause of meningeal hemorrhage is trauma. 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.

Hyperplasia of the epithelium lining gill lamellae is a nonspecific response to irritation. Inciting causes include parasites, bacteria, viruses, and toxins. This case has no obvious organisms, and adjacent lamellae are not fused. This lesion cannot be reliably diagnoses on gills with moderate or severe autolysis.

Histopathology

Formalin-fixed tissues from 10 fish were submitted in 10 cassettes for histopathology. After processing into paraffin, the gills were removed from the submitted cassette and placed in a 'G' cassette with the same # for sectioning. Slide #s 1/1G - 10/10G are labeled in the same order as client #s 1 - 10. Organs included on most slides - heart, liver, spleen, head kidney or trunk kidney, intestinal ceca, mesenteric adipose tissue . All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Liver autolysis: none (slides 4, 5, 7), mild (slides 1, 2, 6, 8, 9, 10), moderate (slide 3). Gill autolysis: none (slide 7G), mild (slides 4G, 5G), moderate (slides 1G, 2G, 3G, 6G, 8G, 9G, 10G). In many of the gill sections, the tips of the filaments are better preserved than the base; this might be a result of poor fixative penetration. To minimize this problem, fix the gill arch in a cassette separate from the other tissues (e.g., label cassettes '1' for tissues and '1G' for gill). The Animal Health Centre does not charge extra for two cassettes per fish. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR-Renibacterium salmoni Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 04/13/10 @ 1:10 PM

Specimen	ID	Test	Result
Tissue	org, fish#1	PCR-Renibacterium salmoninaru	Negative
Tissue	org, fish#2	PCR-Renibacterium salmoninaru	Negative
Tissue	org, fish#3	PCR-Renibacterium salmoninaru	Positive
Tissue	org, fish#4	PCR-Renibacterium salmoninaru	Negative
Tissue	org, fish#5	PCR-Renibacterium salmoninaru	Negative
Tissue	org, fish#6	PCR-Renibacterium salmoninaru	Negative
Tissue	org, fish#7	PCR-Renibacterium salmoninaru	Positive
Tissue	org, fish#8	PCR-Renibacterium salmoninaru	Negative
Tissue	org, fish#9	PCR-Renibacterium salmoninaru	Negative
Tissue	org, fish#10	PCR-Renibacterium salmoninaru	Positive
Tissue	org, pen#101	PCR-Renibacterium salmoninaru	Negative

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 04/13/10 @ 1:10 PM

Specimen	ID	Test	Result
Tissue	org, #1-4	PCR - IHNV	Negative
Tissue	org, #5-8	PCR - IHNV	Negative
Tissue	org, #9-11	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 04/13/10 @ 1:10 PM

Specimen	ID	Test	Result
Tissue	org, #1-4	PCR - VHSV	Negative
Tissue	org, #5-8	PCR - VHSV	Negative
Tissue	org, #9-11	PCR - VHSV	Negative

History of Communication

Date	To	Description
04/13/10 1:32 PM	Mainstream Canada-T - fax	bc report generated
04/13/10 1:32 PM	Dr. Peter McKenzie - e-mail	bc report generated
04/14/10 9:15 AM	Mainstream Canada - e-mail	Case Invoiced



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

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Case: 10-1417

END OF REPORT

Final Report AHC Case: 10-1442

Last Updated: 05/05/10 2:07 PM

Pathologist: Gary D. Marty

Received Date: 04/12/10

Collected Date: 04/06/10

Client Ref No: 7703

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Gerry Burry - 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 formalized Atlantic salmon tissue for Histopathology and fresh tissue for PCR for IHN, VHS and ISA and tissue culture.

Saltwater entry: 2010 S1. Euthanized: No. Prior submission - Yes, 7701 (2010-1368). DOD: April. 6, 2010.

Fresh dead collected from recent mortalities. Samples submitted had hem on liver, pyloric caeca, swim bladder and in muscle. No bacterial growth on samples taken from these fish. Sent 4 histo and 2 PCR/tissue culture.

Final Diagnosis

1a. Trunk kidney: renal tubular epithelial necrosis, with interstitial fibrin, multifocal, subacute, mild (slide 4), moderate (slide 2), severe (slides 1, 3)

1b. Trunk kidney: hydropic degeneration of renal tubular epithelial cells, diffuse, acute, moderate (slides 1, 2), severe (slides 3, 4)

1c. Trunk kidney: intratubular hemorrhage and fibrin, with hemorrhage within the urinary space, multifocal, moderate (slide 1), severe (slide 4)

2a. Liver: hepatic necrosis, acute, focal, moderate (slide 2)

2b. Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions, acute, moderate, focal (slides 2, 4), multifocal (slides 1, 3)

2c. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slide 2), moderate (slides 1, 3, 4)

3. Mesenteric adipose tissue: capillary congestion/hemorrhage, focal, moderate (slide 3), multifocal, moderate (slides 2, 4)

4. Trunk kidney: renal tubular mineralization, multifocal, with dilated tubules and tubular epithelial hyperplasia (nephrocalcinosis), moderate (slide 2)

Final Comment: These fish died from changes that are most severe in the kidney but also include systemic vascular damage. Differentials include infectious disease and toxin exposure; electrocution seems less likely because lesions that might be associated with electrocution (kidney hemorrhage) do not affect all fish. Comments on specific lesions follow:

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). Mild cases are fairly common in debilitated fish. Fibrosis in this case is evidence that the necrosis is at least 48 hours old. Renal tubular epithelial necrosis was fairly common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 12% ; n = 511); the cause was not determined in many cases. Differentials include exposure to toxins (e.g., bacterial toxins, algal toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin). PCR results rule out a common cause in saltwater, VHSV.

Hydropic degeneration of renal tubular epithelial cells is evidence of acute damage. Affected cells are swollen by irregular clear cytoplasmic vacuoles. This type of change is rare in renal tubular epithelial cells of cultured salmonids. Potential causes include exposure to toxins or periods of hypoxia followed by reperfusion.

Renal intratubular hemorrhage and fibrin is evidence of damaged glomeruli or other renal vessels. In this case, the hemorrhage is often within tubules that are lined by deeply basophilic epithelial cells (i.e., regenerative epithelial cells). Hemorrhage in the urinary space is evidence that glomeruli are the source of the hemorrhage. This is an unusual lesion that I have seen only 3 other times (before 2010-1368, MH7701), all with Marine Harvest cases and all about the same time of year: 2008- 1098(MH6605), 2008-1462(MH6615), 2009-1433(MH7214). The fish have no evidence of infectious disease other than hemorrhage; is there any chance that these fish were electrocuted?

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 it is associated with the focus of congestion. 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, in 2009 affecting 12% of the 514 Atlantic examined as part of the Province's Fish Health Auditing and Surveillance Program.

Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV (ruled out in this case by PCR); the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. 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 are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen or fluid). 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, but then decreased back to 43% in 2009. The change in vacuole prevalence might be related changes in the proportion of plant-based components in commercial feeds. At least some types of vacuoles might be normal; their effect on growth and feed conversion is unknown.

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 (ruled out in this case by PCR) and bacterial infections, and sometimes it seems to be associated with a vaccine reaction; the cause is sometimes not determined.

Renal mineralization is common in cultured fish species; when moderate or 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.

Histopathology

Formalin-fixed tissues from 4 juvenile Atlantic salmon were submitted in 4 cassettes for histopathology. Slide #s 1 - 4 are labeled in the same order as client #s 1 - 4. Organs included on most slides - heart, liver, spleen, 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 (slide 3), moderate (slides 2, 4), severe (slide 1). Organs have no postfixation dehydration and no acid hematin deposits.

Measures of physiologic condition:

Hepatocellular glycogen: none (slides 1, 2, 3, 4)

Mesenteric adipose tissue: abundant (all 4 slides)

These measures of physiologic condition are consistent with fish that had been healthy but were not eating full rations at least two days before they died.

Molecular Diagnostics

PCR - IHN Resulted by: A Scouras Verified by: Dr. J. Robinson on 04/14/10 @ 11:26 AM

Specimen	ID	Test	Result
Tissue	7703-1	PCR - IHN	Negative
Tissue	7703-2	PCR - IHN	Negative

PCR - ISA Resulted by: A Scouras Verified by: Dr. J. Robinson on 04/14/10 @ 11:26 AM

Specimen	ID	Test	Result
Tissue	7703-1	PCR - ISA	Negative
Tissue	7703-2	PCR - ISA	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 04/14/10 @ 11:26 AM

Specimen	ID	Test	Result
Tissue	7703-1	PCR - VHSV	Negative
Tissue	7703-2	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Hughes, Giselle Verified by: Dr. J. Robinson on 05/05/10 @ 2:07 PM

Specimen	ID	Isolate	Result	Level
Tissue	7703-1		No viruses isolated	
Tissue	7703-2		No viruses isolated	

Staff Comments:

Interim report (PDF version) with histopathology and PCR results sent to MH fish health staff via Outlook attachment by GD Marty, Thu 2010-04-15 1:54 PM

History of Communication

Date	To	Description
05/06/10 4:31 PM	Morrison, Diane - fax	bc report generated
05/10/10 10:58 AM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-1552

Last Updated: 05/11/10 2:32 PM

Pathologist: Gary D. Marty

Received Date: 04/16/10

Collected Date: 04/14/10

Client Ref No: 10-020; PO 14685

Veterinarian: **Dr. Peter McKenzie**

Clinic:

Phone:

Fax:

Submitter: **Kelly Abel - Mainstream Can.**

Phone:

Fax:

Owner: **Mainstream Canada**

Phone:

Fax:(250) 286-0042

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID: Cypress

Case History

Submitted fresh and formalized Atlantic salmon tissue for Histopathology, Viral culture and PCR for VHS, IHN and BKD.

Saltwater. Vaccinated: Yes. Insurance: No. # dead - 2. DOD: April. 14, 2010.

Swelling in kidneys being observed.

Final Diagnosis

1. Multiple organs (liver, kidney, heart, spleen, gill): disseminated granulomatous inflammation, with intralesional short bacilli characteristic of *Renibacterium salmoninarum* , severe (slide 18A)
2. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 17A)
3. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slide 17A)

Final Comment: Short bacilli in multiple foci of granulomatous inflammation in slide 18A are diagnostic for *Renibacterium salmoninarum* , the cause of bacterial kidney disease, and the associated lesions probably killed this fish. These lesions are consistent with the observation that the affected fish had a swollen kidney. In contrast, the kidney from fish #17 is fairly normal, and the cause of death of this fish is unknown.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe).

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination, handling, or other disease). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 36%; n = 511) and Pacific salmon (prevalence = 42%; n = 71).

Histopathology

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

Slide 17A (Cypress Pen 17 04/14/10) - heart, liver, spleen, trunk kidney (2 pieces), intestinal ceca, mesenteric adipose tissue, brain

Slide 18A (Cypress Pen 18 04/14/10) - heart, liver, spleen, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue, brain

Slides 17B (Cypress Pen 17 04/14/10) and 18B (Cypress Pen 18 04/14/10) - gill

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

Quality control: Liver autolysis: mild (slide 17A), severe (slide 18A). Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR-Renibacterium salmoni Resulted by: Julie Bidulka Verified by: A Scouras on 04/20/10 @ 3:56 PM

Specimen	ID	Test	Result
Tissue	Cypress pen 17	PCR-Renibacterium salmoninaru	Positive
Tissue	Cypress pen 18	PCR-Renibacterium salmoninaru	Positive

PCR - IHN Resulted by: Julie Bidulka Verified by: A Scouras on 04/20/10 @ 8:59 AM

Specimen	ID	Test	Result
Tissue	Cypress pen 17	PCR - IHN	Negative
Tissue	Cypress pen 18	PCR - IHN	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: A Scouras on 04/20/10 @ 8:59 AM

Specimen	ID	Test	Result
Tissue	Cypress pen 17	PCR - VHSV	Negative
Tissue	Cypress pen 18	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Cheryl Cecconi Verified by: Dr. J. Robinson on 05/11/10 @ 2:32 PM

Specimen	ID	Isolate	Result	Level
Tissue	Cypress pen 17		No viruses isolated	
Tissue	Cypress pen 18		No viruses isolated	

History of Communication

Date	To	Description
04/20/10 4:05 PM	Mainstream Canada - e-mail	bc report generated
04/20/10 4:05 PM	Dr. Peter McKenzie - e-mail	bc report generated
05/11/10 3:47 PM	Mainstream Canada - e-mail	bc report generated
05/11/10 3:47 PM	Dr. Peter McKenzie - e-mail	bc report generated
05/13/10 2:40 PM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-1553

Last Updated: 05/11/10 2:32 PM

Pathologist: Gary D. Marty

Received Date: 04/16/10

Collected Date: 04/14/10

Client Ref No: 10-019; PO14685

Veterinarian: **Dr. Peter McKenzie**

Clinic:

Phone:

Fax:

Submitter: **Kelly Abel - Mainstream Can.**

Phone:

Fax:

Owner: **Mainstream Canada**

Phone:

Fax:(250) 286-0042

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID: Simmonds

Case History

Submitted fresh and formalized Atlantic salmon tissue for Histopathology, Viral culture and PCR for VHS, IHN and BKD; also one plate for Bacteriology.

Saltwater. Vaccinated: Yes. # dead - 2. DOD: April. 14, 2010.

Swelling in kidneys being observed. Above test noted Bacteriology identification.

Final Diagnosis

1. Multiple organs (liver, kidney, heart): disseminated granulomatous inflammation, severe (slide 4A), with intralesional short bacilli characteristic of *Renibacterium salmoninarum*, severe (slide 6A)
2. Gill: branchitis, ulcerative, with moderate numbers of filamentous bacteria, multifocal, moderate (slide 6B)
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 4A, 6A)
4. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slides 4A, 6A)

Final Comment: Short bacilli in multiple foci of granulomatous inflammation in slide 6A are diagnostic for *Renibacterium salmoninarum*, the cause of bacterial kidney disease. Identical lesions in slide 4A are probably a result of *Renibacterium salmoninarum* infection, but bacteria are not obvious on H&E (this is fairly common with BKD). In both fish, lesions are most severe in the kidney, consistent with the observation that the affected fish had swollen kidneys.

Contributing to the death of the fish from pen 6 is ulcerative branchitis with filamentous bacteria. Thin rod-shaped to filamentous bacteria are common on the gills of debilitated juvenile salmonids. Although bacterial culture or PCR is required for a definitive diagnosis, the common species in the gill in marine waters is *Tenacibaculum maritimum* (one cause of necrotizing branchitis). Infections are usually associated with crowding, poor water quality, or stress (e.g., concurrent BKD).

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe).

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination, handling, or other disease). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 36%; n = 511) and Pacific salmon (prevalence = 42%; n = 71).

Histopathology

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

Slide 4A (Simmonds Pen 4 04/14/10) - heart, liver, spleen, head kidney, trunk kidney, stomach, intestinal ceca, mesenteric adipose tissue, brain

Slide 6A (Simmonds Pen 6 04/14/10) - heart, liver, spleen, trunk kidney (2pieces), intestinal ceca, mesenteric adipose tissue, brain

Slides 4B (Simmonds Pen 4 04/14/10) and 6B (Simmonds Pen 6 04/14/10) - gill

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

Quality control: Liver autolysis: severe (slides 4A, 6A). Organs have no postfixation dehydration and no acid hematin deposits.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 04/21/10 @ 11:33 AM

Specimen	ID	Isolate	Result	Level
Isolate	Pen 4 Kidney	Vibrio lentus	Positive	
Isolate	Pen 4 Kidney	Aliivibrio wodanis	Positive	
Isolate	Pen 4 Brain	Aliivibrio wodanis	Positive	
Isolate	Pen 6 Kidney	Aliivibrio wodanis	Positive	
Isolate	Pen 6 Brain	Aliivibrio wodanis	Positive	

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 04/21/10 @ 11:33 AM

Organism	ID	e	ffc	s3	sxt	ot
Vibrio lentus	Pen 4 Kidney	s	s	r	s	s
<p>** Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethopri ot = Oxytetracycline</p>						
Aliivibrio wodanis	Pen 4 Kidney					
<p>** Unable to performs sensitivities due to poor growth of organism.</p>						

Molecular Diagnostics

PCR-Renibacterium salmoni Resulted by: Julie Bidulka Verified by: A Scouras on 04/20/10 @ 3:56 PM

Specimen	ID	Test	Result
Tissue	Simmonds Pen 4	PCR-Renibacterium salmoninaru	Positive
Tissue	Simmonds Pen 6	PCR-Renibacterium salmoninaru	Positive

PCR - IHNV Resulted by: Julie Bidulka Verified by: A Scouras on 04/20/10 @ 8:59 AM

Specimen	ID	Test	Result
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Tissue	Simmonds Pen 4	PCR - IHNV	Negative
Tissue	Simmonds Pen 6	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: A Scouras on 04/20/10 @ 8:59 AM

Specimen	ID	Test	Result
Tissue	Simmonds Pen 4	PCR - VHSV	Negative
Tissue	Simmonds Pen 6	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Cheryl Cecconi Verified by: Dr. J. Robinson on 05/11/10 @ 2:32 PM

Specimen	ID	Isolate	Result	Level
Tissue	Simmonds Pen 4		No viruses isolated	
Tissue	Simmonds Pen 6		No viruses isolated	

History of Communication

Date	To	Description
04/20/10 4:07 PM	Mainstream Canada - e-mail	bc report generated
04/20/10 4:07 PM	Dr. Peter McKenzie - e-mail	bc report generated
05/11/10 3:48 PM	Mainstream Canada - e-mail	bc report generated
05/11/10 3:48 PM	Dr. Peter McKenzie - e-mail	bc report generated
05/13/10 2:44 PM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-1625

Last Updated: 04/22/10 2:49 PM

Pathologist: Gary D. Marty

Received Date: 04/20/10

Collected Date: 04/13/10

Client Ref No: PO 14777

Veterinarian: **Dr. Peter McKenzie**

Clinic:

Phone:

Fax:

Submitter: **Zarah Vansnick - Mainstream Canada**

Phone:

Fax:

Owner: **Mainstream Canada-T**

Phone:

Fax:(250) 725-1250

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID: 10-018; PO14777

Case History

Submitted formalized Atlantic salmon tissue for Histopathology.

Age: 80g. Saltwater. Vaccinated: Yes. Prior case: 10-1156. DOD: Apr. 13/10.

Gaping mouths, necrosis in gills. Mortality began 1 day post entry into saltwater. Rule out disease.

Final Diagnosis

1a. Gill: lamellar epithelial hyperplasia and fusion, multifocal, coalescing, with intralesional diatom spines/setae (*Chaetoceros sp.*), subacute, moderate (slide 5B), severe (slides 2B, 3B, 4B), with scattered bacterial rods (slides 3B, 5B) or filament necrosis with bacterial rods, moderate (slides 2B, 4B)

1b. Gill: interfilament diatom spines/setae (*Chaetoceros sp.*), subacute, moderate numbers (slide 8B), abundant (slides 1B, 6B, 7B) and scattered bacterial rods (slides 1B, 6B, 7B)

2a. Liver: hepatic necrosis, acute, multifocal, moderate (slide 3A)

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

2c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 1A)

2d. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slides 5A, 6A), moderate (slide 4A)

3a. Spleen: peritonitis, granulomatous, with fibrocellular fronds, multifocal, mild (slides 1A, 2A, 6A), moderate (slide 7A)

3b. Spleen: granuloma, focal (200 µm in diameter), with a central vacuole (150 µm in diameter), mild (slide 5A)

4. Brain: meningeal and neuropil hemorrhage, focal, mild (slide 1A)

Final Comment: Clinical signs in these fish were a result of accumulation of diatoms in the gills. Overall, changes are very similar to the previous case (2010-1156). Microscopic features of the gills in slides 2G - 5G are fairly characteristic: moderate numbers of diatoms and bacterial rods between pockets of fused lamella and filaments. Microscopic features of the gills in slides 1G and 6B - 8B are unusual: diatoms are abundant but there is no associated hyperplasia; could these fish have been exposed to large numbers of organisms and died before they mounted much of an inflammatory response? 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, 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. [Source: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.] Bacteria that are probably secondary invaders include rods (e.g., a *Vibrio* or related species).

Hepatocellular necrosis can be caused by inadequate vascular perfusion (e.g., as occurs with harmful algal blooms and/or hypoxia, probable in this case) or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Renibacterium salmoninarum*, or *Piscirickettsia salmonis*). 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, in 2009 affecting 12% of the 514 Atlantic salmon but only one of the 72 Pacific salmon examined as part of the Province's Fish Health Auditing and Surveillance Program.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. 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.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen or fluid). 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, but then decreased back to 43% in 2009. The change in vacuole prevalence might be related changes in the proportion of plant-based components in commercial feeds. At least some types of vacuoles might be normal; their effect on growth and feed conversion is unknown.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting spleens of 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe).

The splenic granuloma that has a single large central vacuole is probably a result of vaccine material being entrapped in the splenic parenchyma.

The most common cause of meningeal hemorrhage is trauma. 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.

Histopathology

Formalin-fixed tissues from 8 Atlantic salmon were submitted in 16 cassettes for histopathology. The gills (B cassettes) were immersed 3 h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

Slide #s 1A/1B-8A/8B are labeled in the same order as client #s Fish 1 Binns Apr 13/10 through Fish 8 Binns Apr 13/10

Organs included on most A slides - heart, liver, spleen, head kidney (slide 4 includes trunk kidney; slide 8 has no kidney), intestinal ceca, mesenteric adipose tissue, brain

Organs included on all B slides - gill

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

Quality control: Liver autolysis: none (slides 2A, 3A, 4A, 5A), mild (slide 1A), moderate (slide 6A), severe (slides 7A, 8A). Organs have no postfixation dehydration and no acid hematin deposits.

History of Communication

Case: 10-1625

Date	To	Description
04/22/10 2:50 PM	Mainstream Canada-T - fax	bc report generated
04/22/10 2:50 PM	Dr. Peter McKenzie - e-mail	bc report generated
04/26/10 3:05 PM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-1762

Last Updated: 05/03/10 3:20 PM

Pathologist: Gary D. Marty

Received Date: 04/30/10

Collected Date: 04/21/10

Client Ref No: 7735

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Tiffany MacWilliam - Marine Harvest Can.**

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 Atlantic salmon tissue for Histopathology and PCR for IHN, ISA and VHS.

Saltwater entry: 2009 S1. Vaccinated: Yes. Euthanized: Yes, TMS. Prior submission: No. DOD: April. 21,2010.

Health status check prior to moves. 5 fish for histo N.V.L., gills in separate cassette. 5 PCR samples, all fish had N.V.L. Sample #1 - 5 health fish pooled, #2 - 5 health fish pooled, #3 - 5 health fish pooled, #4 - 5 morts pooled, #5 - 3 morts pooled.

Final Diagnosis

- 1a. Gill: branchitis, granulomatous, lymphocytic, with one multinucleate giant cell, focal (<500 µm in diameter), mild (slide 1A)
- 1b. Gill: lamellar telangiectasis, multifocal, moderate (slide 5A)
- 2a. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slides 1B, 3B, 4B, 5B), moderate (slide 2B)
- 2b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1B, 2B)
- 2c. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slides 3B, 4B, 5B)
- 2d. Liver: hepatitis, perivascular, leukocytic, multifocal, mild (slides 4B, 5B)
3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1B, 4B, 5B)
- 4a. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, moderate (slide 1B)
- 4b. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slide 1B), moderate (slide 2B)
5. Intestine, intestinal ceca and mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 2B, 5B)
- 6a. Heart: epicarditis and endocarditis, lymphohistiocytic, multifocal, mild (slides 3B, 4B, 5B); diffuse, moderate (slide 2B)

Case: 10-1762

6b. Heart: myocardial karyomegaly, multifocal, mild (slide 2B)

7. Skeletal muscle: myonecrosis and myositis, histiocytic, multifocal, mild (slide 4B)

Final Comment: These fish have several lesions that are common among "fresh silvers" sampled as part of the BC Audit and Surveillance Program. However, none of the lesions are of sufficient severity to have killed the fish. These lesions might be evidence that these fish are not as healthy as other 'health status check' fish that I have examined recently. Comments on specific lesions follow:

The focus of granulomatous branchitis (fish 1) is evidence of persistent foreign material. Differentials include vaccine material or an infectious organism (e.g., *Renibacterium salmoninarum*, a common endemic pathogen). The section has no obvious organisms.

Telangiectasis in the gill (lamellar capillary aneurysms or ruptured lamellar capillaries) most commonly results from trauma (e.g., handling).

Pigment in the liver is probably 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.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. 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 or fluid). 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, but then decreased back to 43% in 2009. The change in vacuole prevalence might be related changes in the proportion of plant-based components in commercial feeds. At least some types of vacuoles might be normal; their effect on growth and feed conversion is unknown.

Leukocytic inflammation around vessels in the liver is evidence of chronic immune stimulation. Differentials include a vaccine reaction and a bacterial infection.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe).

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). Mild cases are fairly common in debilitated fish. Renal tubular epithelial necrosis was fairly common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 12% ; n = 511); the cause was not determined in many cases. Differentials include viral hemorrhagic septicemia virus (VHSV, ruled out by PCR in this case) and exposure to toxins (e.g., bacterial toxins, algal toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin).

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination, handling, or other disease). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 36% ; n = 511).

Peritonitis of the intestinal ceca and adjacent mesenteries is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 54% of the 514 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (40% were mild, 12% were moderate, and 2.1% were severe).

Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, with endocarditis affecting 17% of the 512 Atlantic salmon hearts examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 9.3% of the 2181 Atlantic salmon hearts examined as part of the province's Fish Health Auditing and Surveillance Program from 2006 through 2009). 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).

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. Histiocytes in this case are probably cleaning up foci of myonecrosis.

Histopathology

Formalin-fixed tissues from 5 fish were submitted in 10 cassettes for histopathology. Slide #s 1A/1B - 5A/5B are labeled in the same order as client #s 1A/1 - 5A/5.

Organs included on all A slides - gill

Organs included on most B slides - heart, liver, spleen, head kidney, trunk kidney, intestine, intestinal ceca, mesenteric adipose tissue, and (except for fish # 2) skeletal muscle.

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

Quality control: Liver autolysis: none (slide 5B), mild (slides 1B, 2B, 3B, 4B). Large foci of erythrocytes (e.g., spleen in slide 4B) 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). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/03/10 @ 10:45 AM

Specimen	ID	Test	Result
Tissue	7735-1	PCR - IHNV	Negative
Tissue	7735-2	PCR - IHNV	Negative
Tissue	7735-3	PCR - IHNV	Negative
Tissue	7735-4	PCR - IHNV	Negative
Tissue	7735-5	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/03/10 @ 10:45 AM

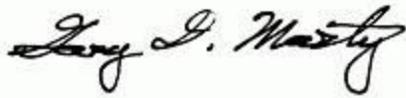
Specimen	ID	Test	Result
Tissue	7735-1	PCR - ISA	Negative
Tissue	7735-2	PCR - ISA	Negative
Tissue	7735-3	PCR - ISA	Negative
Tissue	7735-4	PCR - ISA	Negative
Tissue	7735-5	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/03/10 @ 10:45 AM

Specimen	ID	Test	Result
Tissue	7735-1	PCR - VHSV	Negative
Tissue	7735-2	PCR - VHSV	Negative
Tissue	7735-3	PCR - VHSV	Negative
Tissue	7735-4	PCR - VHSV	Negative
Tissue	7735-5	PCR - VHSV	Negative

History of Communication

Date	To	Description
05/03/10 3:20 PM	Morrison, Diane - fax	bc report generated
05/05/10 8:48 AM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-1763

Last Updated: 05/03/10 10:46 AM

Pathologist: Gary D. Marty

Received Date: 04/30/10

Collected Date: 04/19/10

Client Ref No: 7728

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Tiffany MacWilliam - Marine Harvest Can.**

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 Atlantic salmon tissue for PCR for IHN, ISA and VHS. Saltwater entry: 2009 S1. Vaccinated: Yes. Euthanized:No. Prior submission: No. DOD: April. 19, 2010. 2 PCR samples #1 - 3 fish pooled, #2 - 3 fish pooled. General health check, high mortality due to predation.

Molecular Diagnostics

PCR - IHN Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/03/10 @ 10:45 AM

Specimen	ID	Test	Result
Tissue	7728-1	PCR - IHN	Negative
Tissue	7728-2	PCR - IHN	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/03/10 @ 10:45 AM

Specimen	ID	Test	Result
Tissue	7728-1	PCR - ISA	Negative
Tissue	7728-2	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/03/10 @ 10:45 AM

Specimen	ID	Test	Result
Tissue	7728-1	PCR - VHSV	Negative
Tissue	7728-2	PCR - VHSV	Negative

History of Communication

Date	To	Description
05/03/10 11:17 AM	Morrison, Diane - fax	bc report generated
05/05/10 8:49 AM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-1930

Last Updated: 05/13/10 5:01 PM

Pathologist: Gary D. Marty

Received Date: 05/11/10

Collected Date:

Client Ref No: 10-021

Veterinarian: **Dr. Peter McKenzie**

Clinic:

Phone:

Fax:

Submitter: **Zarah Vansnick - 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 Atlantic salmon tissues for Histopathology and PCR.

Age: 300g. Saltwater. Vaccinated: Yes. Duration of illness 3 months. Insurance: No. Euthanized: No. Prior submission: Yes, 10-1046. DOD: WKY 7/10.

Pool 1 - 4 and 5 - 8 for PCR. 130 - 200 Dead fish each day. Thick gills with bloody mucus. Brain contusions. On feed, Acites, scale loss.

Final Diagnosis

1a. Gill: lamellar capillary thrombosis, focal, acute, mild (slides 4B, 5B), moderate (slides 2B, 6B, 8B), with necrosis, multifocal, severe (slide 3 B)

1b. Gill: lamellar telangiectasis, multifocal, mild (slides 4B, 8B), with thrombosis, mild (slides 6B, 7B)

2a. Brain: meningeal and neuropil hemorrhage, focal, mild (slide 6A), multifocal, moderate (slide 4A), severe (slide 7A)

2b. Brain: meningitis, lymphohistiocytic, focal, mild (slide 5A)

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

3b. Liver: biliary productular cell hyperplasia, diffuse, mild (slides 2A, 4A, 7A, 8A)

3c. Liver: sinusoidal congestion, multifocal, mild (slide 7A), moderate (slide 6A), with acid hematin granules, acute, mild (slide 4A)

3d. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 7A, 8A), moderate (slide 1A)

3e. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slides 7A, 8A), moderate (slides 3A, 4A, 5A)

4a. Heart: epicarditis, multifocal, lymphohistiocytic, mild (slides 1A, 2A, 4A, 6A)

4b. Heart: endocarditis, focal, with endothelial cell hypertrophy, mild (slide 3A)

4c. Heart: endocarditis, focal, lymphohistiocytic, mild (slide 7A)

5a. Spleen: splenitis, granulomatous, focal, mild (slides 1A, 4A)

5b. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles and radiating foreign material, each focus about 50 µm in diameter, moderate (slide 7A)

5c. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 2A, 3A, 5A, 6A), moderate (slide 4A)

Final Comment: These fish have several lesions that are fairly common among BC-farmed Atlantic salmon that die. Lesions of sufficient severity to have killed the fish include moderate to severe branchial thrombosis (4 of 8 fish) and brain hemorrhage (2 of 8 fish). Have these fish been exposed to toxic algal blooms? Lesions in the other 2 fish are not of sufficient severity to have clearly killed the fish. Comments on specific lesions follow:

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; affected gills have no obvious organisms.

Telangiectasis in the gill (lamellar capillary aneurysms or ruptured lamellar capillaries) most commonly results from trauma (e.g., handling).

The most common cause of meningeal hemorrhage is trauma. 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.

Meningitis is evidence of immune stimulation; differentials include viruses, bacteria, or parasites. The section has no obvious organisms.

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 (slide 3A) is evidence against a chronic toxic cause for the hepatic necrosis. Hepatic necrosis is somewhat common in salmon that die in marine net pens, in 2009 affecting 12% of the 514 Atlantic salmon

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 "fresh silvers" that die in marine net pens, affecting 14% of the 514 Atlantic salmon livers examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program. Biliary preductular cell hyperplasia occurred in 8.3% of the 72 farmed Pacific salmon examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

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 or fluid). 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, but then decreased back to 43% in 2009. The change in vacuole prevalence might be related changes in the proportion of plant-based components in commercial feeds. At least some types of vacuoles might be normal; 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. It is common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 23% of the 512 Atlantic salmon hearts examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

Endothelial cell hypertrophy in the heart is evidence of systemic disease. Differentials include a bacterial or viral infection (e.g., VHSV, but unlikely in this case based on PCR results), or exposure to toxins (e.g., algal toxins); the cause is often not determined. Hypertrophic endothelial cells are basophilic and up to 10 µm thick.

Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 17% of the 512 Atlantic salmon hearts examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

The focus of granulomatous inflammation in the spleen is evidence of persistent antigen. The lesion in slide 4A contains a multinucleate giant cell. Vaccine material is the most common cause in farmed Atlantic salmon in BC. The primary differential is a focal infection with *Renibacterium salmoninarum*; the lesion contains no obvious organisms with the routine H&E stain.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe). Vacuoles are probably a result of vaccine material lost during tissue processing.

Histopathology

Formalin-fixed tissues from 8 fish were submitted in 16 cassettes for histopathology. The gills (B cassettes) were immersed 2 h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

Slide #s 1A/1B-16A/16B are labeled in the same order as client #s Fish 1 Mac Lake May 7/10 - Fish 8 Mac Lake May 7/10

Organs included on most 'A' slides - heart, liver, spleen, head kidney, intestinal ceca, mesenteric adipose tissue; all 'B' slides contain gill.

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

Quality control: Liver autolysis: mild (slides 2A, 3A, 4A, 7A, 8A), moderate (slides 1A, 6A), severe (slide 5A). Organs have no postfixation dehydration and no acid hematin deposits (other than in liver lesions on slide 4A).

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 05/13/10 @ 10:34 AM

Specimen	ID	Test	Result
Tissue	#1-4 pool	PCR - IHNV	Negative
Tissue	#5-8 pool	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 05/13/10 @ 10:34 AM

Specimen	ID	Test	Result
Tissue	#1-4 pool	PCR - VHSV	Negative
Tissue	#5-8 pool	PCR - VHSV	Negative

History of Communication

Date	To	Description
05/13/10 5:01 PM	Mainstream Canada-T - fax	bc report generated
05/13/10 5:01 PM	Dr. Peter McKenzie - e-mail	bc report generated
05/14/10 12:51 PM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-2116

Last Updated: 05/29/10 7:56 PM

Pathologist: Gary D. Marty

Received Date: 05/21/10

Collected Date: 05/19/10

Client Ref No: #7743

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Tiffany MacWilliam - 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 tissues for histology and PCR for IHN, VHS and ISA.

One fish presenting with hem in muscle, liver and around kidney.

Species: Atlantic salmon. Saltwater entry: 2008 SO. Vaccinations: unknown. Prior submission: no. Date fish died: Apr. 29/10

Final Diagnosis

1a. Liver: hepatic necrosis, acute, multifocal, with hemorrhage and acid hematin granules, severe

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

1c. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild

2. Intestine: peritonitis, chronic, focal, with fibrocellular fronds and foci of mineral, moderate

3. Kidney: glomerular capillary congestion, generalized, diffuse, moderate

Final Comment: The fish probably died from 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 (PCR results rule out VHSV, IHNV, and ISAV in this case). 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, in 2009 affecting 12% of the 514 Atlantic salmon examined as part of the Province's Fish Health Auditing and Surveillance Program. Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested foci were acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion. Comments on other lesions follow:

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. However, the presence of mineralized foci is not common within vaccine reactions, and vaccine reactions of this magnitude are usual in old fish. Therefore, some type of non-vaccine foreign material might be causing the lesion.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It might be related to increased protein needed as part of an inflammatory response. It is common in juvenile fish with ulcers.

Pigment in the liver is probably 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.

Glomerular capillary congestion is a distinctive lesion that occurs occasionally in Atlantic salmon. The change is not associated with hemorrhage or mesangial changes, but it might be a result of altered glomerular vascular function. It is not described in major fish pathology textbooks (i.e., significance unknown).

Histopathology

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

Slide 1 (7743-1) - liver, spleen, trunk kidney, intestine, intestinal ceca, mesenteric adipose tissue, skin/skeletal muscle

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

Quality control: Liver autolysis: moderate. Large foci of erythrocytes (e.g., liver) 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). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 05/26/10 @ 10:01 AM

Specimen	ID	Test	Result
Tissue	organs, 7743	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Ken Sojonky on 05/26/10 @ 10:01 AM

Specimen	ID	Test	Result
Tissue	organs, 7743	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 05/26/10 @ 10:01 AM

Specimen	ID	Test	Result
Tissue	organs, 7743	PCR - VHSV	Negative

History of Communication

Date	To	Description
05/29/10 7:56 PM	Morrison, Diane - fax	bc report generated
06/02/10 9:21 AM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-2177

Last Updated: 06/01/10 10:28 AM

Pathologist: Gary D. Marty

Received Date: 05/27/10

Collected Date:

Client Ref No: #7758

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Gerry Burry - 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 fish tissue for histology and fresh tissues for PCR for IHN, VHS, and ISA.

Dead fish with signs of petechial hemorrhaging on pyloric caeca. Low mortality on site.

Atlantic Salmon, saltwater entry 2010, S1. Vaccinations: yes. Fish died May 12/10.

Final Diagnosis

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

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

2. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, mild

3. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild

4. Intestine, lamina propria: vascular congestion, multifocal, mild

Final Comment: This fish has several lesions that are common in production fish that die in BC marine net pens. None of the lesions are of sufficient severity to explain the death of this fish, but vascular congestion in the liver and intestine provide evidence of systemic vascular change as a contributing cause. Comments on specific lesions follow:

Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. PCR results rule out VHSV, IHN, and ISAV in this case. 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 to twice the size of hepatocyte nuclei. The inclusions are probably 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 might be related to increased protein needed as part of an

inflammatory response. It is common in juvenile fish with ulcers. Mature females normally have basophilic hepatocytes: needed to produce protein for deposition in their eggs.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe).

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination, handling, or other disease). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 36% ; n = 511)

Distension of capillaries in the intestinal lamina propria is a distinctive change (sometimes called "red gut") that is sometimes attributed to a nutritional (or rather an anti-nutritional) colitis exacerbated by opportunistic colonizing bacteria: mostly vibrios of various types. This type of reaction is recognized in salmon, yellowtail, and *Seriola* (source: Dr. Mark Sheppard, BC-MAL, personal communication).

Histopathology

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

Slide 1 (7758) - liver, spleen, heart, trunk kidney, head 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 05/31/10 @ 12:18 PM

Specimen	ID	Test	Result
Tissue	organ (7758)	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/31/10 @ 12:18 PM

Specimen	ID	Test	Result
Tissue	organ (7758)	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/31/10 @ 12:19 PM

Specimen	ID	Test	Result
Tissue	organ (7758)	PCR - VHSV	Negative

History of Communication

Date	To	Description
06/01/10 10:28 AM	Morrison, Diane - fax	bc report generated
06/02/10 9:53 AM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-2179

Last Updated: 05/31/10 4:40 PM

Pathologist: Gary D. Marty

Received Date: 05/27/10

Collected Date:

Client Ref No: #7772

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Gerry Burry -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 fish tissue for histology and fresh tissue for PCR for IHN, ISA, and VHS.

Fish with petechial hemorrhaging on pyloric caeca, liver, swim bladder, along with leaching of kidney into muscle. Some hemorrhaging in muscle.

Atlantic Salmon; Saltwater entry 2009 S0, Vaccinations: Yes, Euthanized: No.

Final Diagnosis

1a. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, moderate (viral hemorrhagic septicemia virus, VHSV)

1b. Head kidney : nephritis, interstitial, fibrinous, with hematopoietic cell atrophy, moderate (VHSV)

2a. Liver: hepatocellular single cell necrosis (due to VHSV?), disseminated, acute, mild

2b. Liver: pericholangitis, lymphohistiocytic, multifocal, mild

3. Skin: subcutaneous congestion and hemorrhage, multifocal, coalescing, moderate

4. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild

Final Comment: The fish probably died from complications related to VHSV infection. The qPCR Ct value of 25.2 is considered a strong positive, and lesions in several organs are consistent with a VHSV infection. Comments on specific lesions follow:

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). This case is probably related to the presence of viral hemorrhagic septicemia virus (VHSV). Differentials include exposure to toxins (e.g., bacterial toxins, algal toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin). Renal tubular epithelial necrosis was fairly common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 12%; n = 511); the cause was not determined in many cases.

Loss of interstitial hematopoietic cells in the kidney, along with fibrinous inflammation, is an uncommon manifestation of infection with viral hemorrhagic septicemia virus (VHSV).

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, probable in this case).

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.

Congestion and microhemorrhage are nonspecific vascular lesions that can occur in most any organ. In this case (skin), they are most likely related to VHSV. Differentials include algal toxins, and substances released from inflammatory cells or bacteria.

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 23% of the 512 Atlantic salmon hearts examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program .

Histopathology

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

Slide 1 (7772) - liver, spleen, heart, trunk kidney, head kidney, intestine, intestinal ceca, mesenteric adipose tissue, skin/skeletal muscle

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

Quality control: Liver autolysis, mild; intestinal autolysis, severe. Large foci of erythrocytes (e.g., spleen) have a few 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). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/31/10 @ 12:19 PM

Specimen	ID	Test	Result
Tissue	organ (7772)	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/31/10 @ 12:19 PM

Specimen	ID	Test	Result
Tissue	organ (7772)	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/31/10 @ 12:19 PM

Specimen	ID	Test	Result
Tissue	organ (7772)	PCR - VHSV	Positive

History of Communication

Date	To	Description
05/31/10 4:40 PM	Morrison, Diane - fax	bc report generated
06/02/10 9:54 AM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-2277

Last Updated: 06/04/10 2:17 PM

Pathologist: Gary D. Marty

Received Date: 06/03/10

Collected Date:

Client Ref No: 7785

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 formalized fish tissue for histology.

Three gill samples submitted in formalin from site. Site staff noticed gill filaments have a "burned" look to them.

Saltwater entry: 2008 SO, vaccinated, no prior submission.

Final Diagnosis

1. Gill: lamellar epithelial hyperplasia and fusion (synechia), multifocal, moderate (slide 2), severe (slides 1, 3)
2. Gill: filament fusion, focal, chronic, with asymmetric cartilage hyperplasia, moderate (slide 2)

Final Comment: The gross appearance of 'burned' gill is a result of lamellar hyperplasia with fusion (synechia). This combination of lesions might be a result of physical damage from exposure to an irritant (e.g., fine feed particles or toxins in the water, including ammonia or algal toxins); differentials include parasites or diatoms, but the lesions contain no organisms (they might have been lost during processing). Lesions tend to be most prominent at the tips of the filaments, giving affected filaments a clubbed appearance.

In one case (slide 2), three adjacent filaments are fused. Asymmetric cartilage hyperplasia in the fused region supports the conclusion that the filaments had been fused for as long as several weeks.

The extent of the lesion probably did not greatly affect fish health for these normally sedentary farm fish, but the lesions would decrease the ability of the fish to thrive through periods of hypoxia or other stress.

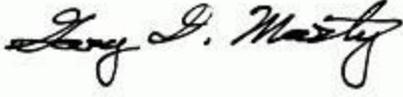
Histopathology

Formalin-fixed gill arches were submitted in 3 cassettes for histopathology. Cassettes were placed in Protocol B (hydrochloric acid solution) for 1.25 hours, rinsed in water, and then processed routinely into Slides 1, 2 and 3 (7785) - gill. All gills were examined; those not listed elsewhere have no significant lesions.

Quality control: Autolysis: none (all slides). Decalcification is complete. Tissues have no postfixation dehydration and no acid hematin deposits.

History of Communication

Date	To	Description
06/04/10 2:18 PM	Morrison, Diane - fax	bc report generated
06/08/10 10:01 AM	Marine Harvest Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-2338

Last Updated: 06/10/10 1:25 PM

Pathologist: Gary D. Marty

Received Date: 06/08/10

Collected Date:

Client Ref No: 10-022

Veterinarian: **Dr. Peter McKenzie**

Clinic:

Phone:

Fax:

Submitter: **Nathan Cassan - Mainstream**

Phone:

Fax:

Owner: **Mainstream Canada - NC**

Phone:

Fax:(250) 286-0042

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

Case History

Submitted formalized tissue for histology.

Constant elevated mortality, fish observed to have both hemorrhage of the brain and heart. Pieces dissected and submitted all exhibited these symptoms.

PO# 15165

580g, Saltwater, Vaccinated, not a legal case, not euthanized, no prior submission, 10 submitted dead, date died: June 2, 2010.

Final Diagnosis

1a. Brain: meningeal and neuropil hemorrhage, focal, mild (slides 4, 7, 8, 9), multifocal, moderate (slides 1, 10), with fibrinoid vasculitis, moderate, focal (slide 10)

1b. Brain: capillary (vascular) congestion, diffuse, mild (slides 7, 9), moderate (slides 6, 10)

2a. Heart: epicarditis, multifocal, lymphohistiocytic, mild (slides 4, 5, 7, 8, 9), moderate (slide 3)

2b. Heart, margin of outer cortical and inner trabecular layers: congestion, multifocal, mild (slide 5)

2c. Heart: endocarditis, multifocal, lymphohistiocytic, mild (slide 8)

Final Comment: Two fish (#s 1 and 10) have cerebral hemorrhage of sufficient severity to explain their death. Four other fish have some cerebral hemorrhage, but microscopic lesions alone cannot readily explain their death (hemorrhage outside of the plane of section might have been more significant, or they might have died from something else). The most common cause of meningeal hemorrhage is trauma. 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. Fibrinoid vasculitis is a rare lesion in Atlantic salmon brains (I don't recall having seen it before without obvious association with infectious organisms); although this type of lesion is not well-described in fish, vasculitis in other animals is often related to auto-immune or infectious diseases. Because the lesion occurred in only one focus and one fish, this change might simply be an artifact. Brain sections have no obvious infectious organisms.

Congestion of brain capillaries, including the meninges, in well-preserved brains can be nonspecific evidence of circulating vasodilators or a
Case: 10-2338

mass -occupying intracranial lesion; hemorrhage sometimes occurs in severe cases. As the postmortem interval increases, the prevalence of passive capillary congestion also increases (i.e., capillary congestion is not reliable for diagnosing cause of death when brain autolysis is moderate or severe; in these cases, congestion is simply evidence that the head was lower than the body after the fish died). Antemortem capillary congestion can be associated with inflammation, 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 of freshly preserved brain, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section contain erythrocytes.]

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 23% of the 512 Atlantic salmon hearts examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program .

Congestion and hemorrhage between the peripheral compact cortical layer and inner trabecular layer of the heart is a distinctive lesion that I started seeing in 2008 in clinical submissions from both Atlantic and Pacific salmon. I noted this change only once among samples examined from 2006 - 2008 as part of the BC Audit and Surveillance Program, but in 2009 I had several cases. The vascular congestion might be a result of a congenital malformation. Alternatively, it might be a result of endothelial damage, with bacterial and viral infections as possible differentials. This lesion occurs in all cases of renal intratubular hemorrhage (but kidney was not included among submitted organs in this case). To rule out other differentials, consider bacteriology and PCR for VHSV.

Lymphohistiocytic inflammation in the heart (endocarditis) is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 17% of the 512 Atlantic salmon hearts examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

If increased mortality continues, consider a broader range of diagnostic tests: bacteriology on kidney, histopathology on multiple organs (e.g., liver, spleen, heart, head kidney, trunk kidney, brain, intestinal ceca, and gill), and PCR for VHSV and IHN (even if negative, it will provide a record that Mainstream routinely tests suspect cases of septicemia).

Histopathology

Formalin-fixed samples of brain and heart from 10 fish were submitted in 10 cassettes for histopathology (tissues on each slide 1 - 10 are from a unique fish). All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Brain autolysis: mild (slides 2, 4, 8), moderate (slides 1, 3, 5, 6, 7, 9, 10). Organs have no postfixation dehydration and no acid hematin deposits.

History of Communication

Date	To	Description
06/10/10 1:32 PM	Mainstream Canada - NC - e-mail	bc report generated
06/10/10 1:32 PM	Dr. Peter McKenzie - e-mail	bc report generated
06/14/10 9:14 AM	Mainstream Canada - e-mail	Case Invoiced
06/21/10 8:59 AM	Mainstream Canada - e-mail	bc report generated



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

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END OF REPORT

Final Report AHC Case: 10-2492

Last Updated: 06/22/10 11:04 AM

Pathologist: Gary D. Marty

Received Date: 06/18/10

Collected Date:

Client Ref No: 7802

Veterinarian:

Clinic:

Phone:

Fax:

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 and formalized tissue for histology and PCR IHN, VHS, and ISA.

One histo of fish with bloody viscera, one sample for PCR.

Saltwater entry 2009, S1, vaccinated, not legal, no prior submission. Fish died on June 9, 2010.

Final Diagnosis

1. Intestinal ceca: enteritis, acute, diffuse, with intraluminal fibrin and bacterial rods, multifocal, mild (slide 1)
2. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 1)
3. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 1)

Final Comment: Intestinal ceca have the most severe lesions in this fish. Intraluminal fibrin, with a few associated bacterial rods, is a pattern of enteritis that occurs sporadically among farmed salmon in BC. It is probably a result of increased vascular leakage of intestinal vessels. Differentials include bacteria and viruses. Enteritis without bacteria is considered characteristic of IPNV infection (pp. 190, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson), but IPNV has never been identified in farmed salmon in BC. PCR results rule out tested viruses as the cause. The gross finding of "bloody viscera" is usually a result of vascular congestion, but the sections have no evidence of visceral capillary congestion.

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 might be related to increased protein needed as part of an inflammatory response. It is common in juvenile fish with ulcers.

Histopathology

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

Slide 1 (Pen 6 D1 9/6/10) - heart, liver, spleen, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue, skeletal muscle

Slide 2 (Pen 6 D1 9/6/10) - gill

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 06/22/10 @ 8:53 AM

Specimen	ID	Test	Result
Tissue	orgs, 7802	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Ken Sojonky on 06/22/10 @ 8:53 AM

Specimen	ID	Test	Result
Tissue	orgs, 7802	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 06/22/10 @ 8:53 AM

Specimen	ID	Test	Result
Tissue	orgs, 7802	PCR - VHSV	Negative

History of Communication

Date	To	Description
06/22/10 11:04 AM	Marine Harvest Canada - e-mail	bc report generated
06/22/10 11:06 AM	Marine Harvest Canada - fax	bc report generated
06/23/10 3:24 PM	Marine Harvest Canada - fax	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-2543

Last Updated: 06/25/10 9:55 AM
Pathologist: Gary D. Marty
Received Date: 06/22/10
Collected Date:
Client Ref No: 10-023 PO#15212

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 fish tissue for histology.

Completed mouth rot treatment end of May. Mortality decreased then increased. Mucus in gills, fluid in swim bladders, operculum deformities, mucus in digestive tract and some hemorrhaging in digestive tract.

Atlantic Salmon 400g, saltwater, vaccinated, sick for 7 days, not euthanized, no prior case, fish died on June 15, 2010.

PO# 15212

Final Diagnosis

- 1a. Gill: lamellar capillary thrombosis, bifocal, acute, mild (slide 4G)
- 1b. Gill: lamellar telangiectasis, focal or multifocal, mild (slide 4G), with thrombosis, mild (slides 1G, 2G, 3G, 7G, 8G)
- 2a. Liver: hepatocellular hydropic degeneration and single cell necrosis, disseminated, acute, mild (slide 4)
- 2b. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 4)
- 2c. Liver: sinusoidal congestion, multifocal, mild (slide 6)
- 2d. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 2, 6)
- 2e. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 6, 7)
4. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slides 2, 5, 6, 7)
- 5a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 2, 4, 7), moderate (slide 5)
- 5b. Spleen: fibrous capsule granuloma, focal (200 µm in diameter), with central necrotic debris and 2 vacuoles, mild (slide 4)

6. Intestine: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 2, 3)

Final Comment: Other than the mouthrot, which was described clinically, these fish have no evidence of underlying life-threatening disease. Most of their lesions could either be (i) secondary to mouthrot or (ii) common background lesions in farmed Atlantic salmon that die. This pattern of no secondary disease is common in fish with mouthrot: the mouth has severe lesions but other organs have mostly routine lesions. Comments on specific lesions follow:

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; affected gills have no obvious organisms.

Telangiectasis in the gill (lamellar capillary aneurysms or ruptured lamellar capillaries) most commonly results from trauma (e.g., handling). Thrombosis sometimes occurs, probably as a result of altered blood flow in the aneurysm.

Hydropic degeneration among small numbers of hepatocytes provides evidence that the liver was being exposed to toxins (e.g., algal toxins or other natural toxins). Potential sources of the inciting toxins include the water or feed, a bacterial infection, or circulating oxygen radicals following a period of hypoxia. 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. Reference: Wolf, J.C., and M.J. Wolfe. 2005. A brief overview of nonneoplastic hepatic toxicity in fish. *Toxicologic Pathology*. 33(1):75-85.

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 "fresh silvers" that die in marine net pens, affecting 14% of the 514 Atlantic salmon livers examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

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 might be related to increased protein needed as part of an inflammatory response. It is common in juvenile fish with ulcers.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination, handling, or other disease). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 36% ; n = 511).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting the spleen of 65% of the 509 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (41% were mild, 19% were moderate, and 4.9% were severe).

Fibrous capsule granulomas form in response to indigestible foreign material. They are common in wild fish, usually in response to dead parasites, but they are unusual in farmed fish. The granuloma in the spleen of fish 4 might be in response to vaccine material. It was probably of little significance for fish health.

Histopathology

Formalin-fixed tissues from 8 fish were submitted in 16 cassettes for histopathology. The gills (G cassettes) were immersed 2 h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

Slide #s 1/1G-8/8G are labeled in the same order as client #s Fish 1 Rant June 15/10 - Fish 8

Organs included on most 'A' slides - heart, liver, spleen, brain, trunk kidney, intestinal ceca, and mesenteric adipose tissue; all 'B' slides contain gill.

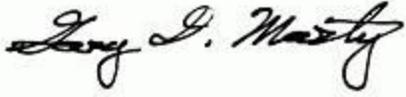
All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Liver autolysis: mild (slide 6), moderate (slides 3, 4, 8), severe (slides 1, 2, 5, 7). Organs have no postfixation dehydration and Case: 10-2543

no acid hematin deposits.

History of Communication

Date	To	Description
06/25/10 10:01 AM	Mainstream Canada-T - fax	bc report generated
06/25/10 10:01 AM	Dr. Peter McKenzie - e-mail	bc report generated
06/29/10 9:44 AM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-2582

Last Updated: 06/28/10 3:36 PM

Pathologist: Gary D. Marty

Received Date: 06/24/10

Collected Date:

Client Ref No: 7819

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

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 5 formalized fish samples for histology.

Suspect water quality issue. Gills appear damaged but no internal lesions.

Saltwater entry 2008, so, vaccinated, sick from June 10, 2010, fish died on June 17, 2010

Final Diagnosis

1. Gill: lamellar aneurysms distended by fibrinous thrombi, multifocal, acute and subacute, mild (slides 4, 7), moderate (slides 1, 2, 9)
2. Gill, tips of filaments: branchitis, granulomatous, with abundant eosinophilic granular cells (EGCs. Slide 9)

Final Comment: Most of these gills have discrete foci of lamellar capillary aneurysms that extend along nearly the entire length of the lamella (~400 µm) and up to 50 µm thick. Aneurysms are most common among lamella on the distal 1/3 of the filaments. Aneurysms are filled with fibrin, some of which is recanalized (indicating that the thrombi are more than a few days old), but they contain few or no erythrocytes. In general, 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; the sections have no obvious organisms.

One gill (slide 9) has distinct thickening of the distal end of the filaments. The granulomatous inflammation and abundant EGCs are distinctive, involving the filaments but not the lamellae, but sections contain no organisms. Consider toxin exposure as a differential.

Histopathology

Formalin-fixed gills from 5 fish were submitted in 5 cassettes for histopathology. All cassettes were immersed 2.75 h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

Slide #s - 1 (7819-1), 4 (7819-4), 7 (7819-7), 8 (7819-8) and 9 (7818-9?)

Case: 10-2582

Quality control: Gill autolysis: mild (slide 7), moderate (slides 1, 4, 8). Decalcification is complete and differential staining is good. Organs have no postfixation dehydration and no acid hematin deposits.

History of Communication

Date	To	Description
06/28/10 3:37 PM	Morrison, Diane - fax	bc report generated
06/30/10 4:25 PM	Marine Harvest Canada - fax	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-2635

Last Updated: 07/02/10 2:29 PM

Pathologist: Gary D. Marty

Received Date: 06/29/10

Collected Date:

Client Ref No: 7831

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 histo samples.

Gills have thick white appearance. Microscopically we see no plankton or other organisms. Condition has become more severe since submission of case 7785. No increase in mortality.

2008 SO, euthanized TMS bled out, yes prior case 7785 (2010-2277), not legal.

Final Diagnosis

1. Gill, tips of filaments: lamellar epithelial hyperplasia and squamous metaplasia, multifocal, severe (slides 1A, 2B, 2C, 3B), with a focus of granulomatous inflammation and multinucleate giant cells (slide 1C), or with intralesional pockets of necrosis (slides 2B, 3B, 3C)

2a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 1A)

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

2c. Liver: pericholangitis, lymphohistiocytic, multifocal, mild (slide 1A)

2d. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slides 1A, 3A), moderate (slide 2A)

3. Heart: endocarditis, multifocal, lymphohistiocytic, mild (slides 2A, 3A)

4a. Mesenteries: adipose tissue atrophy, diffuse, mild (slide 3A), moderate (slide 2A)

4b. Intestinal ceca and mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1A, 2A, 3A)

Final Comment: The thickened appearance of tips of the gill filaments is a result of severe lamellar hyperplasia that fills the interlamellar space. These findings are consistent with the gross photos of the lesion e-mailed to me on 25 June 2010. This combination of lesions might be a result of physical damage from exposure to an irritant (e.g., fine feed particles or toxins in the water, including ammonia or algal toxins); differentials include parasites or diatoms, but the lesions contain no organisms. Gill clubbing is also a classic lesion associated with dietary

pantothenic acid deficiency. The base of affected filaments is normal, so fish probably are able to survive as long as dissolved oxygen levels remain high and they are not unduly stressed. Other lesions are of less concern:

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 "fresh silvers" that die in marine net pens, affecting 14% of the 514 Atlantic salmon livers examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. 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 is probably 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. Hemosiderin accumulation in the liver might result from increased turnover of red blood cells.

Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 17% of the 512 Atlantic salmon hearts examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

The mesenteries in slide 2A have only small amounts of adipose tissue. This is a very unusual finding that never occurs with "fresh silvers" sampled as part of the BC Fish Health Auditing and Surveillance Program. Were these fish poor-doers? Are the gill lesions limited to poor doers at the farm, or do all fish seem to be affected?

Peritonitis of the intestinal ceca and adjacent mesenteries is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 54% of the 514 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (40% were mild, 12% were moderate, and 2.1% were severe).

Histopathology

Formalin-fixed tissues (A slides : liver, head kidney, trunk kidney, heart, spleen, intestine, intestinal ceca, and mesenteric adipose tissue) and gill arches (B and C slides) were submitted in 3 cassettes from each of 3 fish for histopathology. Cassettes with gill were immersed in Protocol B (hydrochloric acid solution) for 2 hours, rinsed in water, and then processed routinely into paraffin. All organs were examined; those not listed elsewhere have no significant lesions.

Quality control: Gill autolysis: none (all gill slides). 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. Decalcification is complete, and differential staining is good. Tissues have no postfixation dehydration.

History of Communication

Date	To	Description
07/02/10 2:30 PM	Morrison, Diane - fax	bc report generated
07/12/10 3:49 PM	Marine Harvest Canada - fax	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-2667

Last Updated: 07/28/10 3:04 PM

Pathologist: Gary D. Marty

Received Date: 06/30/10

Collected Date: 06/21/10

Client Ref No: 10-024/ PO 15088

Veterinarian: **Dr. Peter McKenzie**

Clinic: **Mainstream Canada**

Phone: (250) 286-0022

Fax: (250) 286-0042

Submitter: **Kelly Abel - Mainstream Can.**

Phone:

Fax:

Owner: **Mainstream Canada**

Phone:

Fax:(250) 286-0042

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age:

Premise ID:

Case History

Submitted Atlantic salmon tissues for histopathology; 2 plates for bacteriology, tissues for viral culture and PCR for IHN, VHS and BKD.

Constant elevated mortality with hemorrhaging and internal swelling kidney and spleen. Pen 12, 2007 year class average wt 19.340g females and Pen 13 year class 2008, 11.510g average wt,females. Each fish plated on TSA and TSA with 5% S.B. for bacterial identification.

Environment: Saltwater. Euthanized: no, died June 20/10. Vaccinated: Yes Insurance/Legal: no. Prior submission: Yes.

PO # 15088.

Final Diagnosis

1. Heart: intraluminal colonies of bacterial rods consistent with *Aeromonas salmonicida*, with lytic necrosis, multifocal, mild (slide 4A), abundant, with mild histiocytic endocarditis (slide 1A)
2. Gill: colonies of bacterial rods (*Aeromonas salmonicida*?), small numbers (slide 1A)
3. Spleen and liver (slide 4A) and gill (slide 4B): colonies of bacterial rods (*Aeromonas salmonicida*?), small numbers
 - 3a. Liver: hepatic necrosis, acute, multifocal, severe (slide 2A)
 - 3b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1A, 3A)
 - 3c. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slides 1A, 2A, 4A), moderate (slide 3A)
 - 3d. Liver: cholangitis, proliferative, with biliary ductular hyperplasia, multifocal, moderate (slide 3A)
4. Intestinal ceca, lamina propria: vascular congestion, multifocal, mild (slide 3A)
- 5a. Trunk kidney, urinary space and renal tubular lumen: protein casts, multifocal, mild (slide 4A)

5b. Trunk kidney: interstitial fibrin, disseminated, mild (slide 4A)

Final Comment: These fish have several lesions that might have contributed to mortality. Histopathology fish #s 1 and 4 probably died from furunculosis, fish #2 probably died from complications associated with hepatic necrosis, and the death of fish #3 might be related to biliary lesions. Fish have no evidence of *Renibacterium salmoninarum* infection. Comments on specific lesions follow:

Organs in two fish (#s 1 and 4) have microscopic features characteristic of systemic *Aeromonas salmonicida*, the cause of furunculosis in salmonids. In fish #1, large colonies of bacterial rods in the lumen of the ventricle (and branchial vessels) are associated with lytic necrosis of adjacent structures and small numbers of macrophages. Bacterial culture or PCR are needed for a definitive diagnosis. *Aeromonas salmonicida* infection is most common in freshwater-reared salmonids, but also causes problems in saltwater-reared salmonids (especially Atlantic salmon) and other marine fish species. In my experience it is rare in Atlantic salmon > 1 kg. Infected fish are considered the primary reservoir of the bacterium, but bacteria can survive in fresh and salt water. [Source: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.] The gill in slide 3B has several small colonies of bacterial rods, but severe postmortem autolysis in this specimen means that these bacteria might have all grown after death (if so, they are not significant).

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 (slide 2A) 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, in 2009 affecting 12% of the 514 Atlantic salmon sampled as part of the BC Fish Health Auditing and Surveillance Program.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. It might be related to increased protein needed as part of an inflammatory response. It is common in juvenile fish with ulcers. Mature females normally have basophilic hepatocytes: needed to produce protein for deposition in their eggs.

Pigment in the liver is probably 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.

Hyperplasia of the bile ductules is an unusual lesion in Atlantic salmon. It probably resulted from exposure of the biliary system to toxins. The toxins could be endogenous or exogenous. The affected liver has no obvious infectious organisms.

Distension of capillaries in the intestinal lamina propria is a distinctive change (sometimes called "red gut") that is sometimes attributed to a nutritional (or rather an anti-nutritional) colitis exacerbated by opportunistic colonizing bacteria: mostly vibrios of various types. This type of reaction is recognized in salmon, yellowtail, and *Seriola* (source: Dr. Mark Sheppard, BC-MAL, personal communication). The affected intestinal ceca in this case have no obvious infectious organisms.

Renal tubular protein casts result from glomerular or tubular dysfunction; excess protein leaks through glomeruli, or tubules are unable to reabsorb protein. Renal tubular protein casts are uncommon in Atlantic salmon, in 2009 affecting only 3 of the 514 Atlantic salmon sampled as part of the BC Fish Health Auditing and Surveillance Program.

Renal interstitial fibrin deposits are eosinophilic and acellular. The deposits are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin; toxins in the water or feed are less likely. Renal interstitial fibrin is an uncommon lesion in Atlantic salmon, in 2009 affecting only 3 of the 514 Atlantic salmon sampled as part of the BC Fish Health Auditing and Surveillance Program.

Histopathology

Formalin-fixed tissues from 4 fish were submitted in 4 cassettes for histopathology. Gills were removed from the original (A) cassettes and placed in separate (B) cassettes.

Slide #s - 1A/1B (Cypress Pen 12 Fish 1 06/21/10), 2A/2B (Cypress Pen 12 Fish 2 06/21/10), 3A/3B (Cypress Pen 13 Fish 3 06/21/10), 4A/4B (Cypress Pen 13 Fish 4 06/21/10 - gill, heart, liver, spleen, head kidney, trunk kidney, intestine/intestinal ceca, mesenteric adipose tissue, brain)

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

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

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 07/07/10 @ 11:49 AM

Specimen	ID	Isolate	Result	Level
Isolate	Fish 1 Brain	Psychrobacter sp.	Positive	
Isolate	Fish 1 Kidney	Aeromonas salmonicida	Positive	
Isolate	Fish 2 Brain		No Bacteria Isolated	
Isolate	Fish 2 Kidney		No Bacteria Isolated	
Isolate	Fish 3 Brain	Aeromonas salmonicida	Positive	
Isolate	Fish 3 Kidney	Aeromonas salmonicida	Positive	
Isolate	Fish 4 Brain	Aeromonas salmonicida	Positive	
Isolate	Fish 4 Kidney	Aeromonas salmonicida	Positive	

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 07/05/10 @ 11:43 AM

Organism	ID	e	ffc	s3	sxt	ot
Aeromonas salmonicida	Fish 1 Kidney	s	s	s	s	s
Psychrobacter sp.	Fish 1 Brain	r	s	r	r	s

** Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethoprim, ot = Oxytetracycline

Molecular Diagnostics

PCR-Renibacterium salmoni Resulted by: Ken Sojonyk Verified by: Dr. J. Robinson on 07/05/10 @ 4:38 PM

Specimen	ID	Test	Result
Tissue	Pen 12-1	PCR-Renibacterium salmoninaru	Negative
Tissue	Pen 12-2	PCR-Renibacterium salmoninaru	Negative
Tissue	Pen 13-3	PCR-Renibacterium salmoninaru	Negative
Tissue	Pen 13-4	PCR-Renibacterium salmoninaru	Negative

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/05/10 @ 2:33 PM

Specimen	ID	Test	Result
Tissue	Pen 12-1	PCR - IHNV	Negative
Tissue	Pen 12-2	PCR - IHNV	Negative
Tissue	Pen 13-3	PCR - IHNV	Negative
Tissue	Pen 13-4	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/05/10 @ 2:33 PM

Specimen	ID	Test	Result
Tissue	Pen 12-1	PCR - VHSV	Negative
Tissue	Pen 12-2	PCR - VHSV	Negative
Tissue	Pen 13-3	PCR - VHSV	Negative
Tissue	Pen 13-4	PCR - VHSV	Negative

Tissue Culture Resulted by: Hughes,Giselle Verified by: Cheryl Cecconi on 07/28/10 @ 2:09 PM

Specimen	ID	Isolate	Result	Level
Tissue	Pen 12-1		No viruses isolated	
Tissue	Pen 12-2		No viruses isolated	
Tissue	Pen 13-3		No viruses isolated	
Tissue	Pen 13-4		No viruses isolated	

History of Communication

Date	To	Description
07/05/10 4:21 PM	Mainstream Canada - e-mail	bc report generated
07/05/10 4:22 PM	Dr. Peter McKenzie - e-mail	bc report generated
07/07/10 4:18 PM	Mainstream Canada - e-mail	bc report generated
07/07/10 4:18 PM	Dr. Peter McKenzie - e-mail	bc report generated
07/28/10 3:01 PM	Mainstream Canada - e-mail	bc report generated
07/28/10 3:02 PM	Dr. Peter McKenzie - e-mail	bc report generated
07/28/10 3:04 PM	Mainstream Canada - e-mail	bc report generated
07/28/10 3:04 PM	Dr. Peter McKenzie - e-mail	bc report generated
08/06/10 9:26 AM	Mainstream Canada - e-mail	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-2699

Last Updated: 07/08/10 2:11 PM

Pathologist: Gary D. Marty

Received Date: 07/06/10

Collected Date:

Client Ref No: 7838

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 IHN, VHS, and ISA.

6 fresh mortis for viral screen prior to fish moves; 2 pools of 3 fish.

Saltwater entry 2009 SO, not legal, not euthanized, no prior submission. Vaccinated.

Molecular Diagnostics

PCR - IHN Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/08/10 @ 2:11 PM

Specimen	ID	Test	Result
Tissue	7838 #1-3	PCR - IHN	Negative
Tissue	7838 #4-6	PCR - IHN	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/08/10 @ 2:11 PM

Specimen	ID	Test	Result
Tissue	7838 #1-3	PCR - ISA	Negative
Tissue	7838 #4-6	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/08/10 @ 2:11 PM

Specimen	ID	Test	Result
Tissue	7838 #1-3	PCR - VHSV	Positive
Tissue	7838 #4-6	PCR - VHSV	Negative

History of Communication

Date

To

Description

07/08/10 3:00 PM

Morrison, Diane - fax

bc report generated

Case: 10-2699



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END OF REPORT

Final Report AHC Case: 10-2700

Last Updated: 07/08/10 3:28 PM

Pathologist: Gary D. Marty

Received Date: 07/06/10

Collected Date:

Client Ref No: 7841

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Gerry Burry**

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 PCR for IHN, ISA, and VHS, and histology.

Sample taken from fish with blood in body cavity, stomach, swimbladder, and lower intestine.

Saltwater entry 2009 S1, vaccinated, not legal, not euthanized, no prior case. 1 in group, 1 dead, 1 submitted.

Final Diagnosis

1. Intestinal ceca: enteritis, acute, diffuse, with intraluminal fibrin, moderate

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

2b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate

2c. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild

Final Comment: This fish probably died from complications related to fibrinous enteritis. Intraluminal fibrin, with few associated inflammatory cells, is a pattern of enteritis that occurs sporadically among farmed salmon in BC. In 2009, it affected 5.6% of 514 Atlantic salmon sampled as part of the BC Fish Health Audit and Surveillance Program. It is probably a result of increased vascular leakage of intestinal vessels. Differentials include bacteria and viruses. The lesion is considered characteristic of IPNV infection (pp. 190, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson), but IPNV has never been identified in farmed salmon in BC.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. 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 or fluid). 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, but then decreased back to 43% in 2009. The change in vacuole prevalence might be related changes in the proportion of plant-based components in commercial feeds. At least some types of vacuoles might be normal; their effect on growth and feed conversion is unknown.

Pigment in the liver is probably 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. Hemosiderin accumulation in the liver might result from increased turnover of red blood cells .

Histopathology

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

Slide 1 (7841) - liver, spleen, 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: 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 07/08/10 @ 2:11 PM

Specimen	ID	Test	Result
Tissue	7841	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/08/10 @ 2:11 PM

Specimen	ID	Test	Result
Tissue	7841	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/08/10 @ 2:11 PM

Specimen	ID	Test	Result
Tissue	7841	PCR - VHSV	Negative

History of Communication

Date	To	Description
07/08/10 3:29 PM	Morrison, Diane - fax	bc report generated
07/13/10 9:36 AM	Marine Harvest Canada - fax	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-2701

Last Updated: 07/08/10 5:43 PM

Pathologist: Gary D. Marty

Received Date: 07/06/10

Collected Date:

Client Ref No: 7842

Veterinarian: **Diane Morrison**

Clinic: **Marine Harvest Canada**

Phone: (250) 850-3276

Fax: (250) 850-3275

Submitter: **Gerry Burry**

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 PCR for IHN, ISA, and VHS, and histology.

Pre movement health checks. 9 histo, 3 from each of 3 separate pens. 3 PCR samples - pools of 3 from each of 3 separate pens.

Vaccinated. 9 in group, not euthanized, submitted 9 dead.

Final Diagnosis

1a. Liver: peritonitis, fibrinous, regionally diffuse, moderate (slide 7)

1b. Liver: hepatic necrosis, acute, multifocal, mild (slide 8)

1c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1, 2, 4, 5, 7, 8), moderate (slide 3)

1d. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slides 1, 7, 8), moderate (slides 2, 5, 6, 9)

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

1f. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slides 1, 3)

1g. Liver: pericholangitis, lymphohistiocytic, focal, mild (slide 5)

1h. Liver: sinusoidal congestion, multifocal, moderate (slide 6), with intracytoplasmic spherical golden to amphophilic inclusions, multifocal, moderate (slide 9)

2. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slides 1, 3, 5, 6, 7, 9), moderate (slide 8)

3a. Intestinal ceca and mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1, 4, 9), granulomatous, multifocal, moderate (slide 4), severe (slides 5, 8)

3b. Intestine, lamina propria: vascular congestion, multifocal, moderate (slide 9)

4. Spleen: splenitis, granulomatous, multifocal, with multinucleate giant cells, moderate (slide 5)

5a. Heart: epicarditis, multifocal, histiocytic, mild (slide 7)

5b. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild (slides 8, 9)

5c. Heart: mural thrombosis, multifocal, mild (slide 9)

Final Comment: These fish have several lesions that are fairly common among Atlantic salmon that die in BC marine net pens. None of the lesions are of sufficient severity to explain the death of these fish, but hepatic peritonitis in fish #7 is evidence of systemic inflammation that might have killed this fish. PCR results rule out VHSV, IHNV, and ISAV contributing to lesion development. Comments on specific lesions follow:

Most of the hepatic capsule in fish #7 is covered by a band of fibrin that varies from 50 - 150 µm thick. This might be a result of a bacterial infection or an aberrant reaction to a vaccine; the section contains no obvious organisms.

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, in 2009 affecting 12% of the 514 Atlantic salmon.

Basophilic cytoplasm in hepatocytes is an indication of active protein synthesis. 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 or fluid). 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, but then decreased back to 43% in 2009. The change in vacuole prevalence might be related changes in the proportion of plant-based components in commercial feeds. At least some types of vacuoles might be normal; their effect on growth and feed conversion is unknown.

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

Pigment in the liver is probably 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. Hemosiderin accumulation in the liver might result from increased turnover of red blood cells.

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.

Multifocal sinusoidal congestion in the liver is either a nonspecific vascular lesion or a postmortem artifact. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). 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 (slide 9) are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination, handling, or other disease). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 36% ; n = 511).

Peritonitis of the intestinal ceca and adjacent mesenteries is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 54% of the 514 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2009 as part of the British Columbia Fish Health Auditing and Surveillance Program (40% were mild, 12% were moderate, and 2.1% were severe). In these fish the spleen is sometimes involved (but similar inflammation in the spleen is not diagnosed separately here).

Distension of capillaries in the intestinal lamina propria is a distinctive change (sometimes called "red gut") that is sometimes attributed to a nutritional (or rather an anti-nutritional) colitis exacerbated by opportunistic colonizing bacteria: mostly vibrios of various types. This type of reaction is recognized in salmon, yellowtail, and *Seriola* (source: Dr. Mark Sheppard, BC-MAL, personal communication). Slide 9 has no obvious associated organisms.

Foci of granulomatous inflammation in the spleen are evidence of persistent antigen. Vaccine material is the most common cause in farmed Atlantic salmon in BC. The primary differential is a focal infection with *Renibacterium salmoninarum*, but the lesion contains no obvious organisms with the routine H&E stain.

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. In fish #7, histiocytic epicarditis might have the same underlying cause as hepatic peritonitis. Lymphohistiocytic epicarditis is common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 23% of the 512 Atlantic salmon hearts examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program. The sections contain no obvious organisms.

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

Histopathology

Formalin-fixed tissues from 9 fish were submitted in 9 cassettes for histopathology. Slide #s 1-9 are labeled in the same order as client #s 1 - 9.

Organs included on most slides - heart, liver, spleen, 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: mild (slide 3), moderate (slides 5, 7, 9), severe (slides 1, 2, 4, 6, 8). Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/08/10 @ 2:11 PM

Specimen	ID	Test	Result
Tissue	7842-1	PCR - IHNV	Negative
Tissue	7842-2	PCR - IHNV	Negative
Tissue	7842-3	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/08/10 @ 2:11 PM

Specimen	ID	Test	Result
Tissue	7842-1	PCR - ISA	Negative
Tissue	7842-2	PCR - ISA	Negative
Tissue	7842-3	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/08/10 @ 2:12 PM

Specimen	ID	Test	Result
Tissue	7842-1	PCR - VHSV	Negative
Tissue	7842-2	PCR - VHSV	Negative
Tissue	7842-3	PCR - VHSV	Negative

History of Communication

Date	To	Description
07/08/10 5:44 PM	Morrison, Diane - fax	bc report generated
07/13/10 9:36 AM	Marine Harvest Canada - fax	Case Invoiced



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

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END OF REPORT

Final Report AHC Case: 10-2762

Last Updated: 07/12/10 2:10 PM

Pathologist: Gary D. Marty

Received Date: 07/09/10

Collected Date:

Client Ref No: 10-82

Veterinarian: **Barry Milligan**

Clinic:

Phone:

Fax:

Submitter: **Michael Ness**

Phone:

Fax:

Owner: **Grieg Seafoods BC Ltd.**

Phone: (250) 286-0838

Fax:(250) 286-1883

Animal Data

Species: Atlantic Salmon

Breed:

Sex:

Age: 14 Months

Premise ID:

Case History

Submitted formalized fish tissue for histology.

Red rash on ventral side of fish and on ventral fins.

Barnes Bay, Saltwater, vaccinated, sick for one month, not euthanized, 2 dead on July 7, 2010.

Final Diagnosis

1a. Liver: hepatic necrosis, acute, multifocal, coalescing, severe (slide 2A)

1b. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slide 2A)

2. Trunk kidney: renal tubular epithelial necrosis, focal, acute, mild (slide 2A)

3. Mesenteric adipose tissue: saponification of fat (fat necrosis), with scattered macrophages, bifocal, mild (slide 2A)

Final Comment: Fish #2 died of complications related to hepatic necrosis, whereas fish #1 has no lesions of sufficient severity to explain its death. 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. Consider testing cohorts of these fish for VHSV and bacteria (if not already done). 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, in 2009 affecting 12% of the 514 Atlantic salmon.

Pigment in the liver is probably 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.

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). Mild cases are fairly common in debilitated fish. Renal tubular epithelial necrosis was fairly common among fish sampled in 2009 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 12% ; n = 511); 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). The cause in this case is probably the same as for the hepatic necrosis.

The lipid in the foci of necrotic fat cells has been replaced by wispy, pale, 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.

Histopathology

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

For cassette 2, gills were removed from the original (A) cassette and placed in a separate (B) cassette.

Slide 1 (Fish 1 BB 8.07.10) - gill, liver, spleen, trunk kidney, mesenteric adipose tissue, brain

Slide 2A (Fish 2 BB 8.07.10) - liver, spleen, trunk kidney, intestinal ceca, mesenteric adipose tissue

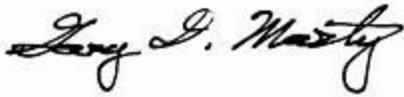
Slide 2B (Fish 2 BB 8.07.10) - gill

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

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

History of Communication

Date	To	Description
07/12/10 2:10 PM	Milligan, Barry - e-mail	bc report generated
07/13/10 1:55 PM	Grieg Seafoods BC Ltd. - e-mail	Case Invoiced



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

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