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Ministry of Agriculture and Lands

Animal Health Centre

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Ministry of Agriculture and Lands
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Final Report AHC Case: 07-1742

Last Updated: 05/07/07 4:13 PM
Pathologist: Gary D. Marty
Received Date: 05/03/07
Collected Date: 05/03/07
Client Ref No: SS-133

Veterinarian: **Dr. Sonja Saksida**
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Submitter:
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Fax:
Owner:
Phone:
Fax:

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

Case History

Sumbitted formalized tissue for histology. Some in the population starting to exhibit exophthalmia (unilateral). No gross evidence of BKD or other infectious agent. Possible rule outs - eye picking, supersaturation. In house lab findings.

Final Diagnosis

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

1b. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 3)

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

3. Eye: peri-choroidal erythrocytes, abundant (slides 1, 3)

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

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

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

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

The connective tissue around the choroid plexus contains abundant free erythrocytes. If the eye was sampled from an anesthetized fish, then this probably is a result of sampling; however, if the fish was dead when sampled, then this represent hemorrhage and is consistent with the clinical signs of exophthalmos.

Renal tubular epithelial protein droplets are normal in some species, or they might be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2006 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 22%; n = 495) and

Pacific salmon (prevalence = 38%; n = 134).

Histopathology

A single overfilled cassette of formalin-fixed tissues was submitted for histopathology. Soft tissues were separated into two cassettes for processing onto two slides (1 and 3); the two pieces of gill were placed in another cassette, decalcified in Protocol B for one hour, and then processed onto slide 2.

Slide 1 - heart (2 pieces), trunk kidney (2 pieces), liver, spleen, choroid plexus (eye), stomach, intestinal ceca, and mesenteric fat

Slide 2 - gill (2 pieces)

Slide 3 - trunk kidney 2 pieces), liver, spleen, choroid plexus (eye), stomach, intestinal ceca, and mesenteric fat

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

Quality control: Tissue preservation is good for most organs; some segments of intestinal ceca have severe autolysis (these parts probably were adjacent to the gallbladder). Gill decalcification is complete. Tissues have no postfixation dehydration and no deposits of acid hematin.

Measures of physiologic condition

Hepatocellular glycogen, none (slide 3), moderate amounts (slide 1)

Lipid in mesenteric adipose tissue, abundant (slides 1, 3)

This pattern in the measures of physiologic condition is consistent with healthy growing fish (abundant mesenteric fat) that were feeding fairly normally until perhaps the day they were sampled (slide 1) or until a few days before they were sampled (slide 3).

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

END OF REPORT

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Final Report AHC Case: 07-3315

Last Updated: 08/31/07 3:56 PM
Pathologist: Stephen Raverty, DVM
Received Date: 08/24/07
Collected Date: 08/24/07
Client Ref No:

Veterinarian: **Dr. Sonja Saksida**
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Submitter:
Phone:
Fax:
Owner: **SS-206**
Phone:
Fax:

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

Case History

Chinook salmon. Age: smolt. Avg weight: 180g. Site type: net pens. Priority: normal. Mortality increased no gross signs of infectious agent. Site had some heterosigma and low DO issues in the last couple of weeks. Request Histology. Please fax or email report o Dr. Sonja Saksida.

Final Diagnosis

COMMENTS:

Post mortem change significant hampered microscopic assessment of multiple sections of gills. There was generalized artefactual exfoliation of respiratory epithelia and randomly interspersed within the primary lamellae, there are individual to small clusters of phytoplankton-like organisms with no apparent hemorrhage or reactive change of secondary lamellae (telangiectasia, respiratory epithelial hyperplasia). The precise contribution of these structures to antemortem morbidity is unknown. There were no other apparent lesions within the examined tissues.

Histopathology

HISTOPATHOLOGY:

1). Gills: Phytoplankton, mild, multifocal, random

There are no apparent lesions within multiple sections of gills, swim bladder, kidney, brain, heart, skeletal muscle, peripheral nerves, peripheral vasculature, stomach, adipose tissue, liver, spleen or small intestine.

Stephen Raverty, DVM
Stephen.Raverty@gov.bc.ca

END OF REPORT

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Final Report AHC Case: 07-4778

Last Updated: 01/02/08 2:46 PM
Pathologist: Gary D. Marty
Received Date: 12/24/07
Collected Date: 12/24/07
Client Ref No: SS208

Veterinarian: **Dr. Sonja Saksida**
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Submitter: **Sea to Sky Vet**
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Owner: **Sea to Sky Veterinary Ser**
Phone:
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Animal Data
Species: Chinook Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 3 cassettes for histology.

Mortality increasing. Mortalities not exhibiting any gross lesions. Some predation (seals, otters).

Chinook, Yearling, Netpen, 200g.

Final Diagnosis

1a. Brain: capillary (vascular) congestion, diffuse, moderate (slides 1B, 2B)

1b. Brain: meningeal hemorrhage, multifocal, moderate (slide 3B)

2a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 3A), moderate (slides 1A, 2A)

2b. Liver: perivascular hematopoiesis, bifocal, small amounts (slide 1A)

3. Intestinal mesenteries: capillary congestion, multifocal, mild (slide 1A)

4. Trunk kidney: glomerulonephritis, membranous, diffuse, mild (slide 2A)

Final Comment: Sections of brain provide the best clues to what killed these fish. Two fish have evidence of septicemia, and one fish died of cerebral trauma. Comments on specific lesions follow:

Congestion of brain capillaries is evidence of circulating vasodilators; differentials include viral, bacterial, and parasitic infections, but the sections contain no obvious organisms. The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section contain erythrocytes.

The most common cause of meningeal hemorrhage is trauma, and the hemorrhage in fish #3 is sufficient enough to have killed the affected fish.

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

Foci of hematopoietic cells in the liver are unusual in Chinook salmon; they are evidence that hematopoiesis in the kidney and spleen are not able to meet demands for these cells. The volume of hematopoietic cells in the trunk kidney seems about normal; the spleen and head kidney were not included for examination in slides from the affected fish.

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

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

Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology. After samples were processed into paraffin, they were split into two cassettes (yielding 2 cassettes). Paraffin blocks 1B and 3A were subjected to additional surface decalcification with 8% formic acid before sectioning.

Slide 1A (SS208) - heart, liver, intestine, trunk kidney, visceral mesenteries (lining of gonad?), mesenteric adipose tissue

Slide 1B - gill, brain, intestinal cecum, and mesenteric adipose tissue

Slide 2A (SS208) - heart (2 pieces), liver, spleen, trunk kidney, and a 2 × 1 mm piece of brain

Slide 2B - brain, intestinal cecum (severely autolyzed), and mesenteric adipose tissue

Slide 3A (SS208) - brain, liver, intestine, trunk kidney, part of the auditory apparatus, intestinal ceca, mesenteric adipose tissue

Slide 3B - gill, brain, intestinal ceca, mesenteric adipose tissue

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

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

Gary D. Marty
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END OF REPORT

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Final Report AHC Case: 07-4779

Last Updated: 01/03/08 1:24 PM
Pathologist: Gary D. Marty
Received Date: 12/24/07
Collected Date: 12/24/07
Client Ref No: SS209

Veterinarian: **Dr. Sonja Saksida**
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Submitter: **Sea to Sky Vet**
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Fax:
Owner: **Sea to Sky Veterinary Ser**
Phone:
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Animal Data
Species: Chinook Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted one cassette for histology.

Some soft flesh complaints. Pitting in muscle observed in sample submitted. R/O Kudoa BKD.

Chinook, Harvest, 3kg.

Final Diagnosis

1a. Skeletal muscle: myositis, lymphocytic, granulomatous, multifocal, with moderate numbers of Gram -positive short bacterial rods consistent with *Renibacterium salmoninarum*, mild (slide 1A)

1b. Skeletal muscle: vasculitis, granulomatous, multifocal, with hemorrhage, a *Loma salmonae* spore, and moderate numbers of Gram-positive bacterial rods consistent with *Renibacterium salmoninarum*, moderate (slide 1A)

2. Spleen and trunk kidney: *Loma salmonae* xenomas (each ~40 µm in diameter), multifocal, mild (slide 1B)

3. Trunk kidney: nephritis, interstitial, granulomatous, focal, radiating, with intracellular Gram -positive short bacterial rods consistent with *Renibacterium salmoninarum*, severe (slide 1B)

3. Spleen: splenitis, granulomatous, diffuse, with intracellular Gram-positive short bacterial rods consistent with *Renibacterium salmoninarum*, severe (slide 1B)

Final Comment: Gross lesions in the skeletal muscle are a result of vasculitis and granulomatous inflammation. The primary differentials for these lesions in Chinook salmon include *Renibacterium salmoninarum* and *Loma salmonae*, and this fish is infected with both organisms. Xenomas of the microsporidian *Loma salmonae* are common in vascular organs of Chinook salmon (here, in the spleen and trunk kidney). These xenomas are usually not associated with lesions, but affected fish often have more severe lesions in the gill or other organs resulting from a reaction to individual spores. Gill from this fish was not included for examination.

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

The skeletal muscle has no evidence of *Kudoa thyrsites*.

Histopathology

Formalin-fixed tissues were submitted in a cassette for histopathology. After samples were processed into paraffin, they were split into two cassettes (yielding 2 cassettes).

Slide 1A - skin/skeletal muscle

Slide 1B - skin/skeletal muscle, spleen, trunk kidney; a section from the same paraffin block was stained with ZN acid-fast and Twort's Gram.

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

Quality control: Trunk kidney autolysis: mild. The large piece of skeletal muscle in slide 1A has severe artefact in the deep tissues; the tissue was brittle when sectioned. Organs have no postfixation dehydration and no acid hematin deposits.

Gary D. Marty
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END OF REPORT

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Final Report AHC Case: 08-533

Last Updated: 02/14/08 3:46 PM
Pathologist: Gary D. Marty
Received Date: 02/12/08
Collected Date: 02/12/08
Client Ref No: SS-211

Veterinarian: **Dr. Sonja Saksida**
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Submitter: **Sea to Sky Veterinary**
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Fax:
Owner:
Phone:
Fax:

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

Case History

Submitted 3 histo cassettes. Follow up from SS-208 (2007- 4778). Brain hemorrhage continues (fish on feed) fish 1- 3. F2 - some jaundice and septicemia. I am questioning the quality of fixative.

Final Diagnosis

1a. Liver: biliary preductular cell hyperplasia, diffuse, moderate (slides 1A, 3A)

1b. Liver: hepatitis, perivascular fibrosis, with lymphohistiocytic inflammation, focal, severe (slide 3A)

1c. Liver: sinusoidal congestion, focal, moderate (slide 3A)

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

2a. Brain: capillary (vascular) congestion, diffuse, moderate (slides 1A, 3A)

2b. Brain: cerebral hemorrhage, focal, moderate (slide 3A)

3. Trunk kidney: interstitial cell degeneration and necrosis, multifocal, moderate (slide 2A)

Final Comment: These fish have several lesions that might provide clues to morbidity.

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins) or come from outside the fish (e.g., from the water or the feed). Biliary preductular cell hyperplasia is rare in farmed Pacific salmon, affecting only 2.4% of the 253 Pacific salmon examined in 2006 and 2007 as part of the Province's Fish Health Auditing and Surveillance Program; only one of those cases had moderate severity.

The liver has a focus of fibrosis, about 4 × 3 mm, that seems to follow vascular and biliary channels. The fibrosis probably is a result of damage to one or both of these channels, and biliary preductular cell hyperplasia is evidence that the cause of the lesion is ongoing. Lack of bacteria on the Gram stain mostly rules out *Renibacterium salmoninarum* as a differential. Lack of hepatic fibrosis in other fish is evidence that this is not a "herd health" issue.

Congestion of brain capillaries is evidence of circulating vasodilators; differentials include viral, bacterial, and parasitic infections, but the sections contain no obvious organisms. [The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section contain erythrocytes.]

The most common cause of meningeal hemorrhage is trauma. The hemorrhage in fish #3 is probably not sufficient enough to have killed the affected fish, but it might be indicative of more severe hemorrhage elsewhere in the brain (i.e., part of the brain not included in the section).

Sinusoidal congestion in the liver is nonspecific evidence of sinusoidal damage. In BC farmed salmon, hepatic sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus and *Listonella anguillarum*. In fish 3 it might be a result of altered blood through the focus of vascular/biliary fibrosis. More diffuse sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. Consider PCR for VHSV and IHNV.

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

The degree of autolysis in the trunk kidney of slide 2A limits the specificity of my diagnosis of interstitial necrosis (autolysis is the primary differential for this change). The renal interstitium contains evidence of nuclear degeneration (pyknosis, karyorrhexis, or karyolysis). Melanin granules are dispersed, presumably as a result of necrosis of melanocytes, and necrotic foci contain small foci of deeply eosinophilic material (probably fibrin). The multifocal nature of this lesions makes me think of a bacterial infection (e.g., *Yersinia ruckeri*), but I also recommend PCR for virus (e.g., VHSV and IHN).

Histopathology

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

Slide 1A (F1) - brain, liver, trunk kidney, head/trunk kidney transition, intestine, intestinal ceca, mesenteric adipose tissue

Slide 2A (F2) - heart, brain, liver, trunk kidney (2 pieces), intestinal ceca, mesenteric adipose tissue

Slide 3A (F3) - heart, brain, liver, trunk kidney, intestinal ceca, and mesenteric adipose tissue; a Twort's Gram stain was done on a section from the same block.

Slides 1B (F1), 2B (F2), and 3B (F3) - gill

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

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

Gary D. Marty
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END OF REPORT

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Final Report AHC Case: 08-571

Last Updated: 02/18/08 1:50 PM
Pathologist: Gary D. Marty
Received Date: 02/14/08
Collected Date: 02/14/08
Client Ref No: D-023

Veterinarian:
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Submitter: **B.C. Centre for Aquatic Health Sciences**
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Owner: **BC Centre Aquatic Hth**
Phone:
Fax:(250) 286-6103

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

Case History

Submitted Chinook Salmon smolt. Mixed visceral tissue. Fish are having high mortalities. Gross BKD. Fish very little visceral fat. Any other lesions beside BKD? Test request: Histopathology.

Final Diagnosis

1a. Heart: epicarditis, diffuse, with abundant intrahistiocytic short bacterial rods consistent with *Renibacterium salmoninarum* (bacterial kidney disease), severe (slide 1A)

1a. Heart: endocarditis and myocardial necrosis, multifocal, with abundant intrahistiocytic short bacterial rods consistent with *Renibacterium salmoninarum* (bacterial kidney disease), severe (slide 4A)

2a. Trunk kidney: interstitial cell necrosis, acute, with abundant short bacterial rods consistent with *Renibacterium salmoninarum* (bacterial kidney disease), severe (slide 4A)

2b. Trunk kidney: interstitial fibrin, multifocal, moderate (slide 1A)

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

2d. Trunk kidney: glomerulonephritis, membranous, diffuse, mild (slide 4A)

3a. Liver: hepatic necrosis, acute, with abundant short bacterial rods consistent with *Renibacterium salmoninarum* (bacterial kidney disease), moderate (slide 4A)

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

3c. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 2A)

4a. Brain: meningoencephalitis, granulomatous, with intrahistiocytic short bacterial rods consistent with *Renibacterium salmoninarum* (bacterial kidney disease), severe (slides 2A, 3A)

4b. Brain: encephalitis, granulomatous, multifocal, with intrahistiocytic short bacterial rods consistent with *Renibacterium salmoninarum* (bacterial kidney disease), mild (slide 4A)

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

5a. Gill vessels (from arch to lamellae): mural thrombi, multifocal, with abundant intrahistiocytic short bacterial rods consistent with *Renibacterium salmoninarum* (bacterial kidney disease), moderate (slide 4B)

5b. Gill: lamellar hyperplasia and fusion, multifocal, mild (slide 2B)

6. Spleen: splenitis, diffuse, with scattered fibrin deposits and abundant intrahistiocytic short bacterial rods consistent with *Renibacterium salmoninarum* (bacterial kidney disease), severe (slide 4A)

7. Mesenteric adipose tissue: peritonitis and steatitis, diffuse, with abundant intrahistiocytic short bacterial rods consistent with *Renibacterium salmoninarum* (bacterial kidney disease), severe (slide 4A)

Final Comment: The presence of *Renibacterium salmoninarum* (the cause of bacterial kidney disease) in multiple organs is consistent with clinical findings. Interestingly, in two of the fish (#s 2 and 3) lesions and organisms are limited to the brain. Only one fish has evidence of another cause: renal interstitial fibrin in fish #1 is evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin (BKD?) or inflammatory cell origin; toxins in the water or feed are less likely. Consider bacteriology and virology (e.g., VHSV). Comments on other lesions follow:

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

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

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

Congestion of brain capillaries is evidence of circulating vasodilators; differentials include viral, bacterial, and parasitic infections. [The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section contain erythrocytes.]

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

Gill lamellar hyperplasia with fusion may be a result of physical damage from exposure to an irritant; differentials include parasites or diatoms, but this lesion contains no organisms (they might have been lost during processing).

Histopathology

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

Slides 1A (12Feb08 #1), 3A (12Feb08 #3), 4A (12Feb08 #4) - spleen, heart, brain, liver, intestine, trunk kidney (3 pieces), intestinal ceca, mesenteric adipose tissue

Slide 2A (12Feb08 #2) - spleen, heart, brain, liver, trunk kidney (3 pieces), intestinal ceca, mesenteric adipose tissue

Slides 1B (12Feb08 #1), 2B (12Feb08 #2), 3B (12Feb08 #3), 4B (12Feb08 #4) - gill

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

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

Gary D. Marty
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END OF REPORT

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Final Report AHC Case: 08-602

Last Updated: 02/21/08 12:26 PM
Pathologist: Gary D. Marty
Received Date: 02/18/08
Collected Date: 02/18/08
Client Ref No: SS-210

Veterinarian: **Dr. Sonja Saksida**
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Phone: (250) 287-2363
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Submitter: **Dave Bailey - Creative Salmon**
Phone: (250) 725-2884
Fax: (250) 725-2885
Owner: **Creative Salmon**
Phone:
Fax:(250) 725-2885

Animal Data
Species: Chinook Salmon
Breed:
Sex: F
Age: 16 Months
Premise ID:

Case History

Submitted three histo samples and 3 slides for histology.

Vaccinated Vibroyen/Remogin. Euthanized. See attached sheet for typed detailed history on samples submitted.

Final Diagnosis

1a. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 1B), moderate (slide 2), severe (slide 1A)

1b. Liver: pericholangitis, lymphocytic, multifocal, mild (slide 1A)

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

2a. Spleen: parenchymal fibrin, multifocal, acute, mild (slide 1A)

2b. Spleen: splenitis, granulomatous, focal, moderate (slide 1B)

3. Blood smear: anemia, mild (slide BQ06-005-2000 Fish 2), moderate (slides BQ06-005-2000 Fish 1, BQ06-005-3000 Fish 1)

4a. Trunk kidney: nephritis, interstitial, granulomatous, with hemorrhage, multifocal, moderate (slide 1B)

4b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 1B)

Final Comment: The clinical signs in this fish are similar to what is thought to be a viral infection in coho salmon cultured in Chile (Smith et al. 2006). Comments on specific lesions follow:

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. Lipidosis accounts for the pale liver in fish BQ06-005- 2000-fish 1 and BQ06-005-2000 Fish 2.

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

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins) or come from outside the fish (e.g., from the water or the feed). Biliary preductular cell hyperplasia is rare in farmed Pacific salmon, affecting only 2.4% of the 253 Pacific salmon examined in 2006 and 2007 as part of the Province's Fish Health Auditing and Surveillance Program.

Multifocal fibrin deposits in the spleen are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin. I have also seen this response in salmon that are PCR positive for VHSV. Lack of remodelling of the fibrin is consistent with these deposits forming just before death. Consider bacteriology and virology, if not already done.

Causes of anemia in fish include viral erythrocytic necrosis virus (VEN), viral hemorrhagic septicemia virus (VHSV), certain dietary deficiencies, and the putative virus described by Smith et al. (2006). Blood cells on the smears from fish 1 (2000 and 3000) are very sparse, consistent with anemia, but the RBCs do not have obvious viral inclusions.

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

Granulomatous splenitis and interstitial nephritis are evidence of chronic immune stimulation. Lack of bacteria on the Gram stain mostly rules out the primary differential, *Renibacterium salmoninarum*, the cause of bacterial kidney disease. The inflammation might be associated with a vaccine or the virus described by Smith et al. (2006)

The lumen of the heart in slide 1A contains a piece of tissue that is about 500 × 100 µm; because it contains blood vessels, I think it might be a piece of spleen that got mixed into the heart during tissue handling or processing (i.e., an artefact).

Literature cited:

Smith, P.A., J. Larenas, J Contreras, J. Cassigoli, C. Venegas, M.E. Rojas, A. Guajardo, S. Perez, and S. Diaz. 2006. Infectious haemolytic anaemia causes jaundice outbreaks in seawater-cultured coho salmon, *Oncorhynchus kisutch* (Walbaum). Journal of Fish Diseases [J. Fish Dis.]. 29(12):709-715.

Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology. For all cassettes, gills were removed from the original cassette and placed in separate (G) cassettes.

Slides 1A (BQ06-005-3000 Fish 1) and 1B (BQ06-005-2000-Fish 1) - spleen, brain, heart, liver, intestine, trunk kidney, intestinal ceca, mesenteric adipose tissue; Twort's Gram stain was done on a section from the same block as slide 1B

Slide 2 (BQ06-005-2000 Fish 2) - spleen, brain, heart, liver, intestinal ceca, trunk kidney (2pieces), mesenteric adipose tissue

Slides 1AG (BQ06-005-3000 Fish 1), 1BG (BQ06-005-2000- Fish 1) and 2G (BQ06-005-2000 Fish 2) - gill

A blood smear was also submitted from each fish.

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

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

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

END OF REPORT

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Final Report AHC Case: 08-985

Last Updated: 03/17/08 1:47 PM
Pathologist: Gary D. Marty
Received Date: 03/12/08
Collected Date: 03/12/08
Client Ref No: PO#2599BM

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

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

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

Case History

Submitted 6 fish, 2 per cassette for histo exam.

Increased mortality, OTC treatment for BKD in February 2008.

Final Diagnosis

1a. Brain: meningeal and neuropil hemorrhage, multifocal, moderate (slide 1A)

1b. Brain: capillary (vascular) congestion, diffuse, mild (slide 2A), moderate (slide 1A)

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

2b. Liver: sinusoidal fibrin, multifocal, acute, moderate (slides 1A, 3A)

2c. Liver: hepatocellular cytoplasmic vacuoles, with intravacuolar eosinophilic material and necrotic cells, diffuse, moderate (slides 2A, 3A)

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

2e. Liver: hepatocellular fatty change (lipidosis), diffuse, moderate (slide 1A), severe (slide 2A)

3. Spleen: parenchymal fibrin, multifocal, acute, moderate (slides 1A, 2A), severe (slide 3A)

4. Heart: endothelial cell hypertrophy and hyperplasia, diffuse, mild (slides 1A, 3A), moderate (slides 2A, 3A)

5. Trunk kidney: renal tubular mineralization, multifocal, mild (slide 1A), with dilated tubules and tubular epithelial hyperplasia (nephrocalcinosis), severe (slide 2A)

6. Gill: congestion of filament vessels, diffuse, moderate (slide 2B, one gill only)

Final Comment: These fish have several lesions, most of which are consistent with an acute inflammatory response. I recommend bacterial culture and PCR for VHSV (if not already done). None of the organs have granulomatous inflammation, and the Gram stain has no organisms,

so *Renibacterium salmoninarum* is low on my list of differentials.

The most common cause of meningeal hemorrhage is trauma, but the small foci of hemorrhage in the neuropil of this fish might be a result of vascular damage (e.g., from a bacterial or viral infection). Trauma often results from fish running into something, including into other fish, and increased activity that leads to brain trauma might be associated with sea lice infestation, avoidance of predators, or stray voltage. Moderate or severe brain hemorrhage is uncommon in cultured Chinook salmon, affecting only 2 of 142 Chinook salmon brains I have examined since January 1, 2007 as part of the BC Fish Health Auditing and Surveillance Program.

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

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

Multifocal fibrin deposits in the liver and spleen are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin. I have also seen this response in fish that are PCR positive for VHSV. Lack of remodelling of the fibrin is consistent with these deposits forming just before death. Consider bacteriology and virology, if not already done.

Hepatocellular vacuoles in slide 2A might contain lipid, fibrin, and/or necrotic epithelial cells. Hepatocytes have limited phagocytic activity, which in this case probably functions to ingest dead adjacent hepatocytes. This is a form of inflammation, and it might be related to VHSV or other infection.

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

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

Hypertrophy and hyperplasia of endothelial cells in the heart might be a response to inflammatory mediators from viral, bacterial, or parasitic infections. The sections have no obvious organisms.

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.

In one gill arch in slide 2B, filament vessels are distended by erythrocytes to about 3× the diameter I would expect for this size gill. I have never seen this type of change [the gills might have been very red grossly]. It might be evidence of circulating vasodilators or it might be a developmental anomaly.

Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology. For all cassettes, gills were removed from the original (A) cassettes and placed in separate (B) cassettes. Paraffin blocks 1B and 2B (gill) were subjected to additional surface decalcification with 8% formic acid before sectioning.

Slide 1A (Ahl 03/07) - heart, spleen (2 pieces), brain (2 pieces), liver (3 pieces), trunk kidney, swimbladder

Slide 2A (Ahl 03/07) - heart, spleen (2 pieces), brain (2 pieces), stomach, liver (2 pieces), trunk kidney (2 pieces), intestinal ceca, mesenteric adipose tissue

Slide 3A (Ahl 03/07) - brain, spleen (3 pieces), heart (3 pieces), liver (3 pieces), trunk kidney, swimbladder (2 pieces), intestinal ceca, mesenteric adipose tissue; Twort's Gram stain was done on a section from the same block.

Slides 1B, 2B and 3B (Ahl 03/07) - gills

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

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

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

END OF REPORT

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Final Report AHC Case: 08-1125

Last Updated: 03/27/08 3:09 PM
Pathologist: Gary D. Marty
Received Date: 03/20/08
Collected Date: 03/20/08
Client Ref No: Ahlstrom Sea Site

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

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

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

Case History

Submitted formalized fish tissues for histopathology.

Chinook. Age: 200g. Saltwater entry. Vaccinated.

Farm: Ahlstrom Sea Site.

Increase mortality. Have been treated with/OTC for BKD.

Final Diagnosis

1. Trunk kidney: renal tubular mineralization, multifocal, with dilated tubules and tubular epithelial hyperplasia (nephrocalcinosis), diffuse, severe (slide 3)

2. Trunk kidney: renal tubular epithelial necrosis, focal, acute, mild (slide 1A)

3. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (slides 1A, 2)

4. Trunk kidney: renal tubular casts of protein and yellow-brown pigment, with tubular epithelial degeneration and regeneration, focal, mild (slide 1A)

Final Comment: Renal mineralization is common in cultured fish species; when severe, the condition is termed nephrocalcinosis. The lesion is not considered fatal, although feed conversion may be adversely affected. The 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.

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

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).

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

Tissues have no evidence of bacterial kidney disease.

Histopathology

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

Slide 1A (Ahl 03/13) - spleen, brain, liver, intestine, trunk kidney (2 pieces), intestinal ceca, mesenteric adipose tissue

Slide 2 (Ahl 03/13) - spleen, brain, liver, trunk kidney, swimbladder, intestinal ceca, mesenteric adipose tissue

Slide 3A (Ahl 03/13) - spleen, brain, liver, intestine, trunk kidney, swimbladder, intestinal ceca, mesenteric adipose tissue

Slides 1B, 3B and 4 (Ahl 03/13) - gill

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

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

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

END OF REPORT

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Final Report AHC Case: 08-4797

Last Updated: 12/17/08 1:44 PM
Pathologist: Gary D. Marty
Received Date: 12/15/08
Collected Date: 12/10/08
Client Ref No:

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

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

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

Case History

Submitted formalized chinook tissues for histopathology.

Saltwater. 85 grams. Vaccinated. Possible BKD.

Final Diagnosis

1a. Trunk kidney: renal tubular mineralization, multifocal, with dilated tubules and tubular epithelial hyperplasia (nephrocalcinosis), moderate (slide 3A), severe (slides 1A, 2A)

1b. Trunk kidney: renal tubular intraluminal protein casts, multifocal, mild (slide 3A)

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

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

3. Heart, stratum compactum: congestion and hemorrhage, diffuse, moderate (slide 1A)

4a. Fin: dermatitis, ulcerative, multifocal, severe, with foci of filamentous bacteria in moderate numbers (slide 2B) or abundant (slide 1B)

4b. Fin: dermatitis, ulcerative, multifocal, fibrinous, severe (slide 3B)

Final Comment: These fish have a several lesions consistent with morbidity, but no evidence of infection with *Renibacterium salmoninarum*. Comments on specific lesions follow:

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.

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

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

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

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 both Atlantic and Pacific salmon. This might be a congenital malformation. Alternatively, it might be a result of endothelial damage, with bacterial and viral infections as the most likely differentials.

Filamentous bacteria commonly invade skin ulcers. Once filamentous bacteria become established, the ulcers often get larger (as in this case). Enlargement of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection). Identification of the bacteria requires culture or PCR. In salt water, *Tenacibaculum maritimum* is most likely. Slide 3B is different in that the ulcers do not contain filamentous bacteria but instead contain abundant fibrin; these ulcers might have a different cause (e.g., a *Vibrio sp*.). Consider bacterial culture, if not already done.

Histopathology

Formalin-fixed tissues from three Chinook salmon were received for histopathology. The gills and skin were decalcified in Protocol B for 3.5 hours before processing routinely into paraffin. Blocks 1A, 2A and 3A were subjected to additional surface decalcification with 8% formic acid before sectioning.

Slide 1A - brain, heart, spleen, liver, ovary, trunk kidney, swimbladder, intestinal ceca

Slide 1B - gill, fin

Slide 2A - brain, heart, spleen (2 pieces), liver, trunk kidney, stomach, intestinal ceca

Slide 2B - skin

Slide 3A - brain, heart, spleen, liver, intestine, swimbladder, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide 3B - gill, skin

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

Quality control: Tissue preservation is excellent for most organs; the gill has mild autolysis. Large foci of erythrocytes (e.g., spleen in slide 1A) have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have no postfixation dehydration.

Measure of physiologic condition:

Mesenteric adipose tissue: none (slide 1A), moderate amounts (slide 2A), or abundant (slide 3A)

Patterns in this measure of physiologic condition vary from a starving fish (slide 1A) to a fish that seems to be eating fairly normally (slide 3A).

Gary D. Marty

Case: 08-4797

END OF REPORT

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Final Report AHC Case: 09-113

Last Updated: 01/20/09 10:49 AM
Pathologist: Gary D. Marty
Received Date: 01/14/09
Collected Date:
Client Ref No:

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

Submitter: **Sea to Sky**
Phone:
Fax:
Owner: **SS-216**
Phone:
Fax:

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

Case History

Submitted formalized Chinook tissue for histopathology.

(DP) Dawley-mortalities -food in gut-no abnormal findings. Indian- severe jaundice-pen 3 blood not coagulating, off feed. (P3)

Final Diagnosis

1a. Trunk kidney: interstitial cell degeneration, necrosis, and hyperplasia (regeneration), diffuse, moderate (slide 1A)

1b. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, moderate (slide 1A)

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

3. Intestine: congestion of the lamina propria, diffuse, mild (slide 2), moderate (slide 1)

4. Intestinal ceca: enteritis, acute, with intraluminal fibrin, moderate (slide 1)

5. Mesenteric adipose tissue: capillary congestion, diffuse, mild (slide 3A)

Final Comment: Causes of anemia in fish include viral erythrocytic necrosis virus (VEN), viral hemorrhagic septicemia virus (VHSV), and certain dietary deficiencies. The clinical signs in fish IndP3 (slide 1) are similar to what is thought to be a viral infection in coho salmon cultured in Chile (Smith et al. 2006). 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). Renal tubular epithelial necrosis was fairly common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program: Atlantic salmon (prevalence = 6.8%; n = 469) and Pacific salmon (prevalence = 4.2%; n = 118); the cause was not determined in many cases. Differentials include viral hemorrhagic septicemia virus (VHSV) and exposure to toxins (e.g., bacterial toxins, algal toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin). Consider bacteriology and virology, if not already done.

Necrotic hematopoietic cells in the renal interstitium have some form of nuclear degeneration (pyknosis, karyorrhexis, or karyolysis). This case might be a result of infection with bacteria (e.g., *Yersinia ruckeri*) or virus (e.g., VHSV, IHN, or some other virus [see Smith et al. 2006]).

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

Congestion of the intestinal lamina propria is an uncommon lesion, and it probably is a result of selective vasodilators in the blood. Cause(s) are probably the same as for the renal lesions.

Intestinal cecal intraluminal fibrin, with few associated inflammatory cells, 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. 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.

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

Literature cited:

Smith, P.A., J. Larenas, J Contreras, J. Cassigoli, C. Venegas, M.E. Rojas, A. Guajardo, S. Perez, and S. Diaz. 2006. Infectious haemolytic anaemia causes jaundice outbreaks in seawater-cultured coho salmon, *Oncorhynchus kisutch* (Walbaum). Journal of Fish Diseases [J. Fish Dis.]. 29(12):709-715.

Histopathology

Formalin-fixed tissues were submitted in 5 cassettes for histopathology. The gills were decalcified in Protocol B (hydrochloric acid solution) for 2 hours before being rinsed and processed routinely into paraffin.

Slide 1A (Ind P3) - heart, liver, spleen, trunk kidney (2 pieces), intestine, intestinal ceca, mesenteric adipose tissue (the gill was removed, processed, and sectioned separately on slide 1B)

Slide 2A (DP 7) - brain, heart, liver, spleen, trunk kidney (2 pieces), intestine, intestinal ceca, mesenteric adipose tissue

Slide 3A (India 1) - brain, heart, liver, spleen, intestine, intestinal ceca, mesenteric adipose tissue

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

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

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

History of Communication

Date	To	Description
01/29/09 9:36 AM	Sea to Sky Veterinary Ser - e-mail	Case Invoiced

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

END OF REPORT

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Final Report AHC Case: 09-126

Last Updated: 02/12/09 9:29 AM
Pathologist: Gary D. Marty
Received Date: 01/15/09
Collected Date:
Client Ref No:

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

Submitter: **Sea to Sky**
Phone:
Fax:
Owner: **SS-217**
Phone:
Fax:

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

Case History

Submitted one plate for bacteriology.

2008 entry. Severe jaundice. Average wt - 1 kg.

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 02/12/09 @ 9:28 AM

Specimen	ID	Isolate	Result	Level
Isolate		Vibrio sp.	Positive	
**: Vibrio sp. was identified using DNA sequencing. The sequence was unable to be discriminate between Aliivibrio wodanis and Aliivibrio salmonicida. The isolate was negative for Aliivibrio salmonicida serology.				
Isolate		Vibrio splendidus	Positive	

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 02/12/09 @ 9:29 AM

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

History of Communication

Date	To	Description
01/21/09 4:32 PM	-	bc report generated
01/21/09 4:33 PM	Sea to Sky Veterinary Ser - e-mail	bc report generated
01/22/09 8:50 AM	-	bc report generated

01/29/09 9:36 AM	Sea to Sky Veterinary Ser - e-mail	Case Invoiced
02/11/09 3:42 PM	-	bc report generated
02/12/09 10:54 AM	Sea to Sky Veterinary Ser - e-mail	bc report generated
02/12/09 10:54 AM	-	bc report generated

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

END OF REPORT

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Final Report AHC Case: 09-469

Last Updated: 02/12/09 11:45 AM
Pathologist: Gary D. Marty
Received Date: 02/10/09
Collected Date: 02/09/09
Client Ref No: 2599BM

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

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

Case History

Submitted formalized Salmon tissues for histopathology. Saltwater. Vaccinated. Farm location; Culloden Point

Inappetence coincident with declining water temperatures. ~0.01%/d mortality. Fish with abundant bile staining and poor body condition.

Concurrent tests include virology and BKD ELISA.

Note: A single submission form was originally submitted with this case, 2009- 0498, and 2009-0499, and tissues from all 3 cases were processed into slides numbered 2009-0469. Block and slide numbers remain as 2009-0469, but results from the appropriate slides are reported in case 2009-0498 or 2009-0499 (see "Histopathology" section for clarification).

Final Diagnosis

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

1b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slide C-1A)

1c. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slide C-1A), moderate (slide C-4A), abundant (slide C-2A)

2a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide C-1A)

2b. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slide C-1A, C-2A, C-3A), moderate (slide C-4A)

3a. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (C-3A), moderate (slide C-2A)

3b. Trunk kidney: renal tubular mineralization, multifocal, mild (slide C-1A)

Final Comment: These fish have several changes that are fairly common in Pacific salmon that die in BC net pens. None of the lesions are of sufficient severity to have killed the fish, but, lesions might provide clues about the cause of mortality:

Hydropic degeneration among small numbers of hepatocytes provides evidence that the liver was being exposed to toxins. Potential sources of the inciting toxins include the water, a bacterial infection, or circulating oxygen radicals following a period of hypoxia. In this case, cytoplasm

of affected hepatocytes is expanded by fine to large foamy vacuoles.

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

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). Their effect on fish health is unknown.

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

The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin is a 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. In the BC Fish Health Auditing and Surveillance Program from 2006 - 2008, splenic lipofuscin deposits were more common among Chinook salmon (38%) than Atlantic salmon (22%).

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

Renal 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.

Histopathology

Formalin -fixed tissues were submitted in 4 cassettes for histopathology. After processing into paraffin, gills were removed from the original (A) cassette and placed in a separate (B) cassette.

Slide C-1A(C.P.) and C-2A(C.P.) - brain, spleen, liver, intestine, trunk kidney

Slide C-3A (C.P.) - brain, spleen, trunk kidney, intestine, intestinal ceca, mesenteric adipose tissue

Slide C-4A (C.P.) - brain, spleen, liver, intestine, trunk kidney, mesenteric adipose tissue

Slides C-1B, C-2B, C-3B, C-4B - gill

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

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

History of Communication

Date	To	Description
02/12/09 11:46 AM	Grieg Seafoods BC Ltd. - e-mail	bc report generated
02/16/09 11:13 AM	Grieg Seafoods BC Ltd. - e-mail	Case Invoiced

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

END OF REPORT

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Toll-Free : 1-800-661-9903

Final Report AHC Case: 09-1136

Last Updated: 03/27/09 4:47 PM
Pathologist: Gary D. Marty
Received Date: 03/26/09
Collected Date:
Client Ref No:

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

Submitter: **Sea to Sky**
Phone:
Fax:
Owner: **SS 218**
Phone:
Fax:

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

Case History

Submitted 2 Salmon cassettes for diagnostic investigation.

Average wt - 1 kg, Age - yearling, Site type - net pen. Fresh mortalities - on feed, no abnormal finding.

Final Diagnosis

1a. Liver: biliary preductular cell hyperplasia, multifocal, mild (slides 1, 2)

1b. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, (lipofuscin and hemosiderin?), mild (slides 1, 2)

1c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 1)

1d. Liver: hepatocellular cytoplasmic vacuoles, multifocal, mild (slide 1)

2a. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (slide 2), moderate (slide 1)

2b. Trunk kidney: renal tubular intraluminal protein casts, multifocal, moderate (slide 2)

2c. Trunk kidney: glomerulonephritis, membranous, diffuse, mild (slide 2)

2d. Trunk kidney: renal tubular mineralization, multifocal, mild (slide 1)

3. Brain: meningeal hemorrhage, multifocal, mild (slide 3)

Final Comment: These fish have several lesions, most of which are fairly common among farmed salmon in British Columbia. Although none of the lesions are of sufficient severity for me to assign a cause of death, renal tubular protein casts in slide 2 might be the most significant. Comments on specific lesions follow.

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

salmon (e.g., it did not occur in the 118 farmed Pacific salmon examined in 2008 as part of the BC Fish Health Auditing and Surveillance Program).

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

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

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These vacuoles are fairly common in Pacific salmon livers sampled as part of the BC Fish Health Auditing and Surveillance Program, and their prevalence has changed little from 76% in 2006 to 84% in 2007 and 86% in 2008.

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

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

Features of glomerulonephritis include thickening of the glomerular basement membranes by homogeneous eosinophilic material. Membranous glomerulonephritis is fairly common in Chinook salmon. Membranous glomerulonephritis is often associated with infections in other parts of the fish, and a link to immune complex deposition has been demonstrated (Lumsden et al. 2008). Membranous glomerulonephritis has been associated with cardiomyopathy syndrome (in Atlantic salmon), nephrocalcinosis, and infections with a number of bacteria and parasitic species (e.g., *Loma salmonae* in Chinook salmon).

Renal mineralization is common in cultured fish species; when severe (not here), the condition is termed nephrocalcinosis. The lesion is not considered fatal, although feed conversion may be adversely affected. The pathogenesis is not fully understood, but renal mineralization has been experimentally reproduced through high carbon dioxide levels, magnesium deficiency, selenium toxicity, and a diet low in minerals (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Clinically, renal mineralization is most commonly associated with high carbon dioxide levels.

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. The hemorrhage might also be a result of blunt trauma to the head (i.e., a euthanasia artifact).

Literature Cited:

Lumsden, J.S., S. Russell, P. Huber, B.A. Wybourne, V.E. Ostland, M. Minamikawa, and H.W. Ferguson. 2008. An immune-complex glomerulonephritis of Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). J. Fish Dis. 31(12): 889-898.

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology. Gills were removed from the original cassette and placed in a separate cassette (3) and then immersed in Protocol B (hydrochloric acid solution) for 1.25 hours before being rinsed and processed with the other cassettes into paraffin.

Slide 1 - heart, spleen, liver, intestine, trunk kidney (2 pieces), intestinal ceca, mesenteric adipose tissue, and a 7-mm -diameter focus of erythrocytes

Slide 2 - brain, gill, heart, spleen, liver, intestine, trunk kidney, intestine, intestinal ceca, and mesenteric adipose tissue

Slide 3 - brain, gill

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

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

Staff Comments:
The final report for this case was sent to Dr. Saksida via Outlook by GDMarty, 2009-03-27 4:51pm.

History of Communication

Date	To	Description
04/08/09 3:34 PM	Sea to Sky Veterinary Ser - e-mail	Case Invoiced

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

END OF REPORT

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Final Report AHC Case: 09-2336

Last Updated: 06/22/09 2:30 PM
Pathologist: Gary D. Marty
Received Date: 06/18/09
Collected Date:
Client Ref No:

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

Submitter: **Sea to Sky Vet**
Phone:
Fax:
Owner: **SS-301**
Phone:
Fax:

Animal Data
Species: Chinook Salmon
Breed:
Sex:
Age: 1 Years
Premise ID:

Case History

Submitted formalized Chinook tissue for histology.

Average wt - approx 1 kg. Site type - net pen. Check for Loma and Rickettsia and BKD (gill and liver only).

Final Diagnosis

1. Liver: hepatitis, granulomatous, fibrinous, multifocal, with intralesional short bacilli characteristic of *Renibacterium salmoninarum* , severe

2. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate

3. Gill: *Loma salmonae* xenomas, multifocal, mild

Final Comment: Although this fish has two of the clinical differentials (*Renibacterium salmoninarum* , and *Loma salmonae*), only the *R. salmoninarum* lesions are of sufficient severity to have killed this fish.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These vacuoles are fairly common in Pacific salmon livers sampled as part of the BC Fish Health Auditing and Surveillance Program, and their prevalence has changed little from 76% in 2006 to 84% in 2007 and 86% in 2008.

Loma salmonae is a microsporidian parasite common in cultured Pacific salmon. As long as the organisms remain confined to their xenomas (as is this case), the inflammatory response by the host is minimal.

Histopathology

Formalin-fixed tissues were submitted in 1 cassette for histopathology (Slide 1 - gills, liver). All organs were examined. Organs not listed elsewhere have no significant lesions.

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

History of Communication

Date	To	Description
06/22/09 2:31 PM	Sea to Sky Veterinary Ser - e-mail	bc report generated
06/24/09 12:42 PM	Sea to Sky Veterinary Ser - e-mail	Case Invoiced

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

END OF REPORT

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Final Report AHC Case: 09-3524

Last Updated: 09/15/09 1:46 PM
Pathologist: Gary D. Marty
Received Date: 09/10/09
Collected Date:
Client Ref No:

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

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: Chinook Salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted formalized tissue from 5 fish for Histopathology.

Increase of mortality in late July at 860g. Mortality increases again in late Aug. Saltwater. Vaccinated - Yes. 1.4Kg

Final Diagnosis

1. Gill: filament vasculitis, fibrinous, necrotizing, multifocal, with intralesional *Loma salmonae* xenomas, severe (slide 3)

2a. Trunk kidney: nephritis, interstitial, granulomatous, multifocal, coalescing, with scattered fibrin deposits, severe (slide 4)

2b. Trunk kidney: glomerulonephritis, membranous, diffuse, mild (slide 4)

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

2d. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slide 4)

3a. Liver: hepatic necrosis, acute, multifocal, with fibrin and intrahistiocytic bacteria (*Piscirickettsia salmonis*), moderate (slide 5)

3b. Liver: pericholangitis, lymphohistiocytic, multifocal, mild (slides 1, 5), moderate (slide 4)

3c. Liver: sinusoidal fibrin, multifocal, acute, mild (slide 4)

3d. Liver: hepatocellular cytoplasmic vacuoles, diffuse, small amounts (slide 4), moderate (slides 1, 2, 5), abundant (slide 3)

4a. Spleen: leukocytic karyorrhexis, disseminated, with intrahistiocytic *Piscirickettsia salmonis*, moderate (slide 5)

4b. Spleen: parenchymal fibrin, multifocal, acute, mild (slide 5)

4c. Spleen: parenchymal golden pigment (lipofuscin?), scattered, intracellular, moderate (slide 4)

Final Comment: These fish have several lesions, a variety of which could have killed the affected fish: *Piscirickettsia salmonis* infection (fish #5), *Loma salmonae* infection (fish #3), and granulomatous interstitial nephritis with fibrin (fish #4). The cause of death in fish #s 1 and 2 is unknown. Variable causes of death at the same farm occurs occasionally among farmed Chinook salmon in BC. For example, one farm visit that was part of the BC Fish Health Audit and Surveillance Program in 2007 included 13 fish with 5 different causes of death, including *Renibacterium salmoninarum* , *Piscirickettsia salmonis*, and *Loma salmonae*. Comments on specific lesions follow:

Moderate numbers of *Loma salmonae* xenomas in the gill--sometimes associated with severe vasculitis and necrosis--is characteristic of infection with *Loma salmonae*. Inflammation and tissue destruction occur when the parasite invades vessels and surrounding tissues of the gill filaments. *Loma salmonae* is directly transmissible from fish to fish by either ingestion of infected tissue or by free spores, and it can easily be transmitted by co-habitation with infected fish (source Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada).

Coalescing foci of granulomatous inflammation with fibrin in the trunk kidney (slide 4) are evidence of active immune stimulation. Differentials include infection with *Renibacterium salmoninarum* , the cause of bacterial kidney disease, *Yersinia ruckeri* infection, or an unusual reaction to a vaccine. The sections contain no obvious organisms with H&E or Gram stain.

Features of glomerulonephritis include thickening of the glomerular basement membranes by homogeneous eosinophilic material. Membranous glomerulonephritis is fairly common in farmed Chinook salmon. Membranous glomerulonephritis is often associated with infections in other parts of the fish, and a link to immune complex deposition has been demonstrated in Chinook salmon (Lumsden et al. 2008). Membranous glomerulonephritis has been associated with cardiomyopathy syndrome (in Atlantic salmon), nephrocalcinosis, and infections with a number of bacteria and parasitic species (e.g., *Loma salmonae* in Chinook salmon).

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.

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 Pacific salmon (prevalence = 31% ; n = 118).

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 fibrin deposits in the liver are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin. I have also seen this response in fish that are PCR positive for VHSV. Lack of remodelling of the fibrin is consistent with these deposits forming just before death. Consider bacteriology and virology, if not already done.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These vacuoles are fairly common in Pacific salmon livers sampled as part of the BC Fish Health Auditing and Surveillance Program, and their prevalence has changed little from 76% in 2006 to 84% in 2007 and 86% in 2008.

The presence of degenerating nuclei (karyorrhexis) in the spleen is evidence of increased cell turnover, possibly as part on an active inflammatory response (e.g., related to *Piscirickettsia salmonis* infection).

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

The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish. In the BC Fish Health Auditing and Surveillance Program from 2006 - 2008, splenic lipofuscin deposits were more common among Chinook salmon (38%) than Atlantic salmon (22%). In this case, increased deposits in fish #4 might be related to inflammation in the kidney. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Literature Cited:

Lumsden, J.S., S. Russell, P. Huber, B.A. Wybourne, V.E. Ostland, M. Minamikawa, and H.W. Ferguson. 2008. An immune-complex glomerulonephritis of Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). J. Fish Dis. 31(12): 889-898.

Histopathology

Formalin-fixed tissues from 5 fish were submitted in 5 cassettes for histopathology. Cassette #s 1 - 4 were labelled V. Aug. 31.

Slide 1 - liver, spleen, head kidney, trunk kidney, brain, intestine, intestinal ceca, mesenteric adipose tissue

Slide 2 - liver, spleen, gill, brain, intestinal ceca, mesenteric adipose tissue

Slides 3 and 5 - liver, spleen, gill, brain, intestine, intestinal ceca, mesenteric adipose tissue; a section from block 5 was stained with specific immunohistochemical reagents for *Piscirickettsia salmonis*.

Slide 4 - liver, spleen, brain, trunk kidney, intestine, intestinal ceca, mesenteric adipose tissue; a Twort's Gram stain was done on a section from block 4

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

Quality control: Liver autolysis: none (slide 4), mild (slides 1, 2, 3, 5). The spleen in slide 5 has precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have no postfixation dehydration.

History of Communication

Date	To	Description
09/15/09 1:46 PM	Milligan, Barry - e-mail	bc report generated
09/21/09 1:23 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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Final Report AHC Case: 09-3594

Last Updated: 09/18/09 10:23 AM
Pathologist: Gary D. Marty
Received Date: 09/15/09
Collected Date:
Client Ref No:

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

Submitter: **Sea to Sky Veterinary Service**
Phone:
Fax:
Owner: **Sea to Sky Veterinary Ser**
Phone:
Fax:(250) 287-2430

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

Case History

Submitted 2 formalized Chinook fish tissues for Histopathology.

Average wt: 80g. Site type - net pen.

Substantial mortality during off-loading smolt from shipping container into net pens. Fish - not scale loss, no signs of septicemia or granulomas. Only gills and pseudobranch appeared darker than would be expected. Bacterial culture - negative. In house lab findings. Please fax or email report to Dr. Sonja Saksida.

Final Diagnosis

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

2. Heart, ventricle: mineralization, focal, mild (slide 1A)

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

Final Comment: These fish have no lesions to explain elevated post-transfer mortality. The lack of lesions is consistent with an acute process, which would not produce morphologic lesions (e.g., stress derived from transportation, acute hypoxia, or acute toxin exposure). Comments on minor lesions follow:

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.

Heart mineralization is an unusual finding. The one focus of mineralization in the affected fish probably was of no significance for its health.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These vacuoles are fairly common in

Pacific salmon livers sampled as part of the BC Fish Health Auditing and Surveillance Program, and their prevalence has changed little from 76% in 2006 to 84% in 2007 and 86% in 2008. The vacuoles might be normal for farmed fish.

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology. After processing into paraffin, tissues were split into separate cassettes (labeled A and B). Gills were removed from the original (A) cassette and placed in separate (G) cassettes.

Slide 1A - brain, heart, liver, trunk kidney, skin/skeletal muscle

Slide 1B - brain, heart, spleen (2 pieces) liver, trunk kidney, intestinal ceca, skin/skeletal muscle, mesenteric adipose tissue

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

Slide 2B - brain (2 pieces), heart (2 pieces), trunk kidney (2 pieces) skin/skeletal muscle

Slides 1G and 2G - gill

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

Quality control: Tissue preservation is good for most organs; tissues adjacent to the gallbladder often have severe autolysis. The livers have several foci of "squish" artifact; this probably occurred when the tissues were sampled. Organs have no postfixation dehydration and no acid hematin deposits.

Staff Comments:
Fish Histopathology Fellow: Meritxell Diez Padrisa, DVM

History of Communication

Date	To	Description
09/18/09 10:24 AM	Sea to Sky Veterinary Ser - e-mail	bc report generated
09/21/09 1:48 PM	Sea to Sky Veterinary Ser - e-mail	Case Invoiced

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

END OF REPORT

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Final Report AHC Case: 10-417

Last Updated: 01/29/10 2:23 PM
Pathologist: Gary D. Marty
Received Date: 01/28/10
Collected Date:
Client Ref No: SS-303

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

Submitter: **Sea to Sky Vet**
Phone:
Fax:
Owner: **Sea to Sky Veterinary Ser**
Phone:
Fax:(250) 287-2430

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

Case History

Submitted formalized Chinook tissues for Histopathology. Age: >1 year in saltwater. Site type: SW.

Boxler 1 - no abnormal findings.

Worne 1 - rule out marine anemia; Worne 2 - rule out Loma.

Final Diagnosis

1a. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (slide 1B), moderate (slide 2B)

1b. Trunk kidney: glomerulonephritis, membranous, diffuse, mild (slide 3B)

1c. Trunk kidney: fibrous capsule granuloma, focal (~90 µm in diameter), with central necrotic mineralized debris, mild (slide 3B)

2. Gill: filament vasculitis, fibrinous, multifocal, moderate (slide 3C)

3a. Liver: hepatocellular hydropic degeneration, disseminated, acute, mild (slide 2A)

3b. Liver: pericholangitis, lymphohistiocytic, diffuse, mild (slide 2A)

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

3d. Liver: hepatocellular cytoplasmic lipid, diffuse, moderate amounts (slide 3A)

4. Brain: encephalitis, multifocal to diffuse, lymphocytic, with gliosis, mild (slide 2A)

5. Mesenteric adipose tissue: capillary congestion/hemorrhage, multifocal, mild (slide 2A)

6. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild (slides 2B, 3B)

Final Comment: These fish have several lesions that might provide clues to morbidity, but only one fish (in slide 3B) has lesions that might be

a result of a specific infections organism (i.e., *Loma salmonae*). Comments on specific lesions follow:

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

Features of glomerulonephritis include thickening of the glomerular basement membranes by homogeneous eosinophilic material. Membranous glomerulonephritis is fairly common in Chinook salmon, affecting 32% of 441 Pacific salmon trunk kidneys examined from 2006 -2009 as part of the BC Fish Health Auditing and Surveillance Program. Membranous glomerulonephritis is often associated with infections in other parts of the fish, and a link to immune complex deposition has been demonstrated in Chinook salmon (Lumsden et al. 2008). Membranous glomerulonephritis has been associated with cardiomyopathy syndrome (in Atlantic salmon), nephrocalcinosis, and infections with a number of bacteria and parasitic species (e.g., *Loma salmonae* in Chinook salmon).

Fibrous capsule granulomas are fairly common in wild fish, but I do not recall having seen one before in farmed Chinook salmon. The granuloma might represent a focus where a parasite died long ago; all that is left is the indigestible remnants. This focus is probably of little significance for fish health.

Multifocal fibrinous vasculitis in the gill of Chinook salmon is most commonly a result of infection with *Loma salmonae*. Although the affected section contains no xenomas, most inflammation and tissue destruction is usually associated with individual spores (which are not readily identified on H&E- stained sections). *Loma salmonae* is directly transmissible from fish to fish by either ingestion of infected tissue or by free spores, and it can easily be transmitted by co-habitation with infected fish (source Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada).

Hydropic degeneration among small numbers of hepatocytes provides evidence that the liver was being exposed to toxins (e.g., algal toxins or other marine biotoxins). 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.

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.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen or fluid). At least some types of vacuoles might be normal; their effect on growth and feed conversion is unknown. 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.

Some degree of lipid accumulation in the cytoplasm of hepatocytes might be normal. Abnormal accumulation of hepatocellular lipid (lipidosis) occurs when fish are not feeding and in cases of inadequate nutrition.

Encephalitis is evidence of immune stimulation. Differentials include infections with viral, bacterial, or parasitic pathogens. Meningoencephalitis of unknown cause occurs fairly commonly in fish sampled as part of the BC Fish Health Auditing and Surveillance Program, affecting 22 of the 72 Pacific salmon examined in 2009 (21% were mild, and 1.4% were moderate; none were severe).

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

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is somewhat common in Pacific salmon "fresh silvers" that die in marine net pens, affecting 12% of the 437 Pacific salmon hearts examined from 2006 - 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

Literature Cited:

Lumsden, J.S., S. Russell, P. Huber, B.A. Wybourne, V.E. Ostland, M. Minamikawa, and H.W. Ferguson. 2008. An immune-complex glomerulonephritis of Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). J. Fish Dis. 31(12): 889-898.

Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology. For all cassettes, tissues were separated from the original (A)

cassettes and placed in separate (B) cassettes and gills were placed in separate (C) cassettes, for a total of 9 cassettes.

Slides 1A (Baxter 1), 2A (Warne 1?) and 3A (Warne 2?) - brain, liver, intestinal ceca, mesenteric adipose tissue; slide 2A also has intestine

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

Slide 2B (Warne 1?) - trunk kidney, heart (2 pieces), spleen (3 pieces)

Slide 3B (Warne 2?) - heart, spleen, trunk kidney, intestine

Slides 1C (Baxter 1), 2C (Warne 1?) and 3C (Warne 2?) - gill

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

Quality control: Liver autolysis: none (slides 2A, 3A), mild (slide 1B), moderate (slide 1A); intestinal ceca autolysis: none (slide 2A), mild (slide 3A), moderate (slide 1A). Large foci of erythrocytes (e.g., liver in slide 1B) 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
01/29/10 2:24 PM	Sea to Sky Veterinary Ser - e-mail	bc report generated
02/08/10 10:52 AM	Sea to Sky Veterinary Ser - e-mail	Case Invoiced

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

END OF REPORT

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Ministry of Agriculture and Lands

Animal Health Centre

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Final Report AHC Case: 10-965

Last Updated: 03/09/10 1:56 PM
Pathologist: Gary D. Marty
Received Date: 03/05/10
Collected Date:
Client Ref No: F-027

Submitter: **BC Centre for Aquatic Health**
Phone:
Fax:
Owner: **BC Centre Aquatic Hth**
Phone:
Fax:(250) 286-6103

Veterinarian:
Clinic:
Phone:
Fax:

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

Case History

Submitted formalized Chinook salmon tissue for Histopatholgy.

Saltwater. Cultured. Age: harvest time.

8 muscle samples for histo.

Final Diagnosis

1. Skeletal muscle, blood vessel: vasculitis, lymphocytic, segmental, focal, mild (slides 3, 4)

2. Skeletal muscle, interfascicular adipose tissue: steatitis, lymphocytic, focal, mild (slides 3, 4)

3. Skeletal muscle: myonecrosis, acute, bifocal (2 myofibres affected), mild (slide 5)

Final Comment: A few of these skeletal muscle samples have mild lesions, but none are of sufficient severity to have caused morbidity. Comments on specific lesions follow:

Vasculitis in Chinook salmon is most commonly associated with *Loma salmonae* infection, but affected vessels contain no obvious organisms.

Lymphocytic steatitis in the interfascicular connective tissue is evidence of chronic immune stimulation ; differentials include a low grade bacterial infection and reaction to a vaccine.

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

Histopathology

Formalin -fixed tissues from 8 fish were submitted in 8 cassettes for histopathology. Slide #s 1-8 are labeled in the same order as client #s 1 - 8

Organs included on all slides - skeletal muscle

All slides were examined; those not listed elsewhere have no significant lesions.

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

History of Communication

Date	To	Description
03/09/10 1:59 PM	BC Centre Aquatic Hth - e-mail	bc report generated
03/11/10 9:57 AM	BC Centre Aquatic Hth - e-mail	Case Invoiced

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

END OF REPORT

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Toll-Free : 1-800-661-9903

Final Report AHC Case: 10-1347

Last Updated: 04/06/10 3:08 PM
Pathologist: Gary D. Marty
Received Date: 04/01/10
Collected Date:
Client Ref No:

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

Submitter: **Sea to Sky Vet Service**
Phone:
Fax:
Owner: **Sea to Sky Veterinary Ser**
Phone:
Fax:(250) 287-2430

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

Case History

Submitted formalized Chinook tissue for Histopathology.

Age: 2009 entry. Average wt: 1.3kg. Site type - net pen.

Low overall mortality rate - mixed presentation in mortalities - several fish with jaundice and anemia. Few with signs of septicemia. In house lab findings.

Final Diagnosis

1a. Liver: hepatocellular fatty change (lipidosis), diffuse, severe (slides 1, 2)

1b. Liver: pericholangitis, lymphohistiocytic, multifocal, mild (slides 1, 2)

1c. Liver: hepatic necrosis, acute, multifocal, mild (slide 2)

2a. Trunk kidney: interstitial cell degeneration and necrosis, diffuse, moderate (slides 1, 2)

2b. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (slides 1, 2)

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

3. Spleen: parenchymal fibrin, multifocal, acute, with leukocytic karyorrhexis, moderate (slides 1, 2)

4. Heart: endocarditis, multifocal, lymphohistiocytic, mild (slide 2)

Final Comment: The clinical signs in these fish are similar to what is thought to be a viral infection in coho salmon cultured in Chile (Smith et al. 2006). However, microscopic features are variable: hepatic lipidosis and necrosis are similar to Smith et al. (2006), but these fish lack erythrophagocytosis and epicarditis described by Smith et al. (2006). Note, however, that Smith et al. provides no useful micrographs, and I don't know the skill level of their histopathology. Comments on specific lesions follow:

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

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.

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 uncommon in salmon that die in marine net pens, in 2009 affecting only one of the 72 Pacific salmon examined as part of the Province's Fish Health Auditing and Surveillance Program.

Necrotic hematopoietic cells in the renal interstitium have some form of nuclear degeneration (pyknosis, karyorrhexis, or karyolysis). This case might be a result of infection with bacteria (e.g., *Yersinia ruckeri*) or virus (e.g., VHSV or IHNV). This lesion is consistent with the clinical finding of anemia.

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

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

Multifocal fibrin deposits in the spleen are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin. I have also seen this response in salmon that are PCR positive for VHSV. Lack of remodelling of the fibrin is consistent with these deposits forming just before death. The presence of degenerating nuclei (karyorrhexis) in the spleen is evidence of increased cell turnover, possibly as part of an active inflammatory response. Consider bacteriology and virology, if not already done.

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

Literature cited:

Smith, P.A., J. Larenas, J Contreras, J. Cassigoli, C. Venegas, M.E. Rojas, A. Guajardo, S. Perez, and S. Diaz. 2006. Infectious haemolytic anaemia causes jaundice outbreaks in seawater-cultured coho salmon, *Oncorhynchus kisutch* (Walbaum). Journal of Fish Diseases [J. Fish Dis.]. 29(12):709-715.

Histopathology

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

Slide 1 (BAX) - liver, spleen, trunk kidney (2 pieces), brain, intestinal ceca, mesenteric adipose tissue

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

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

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

History of Communication

Date	To	Description
04/06/10 3:08 PM	Sea to Sky Veterinary Ser - e-mail	bc report generated
04/12/10 2:24 PM	Sea to Sky Veterinary Ser - e-mail	Case Invoiced

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

END OF REPORT

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Final Report AHC Case: 08-2143

Last Updated: 06/05/08 2:44 PM
Pathologist: Gary D. Marty
Received Date: 06/03/08
Collected Date: 06/03/08
Client Ref No: PO 2599BM

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

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

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

Case History

Submitted formalized tissue for histology.

Suspect low dissolved oxygen levels. Background BKD.

PO 2599BM

Final Diagnosis

1a. Liver: hepatic necrosis, acute, multifocal, moderate (slide 2A)

1b. Liver: sinusoidal congestion, multifocal, mild (slide 1A, one liver), moderate (slide 1A, one liver), severe (slides 1A, one liver; slide 2A)

1c. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, mild (slide 2A)

1d. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate (slide 2A)

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

2b. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (slide 2A)

3. Head kidney: interstitial vascular congestion, diffuse, moderate (slide 2A)

4. Brain: capillary (vascular) congestion, diffuse, mild (slide 2A, one brain), moderate (slide 1A; slide 2B, other brain)

5. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slide 1A), moderate (slide 2A)

Final Comment:

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*). Hypoxia is most likely in slide 2A (based on history and lack of organisms in section). 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 2007 affecting 11% of the 645 Atlantic salmon and 6% of the 119 Pacific salmon examined as part of the BC Fish Health Auditing and Surveillance Program.

Vascular congestion in the liver, kidney, and brain is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. I have not associated this change with hypoxia, but hypoxia could provide the stress needed to allow an infectious organism to replicate and cause disease. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. Severe congestion is rare in the liver of farmed Pacific salmon in BC; indeed, among the 310 Pacific salmon livers I have examined as part of the BC Fish Health Auditing and Surveillance Program since January 2006, none have had severe congestion, and only 6 fish have had moderate congestion. Consider bacteriology and PCR for VHSV and IHNV.

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

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These types of vacuoles were rare before 2007 and probably are a result of the significant increase in the proportion of plant-based components in commercial feeds that occurred in 2007. The vacuoles probably are normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown.

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).

Histopathology

Formalin -fixed tissues were submitted for histopathology. The gills were not decalcified before being processed routinely into paraffin.

Slide 1A - liver, brain, spleen, trunk kidney

Slide 1B - gill (several filaments)

Slide 2A - liver, brain, spleen, head kidney, trunk kidney

Slide 2B - gill (several filaments)

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

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

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

END OF REPORT

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Final Report AHC Case: 08-3183

Last Updated: 08/20/08 4:58 PM
Pathologist: Gary D. Marty
Received Date: 08/18/08
Collected Date: 08/18/08
Client Ref No:

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

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

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

Case History

Submitted formalized fish tissue for histology.

Fish are seen with skin ulcerations ranging from mild to severe. Vaccinated with Vibrogen 2 IP. BKD treatment (OTC) July 1-14, 2008.

Coho. Saltwater. Vaccinated.

Final Diagnosis

1. Skin: subdermal granulation tissue, focal, subacute, severe (slide 3A)

2. Adipose tissue adjacent to skeletal muscle: steatitis, granulomatous, lymphoplasmacytic, diffuse, moderate (slide 5A), with superficial fibrin, severe (slide 4A)

3a. Mesenteric adipose tissue: peritonitis, granulomatous, focal, with an intralesional vacuole about 60 µm in diameter, mild (slide 1A)

3b. Mesenteric adipose tissue: peritonitis, granulomatous, multifocal, with intralesional vacuoles about 60 µm in diameter, mild (slide 5A), moderate (slide 4A)

4. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1A, 2A, 4A, 5A)

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

6a. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slide 3A), moderate (slides 2A, 3A)

6b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 3A)

6c. Spleen: peritonitis, granulomatous, focal, with occasional fine fibrocellular fronds, mild (slide 3A)

Final Comment: The most significant lesions in these fish are consistent with a vaccine reaction, but in my experience ulcers are unusual

manifestation of a vaccine reaction. Were the skin lesions at vaccine sites? Is this vaccine different is some way? Ulcers are more commonly associated with filamentous bacteria (e.g., *Tenacibaculum maritimum*). The sections have no evidence of filamentous bacteria. Either bacteria were lost during processing, or bacteria were not involved in development of the lesion. Bacteria-associated ulcers tend to develop when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection). Comments on specific lesions follow:

The granulomatous inflammation in the adipose tissue (subdermal or retroperitoneal?) adjacent to the skeletal muscle in slide 4A is 1 mm thick. In slide 5A, the inflammation involves adipose tissue on both sides of the skeletal muscle (and is <0.5 mm thick). The pattern of inflammation in both slides is more consistent with an ulcer or vaccine reaction than with a reaction to *Renibacterium salmoninarum*.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 71% of 1115 Atlantic salmon fresh mortalities ("silvers") examined in 2006 and 2007 as part of the British Columbia Fish Health Auditing and Surveillance Program (44% were mild, 22% were moderate, and 5.0% were severe). Peritonitis is less common in Pacific salmon, in 2007 affecting only 14% of the spleen samples and 5.8% of the mesenteric adipose tissue samples. Vacuoles probably are a result of vaccine material lost during tissue processing (Mutoloki et al. 2004).

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

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

The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish. Small amounts of splenic lipofuscin are fairly common in pen-reared Chinook salmon. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Literature cited:

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

Histopathology

Formalin-fixed tissues were submitted in 5 cassettes for histopathology. For all cassettes, gills were removed from the original (A) cassette and placed in separate (B) cassettes. Writing on the original cassettes dissolved during processing and was unreadable when tissues were embedded in paraffin. Histocassettes must be labeled with a marker that will not dissolve in water, alcohol, or xylene. Pencil works well, but can smudge. We prefer STATMARK PENS [available from VerSci Inc. Their phone number is # 1-888-293-5127 and the fax is #1-519-780-0919. The address is 199 Victoria Rd South, Unit 5C, Guelph, Ontario, N1E 6T9. The catalogue number is VS-SMP. There are 12 pens/box and they are about $40/box.] When the pen is used, the mark must be completely dry before the cassette is placed in fluid (i.e., sometimes pencil is the best alternative under field conditions).

Slides 1A, 2A, and 5A - spleen, brain, liver, trunk kidney, skeletal muscle, intestinal ceca, mesenteric adipose tissue

Slide 3A - spleen, brain, liver, skeletal muscle, intestinal ceca, mesenteric adipose tissue

Slide 4A - spleen, brain, liver, trunk kidney, skeletal muscle, stomach, intestinal ceca, mesenteric adipose tissue

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

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

Quality control: Liver autolysis: mild (slides 1A, 2A, 3A, 4A, 5A). Large foci of erythrocytes (e.g., spleen in slide 2A) have precipitates of acid hematin. Acid hematin accumulates when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue). Organs have no postfixation dehydration.

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

END OF REPORT

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Final Report AHC Case: 08-3664

Last Updated: 09/29/08 3:49 PM
Pathologist: Gary D. Marty
Received Date: 09/23/08
Collected Date: 09/23/08
Client Ref No:

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

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

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

Case History

Submitted formalized tissue for histology. External lesions present. Range from mild to severe.

Previous case = 2008-3183.

Final Diagnosis

[Note: because slides each contain tissues from two fish, some diagnoses have two levels of severity; in such cases, one fish had one level of severity, and the other fish had the other level of severity]

1a. Skin: dermatitis, ulcerative, focal, dissecting, with superficial fibrin and peripheral histiocytic inflammation, severe (slide 5+6A)

1b. Skin: dermatitis, ulcerative, diffuse, severe, with scattered superficial filamentous bacteria (slides 1+2A, 7+8A, 9+10A, 11+12A)

1c. Skin: panniculitis, superficial, histiocytic, focal, moderate (slide 3+4A)

2a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 7+8A), moderate (slides 1+2A, 3+4A, 7+8A, 11+12A)

2b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slides 3+4A, 7+8A, 11+12A)

2c. Liver: peritonitis, chronic, diffuse, with fibrocellular fronds, moderate (slide 5+6A)

3a. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slides 1+2A, 7+8A), moderate (slides 3+4A, 5+6A, 9+10A, 11+12A)

3b. Spleen (and mesenteric adipose tissue): peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1+2A, 3+4A, 7+8A), moderate (slide 5+6A)

4. Intestinal ceca: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 3+4A, 5+6A, 7+8A)

5a. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (slides 3+4A, 5+6A)

5b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 1+2A, 5+6A, 7+8A, 11+12A)

5c. Trunk kidney: renal tubular mineralization, multifocal, mild (slide 5+6A)

5d. Trunk kidney: renal tubular casts of protein and yellow-brown pigment, with tubular epithelial degeneration and regeneration, multifocal, mild (slide 5+6A)

6a. Brain: meningitis, lymphoplasmacytic, with eosinophilic granular cells, focal, mild (slide 1+2A)

6b. Brain: capillary (vascular) congestion, diffuse, mild (slide 3+4A), moderate (slides 5+6B, 11+12A)

7. Gill, filament vessel: branchial thrombosis, focal, occlusive, moderate (slide 5+6A)

Final Comment: Consistent with clinical findings, the most significant lesions in these fish are in the skin. Many of the samples contain parts of the reaction, but most parts seem to be present in slide 5+6A. Inflammation dissects peripherally from the margins of the ulcer, with a band of macrophages/melanomacrophages extending peripherally along the margin of the deep dermis and superficial panniculus. This type of ulcer is not common in the fish I examine. Consider the possibility that part of the pathogenesis involves an adverse reaction to a vaccine. Has this vaccine been used before without these problems? Other things that can exacerbate ulcers include filamentous bacteria (*Tenacibaculum maritimum* is likely) and stress (e.g., crowding, suboptimal water quality, or other infection). Specific comments on other lesions follow:

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

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These types of vacuoles were rare before 2007 and might be related to the significant increase in the proportion of plant-based components in commercial feeds that has occurred since 2006. The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown.

The golden pigment in the spleen and kidney most likely is lipofuscin. Accumulation of lipofuscin is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish (and uncommon in younger fish, especially moderate accumulations). Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

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

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

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

Renal mineralization is common in cultured fish species. The lesion is not considered fatal, although feed conversion may be adversely affected. The pathogenesis is not fully understood, but renal mineralization has been experimentally reproduced through high carbon dioxide levels, magnesium deficiency, selenium toxicity, and a diet low in minerals (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Clinically, renal mineralization is most commonly associated with high carbon dioxide levels.

Meningitis is evidence of immune stimulation; differentials include viruses, bacteria, or parasites (e.g. in Pacific salmon, cases of *Loma salmonae* in which the organisms are not seen in the section examined). The section from the affected fish has no obvious organisms.

Congestion of brain capillaries, including the meninges, is nonspecific evidence of circulating vasodilators or a mass-occupying intracranial lesion. Capillary congestion can be associated with bacterial infections (e.g., mouthrot in smolts), but it also results when venous return is blocked (e.g., with thrombi and massive intracranial hemorrhage or inflammation). Congestion of brain capillaries is not common with VHSV. [The neuropil normally contains a rich network of capillaries, but in any given section, the majority of capillaries contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section contain erythrocytes.] Congestion in slide 11+12A is unusual in that the affected vessels also contain large numbers of immature white blood cells.

Thrombosis in the gill is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection; the section has no obvious organisms, but the skin ulcer might have led to changes in homeostasis that led to the thrombus.

Histopathology

Formalin-fixed tissues were submitted in 6 cassettes for histopathology. Brains and gills were removed from the original (A) cassettes and placed in separate (B) and (C) cassettes prior to processing into paraffin. The gills were decalcified in Protocol B (hydrochloric acid solution) for about 2 hours before being rinsed in water and processed routinely with the other tissues into paraffin.

Slide 1+2A (Ahlstrom Fish 1+2), Slide 3+4A (Ahlstrom Fish 3+4), Slide 5+6A (Ahlstrom Fish 5+6), Slide 7+8A (Ahlstrom Fish 7+8) and Slide 9+10A (Ahlstrom Fish 9+10) - spleen (2 pieces), liver (2 pieces), intestinal ceca, trunk kidney (2 pieces), skin/skeletal muscle (2 pieces)

Slide 11+12A (Ahlstrom Fish 11+12) - spleen, liver (2 pieces), intestinal ceca, head kidney, trunk kidney, skin/skeletal muscle (2 pieces)

Slide 1+2B (Ahlstrom Fish 1+2), Slide 3+4B (Ahlstrom Fish 3+4), Slide 5+6B (Ahlstrom Fish 5+6), Slide 7+8B (Ahlstrom Fish 7+8), and Slide 11+12 B (Ahlstrom Fish 11+12) - brain

Slide 9+10B (Ahlstrom Fish 9+10) - brain and intestinal ceca

Slide 1+2C (Ahlstrom Fish 1+2), Slide 3+4C (Ahlstrom Fish 3+4), Slide 5+6C (Ahlstrom Fish 5+6), Slide 7+8C (Ahlstrom Fish 7+8), Slide 9+10C (Ahlstrom Fish 9+10) and Slide 11+12C (Ahlstrom Fish 11+12) - gill

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

Quality control: Liver autolysis: mild (slides 3+4A, 5+6A, 7+8A, 11+12A), moderate (slide 1+2A, 9+10A), or severe (slide 5+6A). Large foci of erythrocytes (e.g., spleen in slide 7+8A) have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have no postfixation dehydration.

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

END OF REPORT

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Final Report AHC Case: 08-3826

Last Updated: 10/07/08 2:32 PM
Pathologist: Gary D. Marty
Received Date: 10/03/08
Collected Date: 10/03/08
Client Ref No:

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

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

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

Case History

Submitted five coho fish for histology exam.

Coho with severe skin lesions caused by Vibrio parahemolyticus. 0.1% mortality/day. Currently on Florfenicol treatment.

Saltwater. Vaccinated. Age: 400 g.

Final Diagnosis

1a. Liver: sinusoidal fibrin, multifocal, acute, mild (slide 1A)

1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 3A), moderate (slides 1A, 4A, 5A)

1c. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate (slide 2A)

1d. Liver: hepatitis, lymphocytic, perivascular, focal, mild (slide 2A)

1e. Liver: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 4A)

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

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

4. Intestinal ceca: peritonitis, chronic, multifocal, with fibrocellular fronds, mild (slides 4A, 5A)

5a. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (slides 3A, 4A)

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

6. Brain: capillary (vascular) congestion, diffuse, mild (slide 5A)

Final Comment: These fish have many of the same lesions described for the previous case (2008-3664). Most of the lesions are consistent

with ongoing inflammation related to skin ulcers or a vaccine. Comments on specific lesions follow:

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

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

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These types of vacuoles were rare before 2007 and might be related to the significant increase in the proportion of plant-based components in commercial feeds that has occurred since 2006. The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown.

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

The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish (and uncommon in younger fish, especially moderate accumulations). Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

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

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

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

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

Histopathology

Formalin-fixed tissues from 5 Coho salmon were received for histopathology. After processing into paraffin, gills were removed from the original (A) cassettes and placed in separate (B) cassettes.

Slide 1A and Slide 3A - brain, spleen, liver, trunk kidney (2 pieces), intestinal ceca, mesenteric adipose tissue

Slide 2A - spleen, liver (2 pieces), trunk kidney (2 pieces), intestinal ceca, mesenteric adipose tissue

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

Slide 5A - brain, spleen, liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

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

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

Quality control: Liver autolysis: mild (slides 1A, 2A, 3A, 4A), moderate (slide 5A), or severe (slide). Gill autolysis: mild (slides 2B, 3B), moderate (slide 4B), severe (slide 1B). The gills were not decalcified, but mineralized filaments are thin and contributed little to sectioning artifact. Large foci of erythrocytes (e.g., spleen in slide 3A) have precipitates of acid hematin. Acid hematin accumulates as brown birefringent

deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have no postfixation dehydration.

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

END OF REPORT

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Final Report AHC Case: 09-2876

Last Updated: 07/31/09 5:24 PM
Pathologist: Gary D. Marty
Received Date: 07/29/09
Collected Date:
Client Ref No: 09-2716

Veterinarian: **Tim Hewison**
Clinic: **Microtek International In**
Phone: (250) 652-4482
Fax: (250) 652-4802

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

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

Case History

Submitted 4 formalized Coho samples for histopathology.

Please find enclosed tissue samples taken from 4 Coho salmon for histopathology testing. The samples were preserved in a 10% formalin buffered solution then transferred to tap water for transportation. Please reference case file 9-2716 on all reports and invoices. if you require any further information please contact me either by phone (250-652-4482 ext 201) or e-mail (thewisonatmicrotek-intl.com).

Final Diagnosis

1. Skin: dermatitis, ulcerative, diffuse, severe, with small (slide 4) to moderate (slide 1) numbers of plump bacterial rods

2. Skin: dermatitis, ulcerative, diffuse, severe, neutrophilic, lymphocytic, with small numbers of bacterial rods (slide 2)

3. Skin: dermatitis, ulcerative, diffuse, severe, with small numbers of bacterial rods (slide 3)

4. Skeletal muscle: myonecrosis, acute, multifocal, mild (slide 2)

Final Comment: Microscopic examination confirms the gross finding of cutaneous ulcers, and bacterial rods consistent with a *Vibrio* or *Alivibrio* species invade into the underlying skeletal muscle. [The actual species of bacteria need to be confirmed by culture or PCR.] In my experience (mostly with Atlantic salmon), ulcers are more commonly filled with filamentous bacteria (*Tenacibaculum maritimum*).

Because the morphology of the bacteria in slides 1 and 4 is different from the bacteria in slides 2 and 3, consider the possibility that the bacteria are not the primary cause of the ulcers, but only opportunists colonizing ulcers caused by something else.

Myonecrosis in slide 2 is limited to scattered individual fibres with flocculent to hypereosinophilic cytoplasm and loss of cross striations. This pattern is consistent with capture myopathy or agonal contractions. It might also be related to the nearby ulcer, or be evidence of underlying deficiency of vitamin E or selenium (reference: Fish Pathology, 3rd Edition. 2001. R.J. Roberts).

Histopathology

Formalin-fixed skin and skeletal muscle from 4 coho salmon were submitted in 4 cassettes for histopathology (slide #s 1 - 4).

All tissues were examined. Tissues not listed elsewhere have no significant lesions.

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

History of Communication

Date	To	Description
07/31/09 5:24 PM	Hewison, Tim - e-mail	bc report generated
08/06/09 3:00 PM	Microtek International In - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-2932

Last Updated: 08/07/09 8:49 AM
Pathologist: Gary D. Marty
Received Date: 08/04/09
Collected Date: 07/23/09
Client Ref No:

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

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

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

Case History

Submitted formalized fish tissue for histopathology.

400000 in group. Smolt. Saltwater. Vaccinated - Vibiogen 2. Duration of illness - 1 week. 400k Coho smolts entered April 2009. Doing well till mid July. Affected fish off-feed and hanging in corners. "Blistering" followed by sloughing of skin. Virology/Bacteriology submitted to Microtek. Similar presentation as Ahlstrom Coho summer 08. Skin /skeletal muscle samples submitted through Microtek (Animal Health Centre case # 2009-2876).

Final Diagnosis

1. Liver: hepatocellular single cell necrosis, acute, multifocal, moderate (slide 1)

2. Spleen: peritonitis, chronic, multifocal, with fibrocellular fronds, mild (slide 2)

3. Mesenteric adipose tissue: steatitis and peritonitis, granulomatous, multifocal, mild (slides 2, 4)

4. Swimbladder: peritonitis, granulomatous, multifocal, moderate (slide 2)

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

4b. Trunk kidney: renal tubular mineralization, multifocal, mild (slide 3), moderate (slide 4)

5. Trunk kidney: interstitial cell hyperplasia, diffuse, moderate (slide 4)

Final Comment: These fish have several changes that are fairly common in cultured Pacific salmon that die in net pens in British Columbia. However, none significantly increase our understanding of the pathogenesis of the external ulcers. 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. This case has multiple foci of hepatocytes that have various degrees of pyknosis but minimal cytoplasmic hypereosinophilia. Most of the affected cells are separated from adjacent cells (loss of cell junctions) and rounded. 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 2008 affecting 10% of the 482 Atlantic salmon and 3.4% of the 118 Pacific salmon examined as part of the Province's Fish Health Auditing and Surveillance Program.

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

Renal tubular epithelial protein droplets 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 Pacific salmon (prevalence = 31% ; n = 118).

Renal mineralization is common in cultured fish species; when severe (not here), the condition is termed nephrocalcinosis. The lesion is not considered fatal, although feed conversion may be adversely affected. The pathogenesis is not fully understood, but renal mineralization has been experimentally reproduced through high carbon dioxide levels, magnesium deficiency, selenium toxicity, and a diet low in minerals (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Clinically, renal mineralization is most commonly associated with high carbon dioxide levels.

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).

Histopathology

Formalin-fixed tissues from 4 fish were submitted in 4 cassettes (all labelled 'Kun') for histopathology.

Organs included on most slides - gill, liver, spleen, trunk kidney; slides 1 and 2 also include intestinal ceca, mesenteric adipose tissue, and swimbladder

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

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

History of Communication

Date	To	Description
08/06/09 4:50 PM	Milligan, Barry - e-mail	bc report generated
08/12/09 10:00 AM	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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Final Report AHC Case: 09-3135

Last Updated: 08/20/09 4:27 PM
Pathologist: Gary D. Marty
Received Date: 08/19/09
Collected Date: 08/17/09
Client Ref No:

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

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

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

Case History

Submitted one formalized fish tissue for histopathology.

Smolt. Saltwater. # In group - 382,782, sick - 18, dead - 18. Duration of illness - 30 days +. # Submitted dead - 4. First sign - liquid under the skin, then open to the flesh. Have treated but increase of mortality.

Final Diagnosis

1a. Skin: dermatitis, ulcerative, with small numbers of filamentous and rod-shaped bacteria, dissecting, moderate (slide 3A), severe (slide 2A)

1b. Skin: panniculitis, diffuse, histiocytic, mild (slide 4A), with thrombosis of the subcuticular vessel on the lateral line, moderate (slide 3A)

2a. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate (slide 4A), abundant (slide 1A)

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

3. Intestine, lamina propria: vascular congestion, multifocal, mild (slide 1A), moderate (slide 2A)

4. Mesenteric adipose tissue: capillary congestion, multifocal, mild (slide 2A), moderate (slide 4A)

5. Abdominal mesenteries: peritonitis, chronic, focal to multifocal, with fibrocellular fronds, mild (slides 1A, 3A), moderate (slide 2A)

Final Comment: Lesions in the skin are consistent with the clinical description. The skin ulcer in slide 2A spans the entire dermis, and vascular congestion and small numbers of rod-shaped bacteria occur along the deep margin of the dermal collagen up to 3mm away from the perforated dermis. Filamentous bacteria (probably *Tenacibaculum maritimum*) are more numerous within 0.5 mm of the dermal perforation. Bacterial numbers are low in relation to the severity of lesions. The bacteria might be producing collagenolytic substances, or the ulceration might be partly or completely due to another cause (e.g., algal toxins in the water). A nutritional deficiency seems an unlikely differential.

In slide 3A, the ulcer is limited to the superficial dermis, but dissecting histiocytic inflammation along the deep margin of the dermis is more prominent than in slide 2A. The thrombus at the level of the lateral line is evidence that vascular lesions might be contributing to ulcer development.

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; vacuoles are more common in the summer. The increase in vacuole prevalence might be related to the increase in the proportion of plant-based components in commercial feeds that has occurred since 2006. The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown.

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

Distension of capillaries in the intestinal lamina propria is sometimes part of the inflammatory response to many infectious diseases; hemorrhage sometimes occurs in severe cases. Differentials include VHSV, a bacterial infection, an unknown vasodilator, or passive congestion (a postmortem change).

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

Peritonitis of the abdominal mesenteries is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 51% of the 470 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (36% were mild, 12% were moderate, and 3.2% were severe).

Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology. Prior to processing, gills were removed from the original (A) cassettes and placed in separate (G) cassettes. The gills were then immersed in Protocol B (hydrochloric acid solution) for 2.5 hours for decalcification before being rinsed in water and processed routinely into paraffin with the other cassettes.

Slide 1A (Kunechin 8-17-09) - brain, spleen, liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slides 2A (Kunechin 8-17-09) and 3A (Kunechin 8-17-09) - brain, spleen, liver, trunk kidney, stomach (slide 3A only), intestine, intestinal ceca, mesenteric adipose tissue, and skin/skeletal muscle

Slide 4A (Kunechin 8-17-09) - brain, spleen, liver, intestinal ceca, skin/skeletal muscle, mesenteric adipose tissue

Slides 1G, 2G, 3G and 4G (Kunechin 8-17-09) - gill

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

Quality control: Liver autolysis: mild (slides 1A, 3A, 4A), moderate (slide 2A). Large foci of erythrocytes (e.g., spleen in slide 2A) have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Decalcification is complete and differential staining is excellent. Organs have no postfixation dehydration.

Staff Comments:
Original submission form listed the species as Atlantic salmon, but Dr. Milligan informed Dr. Marty via a 2009-08-21 6:10 PM e-mail that the correct species is coho salmon.

History of Communication

Date	To	Description
08/24/09 10:53 AM	Milligan, Barry - e-mail	bc report generated
08/25/09 8:47 AM	Grieg Seafoods BC Ltd. - e-mail	bc report generated
08/25/09 1:51 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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Final Report AHC Case: 10-3488

Last Updated: 08/27/10 3:00 PM
Pathologist: Gary D. Marty
Received Date: 08/26/10
Collected Date: 08/25/10
Client Ref No: 10-96

Veterinarian: **Barry Milligan**
Clinic:
Phone:
Fax:

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

Animal Data
Species: Coho Salmon
Breed:
Sex:
Age: 3 Months
Premise ID:

Case History

Submitted formalized Coho salmon tissues for histopathology.

Bubbling under scales of fish leading to pen ulcers.

Environment: Saltwater. Vaccinated: Yes. Duration of illness: 1 month. Euthanized: Yes. Prior submission: Date fish died: Aug. 23/10.

Farm name: Newcombe point. Ref. Number: 10-96

Final Diagnosis

1. Skeletal muscle: myonecrosis, acute, with moderate numbers of superficial filamentous bacteria (*Tenacibaculum maritimum* ?), focal, severe (slide 3)

2. Skin/skeletal muscle: dermatitis, panniculitis, and superficial myositis, ulcerative, lymphohistiocytic, with separation at the margin of the dermis and subcutis, regionally diffuse, severe (slide 4)

3. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 3), moderate (slide 4)

4a. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (slide 4)

4b. Trunk kidney: interstitial granuloma, focal, with intralesional mineral, mild (slide 4)

Final Comment: The ulcers in these sections are consistent with the gross findings. Ulcers like these are notorious for their severity without obvious morphologic cause. The lesions in fish #2 (slide 3) contain filamentous bacteria (probably *Tenacibaculum maritimum*), but the lesions in fish #1 (slide 4) contain no obvious bacteria, although they were almost certainly colonized by one of more species of vibrios. Although the section of necrotic muscle in slide 3 has no overlying skin, I suspect that it was sampled from a deep ulcer in which the skin had already been completely denuded (i.e., a more severe form of the lesion in slide 4). In general, ulcers are associated with stressful conditions (crowding, handling, suboptimal water quality, etc.) or underlying disease. Other lesions in these fish are consistent with ulcers, but they do not provide clues about the cause of 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.

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).

The granuloma in the kidney is evidence of persistent antigen. It might be in response to the small focus of mineral, or it might be part of an aberrant vaccine reaction.

Histopathology

Formalin -fixed tissues were submitted in 4 cassettes for histopathology. Gills were immersed 1.5 h in Protocol B (hydrochloric acid solution) for decalcification and the rinsed in water before being processed with other cassettes into paraffin.

Slides 1 (Fish #2 gills) and 2 (Fish #1 gills) - gill

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

Slide 4 (Fish #1) - spleen, liver, trunk kidney, intestinal ceca, skin/skeletal muscle, mesenteric adipose tissue

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

Quality control: Liver autolysis: severe but readable (slides 3, 4). Organs have no postfixation dehydration and no acid hematin deposits.

History of Communication

Date	To	Description
08/27/10 3:01 PM	Milligan, Barry - e-mail	bc report generated
09/09/10 9:45 AM	Grieg Seafoods BC Ltd. - e-mail	Case Invoiced

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

END OF REPORT

BRITISH COLUMBIA
The Best Place on Earth

Ministry of Agriculture and Lands

Animal Health Centre

AAVLD - Accredited Laboratory

Ministry of Agriculture and Lands
1767 Angus Campbell Road
Abbotsford BC V3G 2M3
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Toll-Free : 1-800-661-9903

Final Report AHC Case: 08-3897

Last Updated: 10/10/08 8:50 AM
Pathologist: Gary D. Marty
Received Date: 10/09/08
Collected Date:
Client Ref No: 6905

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

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

Animal Data
Species: sockeye salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted fresh tissue for PCR for IHN and VHS.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: A Scouras on 10/10/08 @ 8:50 AM

Specimen	ID	Test	Result
Tissue	#6905	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: A Scouras on 10/10/08 @ 8:50 AM

Specimen	ID	Test	Result
Tissue	#6905	PCR - VHSV	Negative

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

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Final Report AHC Case: 09-1801

Last Updated: 05/12/09 9:47 AM
Pathologist: Gary D. Marty
Received Date: 05/11/09
Collected Date:
Client Ref No: 0865-3902

Veterinarian: **William Bennett**
Clinic: **Pacific Biological Statio**
Phone: (250) 756-7032
Fax: (250) 756-7053

Submitter: **W. R. Bennett**
Phone:
Fax:
Owner: **Bennett, William**
Phone:
Fax:(250) 756-7053

Animal Data
Species: sockeye salmon
Breed:
Sex:
Age: 4 Years
Premise ID:

Case History

Submitted Sockeye fish slide for histology.

Freshwater. No vaccinations. Euthanized - MS222. Small piece of brain tissue from returning adult sockeye.

Final Diagnosis

1. none

Final Comment: Severe artifact renders impossible diagnostic histopathology on this preparation.

Histopathology

One slide of paraffin-embedded sockeye salmon tissues was submitted for histopathology. The slide contains sections of eosinophilic material (brain, per contributor); one piece is about 1.5 × 0.75 mm, and the other piece is about 3 × 2 mm. The sections have no normal cellular structure consistent with brain. Instead, the sections are composed of sheets of eosinophilic material with a scale-like pattern. The scales are irregularly angular, varying from 5 - 10 µm wide and 7 - 15 µm long. Overlying and between the scales are moderate numbers of fine acid-hematin granules (they are birefringent under polarized light).

Quality control: Tissue preservation is poor for the entire section, with no recognizable nuclei. The nuclei might have been lysed as a result of immersion of the brain in fresh water (or some other nuclease) before fixation. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification).

History of Communication

Date	To	Description
05/12/09 9:51 AM	Pacific Biological Statio - e-mail	bc report generated
05/21/09 2:25 PM	Pacific Biological Statio - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-1802

Last Updated: 05/12/09 11:05 AM
Pathologist: Gary D. Marty
Received Date: 05/11/09
Collected Date:
Client Ref No: 0861-3120

Veterinarian: **William Bennett**
Clinic: **Pacific Biological Statio**
Phone: (250) 756-7032
Fax: (250) 756-7053

Submitter: **W. R. Bennett**
Phone:
Fax:
Owner: **Bennett, William**
Phone:
Fax:(250) 756-7053

Animal Data
Species: sockeye salmon
Breed:
Sex:
Age: 4 Years
Premise ID:

Case History

Submitted Sockeye fish slide for histology.

Freshwater. No vaccinations. Euthanized - MS222. Unstained slide for QA/QC. Please stain with H&E and compare with others (ie 0865 3902, 0927 2,4,8)

Final Diagnosis

1. none

Final Comment: Section thickness is excellent for paraffin-embedded tissues, and the H&E staining protocol used at the Animal Health Centre provides excellent differentiation of subtle details. I recommend trying the Animal Health Centre protocol on additional PBS sections. To start, stain adjacent serial sections: one with the current PBS protocol and one with the protocol (below) currently used at the Animal Health Centre.

Histopathology

One unstained slide of paraffin-embedded sockeye salmon tissues (gill and trunk kidney) was submitted for staining and quality control analysis.

Quality control: Tissue preservation is fair for both organs. The section is thin, providing excellent resolution that is particularly noticeable in the kidney. Moderate autolysis is associated with some loss of staining intensity. An area that demonstrates good differential eosin staining in the gill is encircled in green. Eosinophilic structures within and around the filament vessels are easily differentiated based on staining hue and intensity: homogeneous orange erythrocyte cytoplasm, dull orange smooth muscle cell cytoplasm, and peripheral red-orange collagen fibrils. Compare this staining with that of slide 09-27-2; in the centre of the sample from fish #3 is a section of pancreatic duct and arterioles adjacent to the wall of the stomach. Note that here, differentiation between erythrocytes, smooth muscle cells, and collagen is limited to structural differences; their staining hue and intensity are nearly identical.

In the kidney, a small artery and ganglion are fairly near the green dot. Erythrocytes, smooth muscle, and collagen can be differentiated based on hue and saturation, although the differentiation is not as good as in the gill. The pale wispy nature of the neuronal membrane is clearly differentiated from collagen of arterioles. Compare this with the nerve on the peripheral margin of the stomach of fish #3 (slide 09-27-2, near the green arrow). The hue of the nerve and adjacent smooth muscle cytoplasm is nearly the same; note also loss of nuclear differentiation

(compared with nuclei in the slide stained at the Animal Health Centre).

Staining protocol: We use commercial alcoholic eosin from Surgipath, and commercial Harris's Hematoxylin from VWR. The acid alcohol is a 0.5% HCl in 80% ethanol, and the base is 1.25% sodium bicarbonate in water. Staining is done on an automatic stainer.

Step	*Reagent*	*Time Min:Sec*	*Limit*	*Agitate*
1	Dry Storage	0:00	No Max.	None
2	Xylene	2:00	No Max.	None
3	Xylene	1:00	No Max.	None
4	Xylene	1:00	No Max.	None
5	100% Ethanol	1:00	No Max.	None
6	100% Ethanol	1:00	No Max.	None
7	100% Ethanol	1:00	No Max.	None
8	Wash	1:00	No Max.	None
9	Hematoxylin	4:15	Critical	None
10	Wash	1:00	No Max.	None
11	Acid Alcohol	0:15	Critical	None
12	Wash	1:00	No Max.	None
13	Bluing/Base	1:00	Standard	None
14	Wash	1:00	No Max.	None
15	95% Ethanol	2:00	Standard	None
16	Alcoholic Eosin	0:27	Standard	None
17	100% Ethanol	0:30	No Max.	None
18	100% Ethanol	0:30	No Max.	None
19	100% Ethanol	1:00	No Max.	None
20	Xylene	1:00	No Max.	None
21	Xylene	1:00	No Max.	None
22	Xylene	1:00	No Max.	None
23	Xylene	1:00	No Max.	None

History of Communication

Date	To	Description
05/12/09 11:08 AM	Pacific Biological Statio - e-mail	bc report generated
05/21/09 2:26 PM	Pacific Biological Statio - e-mail	Case Invoiced

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

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Toll-Free : 1-800-661-9903

Final Report AHC Case: 09-4176

Last Updated: 11/03/10 10:52 AM
Pathologist: Gary D. Marty
Received Date: 10/23/09
Collected Date:
Client Ref No: 09-75

Veterinarian: **William Bennett**
Clinic: **Pacific Biological Statio**
Phone: (250) 756-7032
Fax: (250) 756-7053

Submitter: **W. R. Bennett**
Phone:
Fax:
Owner: **Bennett, William**
Phone:
Fax:

Animal Data
Species: sockeye salmon
Breed:
Sex:
Age:
Premise ID:

Case History

Submitted 22 Sockeye Salmon (Oncorynchus nerka) slides for Histopathology.

Freshwater. Vaccinations: Unknown. # in group - 12.

22 slides - 11 H & E, 11 Gram.

Final Diagnosis

1. Brain: multiple foci of myxosporean spores that each contain two elongate polar capsules (*Myxobolus arcticus*?), 10 of 11 fish, small to moderate numbers

2. Brain hemorrhage: multifocal, 7 of 11 fish, mild to severe

Final Comment: The abnormalities observed grossly are probably a result of traumatic hemorrhage that occurred when fish were killed with a blow to the head. Blood seeping from vessels into meninges might appear grossly as red or pink masses. Sections have no evidence of the two primary differentials: neoplasia (cancer) or inflammation. The Gram stain on slide 3615 contains yellow to brown acid-hematin deposits and multiple foci of fine dark-blue stain precipitates, but no bacteria.

Ten of 11 brains contain clusters of myxosporean spores that are consistent with *Myxobolus arcticus*, a myxosporean common as an incidental finding in the brains of Pacific salmon. Most clusters of spores are limited to the brain stem. Because the spores are only rarely associated with inflammation, their main effect seems to be as a space-occupying lesion.

Histopathology

Slides from 11 blocks (2 sections per block: 1 each stained with H&E and a Gram stain) of sockeye salmon (*Oncorhynchus nerka*) were received from Bill Bennett from PBS-DFO on 26 October 2009. Microscopic findings were scored using criteria for farmed Pacific salmon examined as part of the BC Fish Health Auditing and Surveillance Program; several of the scored lesions did not occur in this group of fish.

Details for each fish are included on the spreadsheet that accompanies this report <2009-4176.xls>. Information provided by e-mail included the following table:

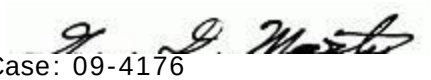
Slide #	Gross score	Preservation and notes	Source
3312	0	RNA later preserved: Clean	Harrison River
3602	1	Histology; R lobes with "nylons" and black dot attached to R	Horsefly Spawning Channel
3605	3	RNA later preserved: small pink mass at top of lobe L	Horsefly Spawning Channel
3608	3	Histology: L very sm. Pink; R pink mass not connected, Nylons	Horsefly Spawning Channel
3612	4	Histology: Haemorrhagic brain, pink mass in R	Horsefly Spawning Channel
3615	3	RNA later preserved: R lobe 1 med and 3 sm masses; bloody/spots outside	Horsefly Spawning Channel
3616	4	Histology; gelatinous texture; bloody/haemorrhagic; mass in R sm to med	Chilko River
3618	0	Histology; Clean	Chilko River
3620	3	Histology; Nylons both sides; outside mass on cerebrum	Chilko River
3622	0	Histology; clean	Chilko River
3623	2	RNA later preserved L major amounts of nylon and webbing	Chilko River
3632	4	Histology; haemorrhagic; mass inside R at centre lots of nylons in L	Chilko River

Quality control: Fixation with RNA later is fairly good; swollen erythrocyte nuclei are the most significant difference from tissues preserved immediately in 10% neutral buffered formalin. Large foci of erythrocytes (e.g., several foci in slide 3615 Gram stain) have precipitates of acid hematin. Acid hematin accumulates as yellow to brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have minimal postfixation dehydration.

Staff Comments:
The original final report (completed 10/27/09 3:30 PM) did not correctly report the prevalence of brain hemorrhage and myxosporeans from the spreadsheet. Those values are corrected in this revised final report; the original spreadsheet remains unchanged.

History of Communication

Date	To	Description
10/27/09 3:32 PM	Pacific Biological Statio - e-mail	bc report generated
11/04/09 3:27 PM	Pacific Biological Statio - e-mail	Case Invoiced
11/03/10 12:09 PM	Pacific Biological Statio - e-mail	bc report generated



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END OF REPORT