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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Final Report AHC Case: 09-24

Last Updated: 01/08/09 9:36 AM
Pathologist: Gary D. Marty
Received Date: 01/06/09
Collected Date: 12/29/08
Client Ref No: 7052

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

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

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

Case History

Submitted one Atlantic Salmon fresh tissue for PCR.

Older fish with hemorrhaging in muscle, body wall, swim bladder, p.c, liver. No mechanical damage. Vaccinated. Saltwater entry 07 S1.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/08/09 @ 9:36 AM

Specimen	ID	Test	Result
Tissue	org 7052-1	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/08/09 @ 9:36 AM

Specimen	ID	Test	Result
Tissue	org 7052-1	PCR - VHSV	Negative

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

END OF REPORT

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Final Report AHC Case: 09-25

Last Updated: 01/08/09 9:37 AM
Pathologist: Gary D. Marty
Received Date: 01/06/09
Collected Date: 12/17/08
Client Ref No: 7046

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

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

Case History

Submitted one fresh Atlantic Salmon tissue for PCR (IHN and VHS).

Mortality at site is low. One fish sampled with hem on pc and no other abnormalities. Vaccinated. Saltwater entry 2007 S0. Not euthanized (fresh morts).

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/08/09 @ 9:36 AM

Specimen	ID	Test	Result
Tissue	org 7046	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/08/09 @ 9:37 AM

Specimen	ID	Test	Result
Tissue	org 7046	PCR - VHSV	Negative

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

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Final Report AHC Case: 09-26

Last Updated: 01/09/09 1:30 PM
Pathologist: Gary D. Marty
Received Date: 01/06/09
Collected Date: 12/16/08
Client Ref No: 7043

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

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

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

Case History

Submitted one formalized Atlantic Salmon tissue for histopathology and one fresh tissue for PCR.

Mortality at site was higher than expected. Histo and tissues for PCR were collected from one fish with eye damage and hem on all organs.

Final Diagnosis

1. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, with attenuated epithelium, moderate

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

3. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate

4. Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, mild

5. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages, disseminated, with intracellular lipofuscin and hemosiderin, mild

Final Comment: This fish has microscopic lesions consistent with clinical findings. The eye lesions might have been the ultimate cause of death in this fish (eye was not included among the tissues submitted for histopathology). 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). Attenuated epithelium results when viable cells spread out to replace dead cells along the basement membrane. 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 exposure to toxins (e.g., bacterial toxins, algal toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin). PCR results rule out a common differential: VHSV.

Granulomas inflammation in the kidney is probably a result of exposure to persistent foreign material. A reaction to a vaccine seems most likely. Lack of organisms on the Gram stain makes bacteria less likely (e.g., *Renibacterium salmoninarum* or *Yersinia ruckeri*).

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

Sinusoidal congestion in the liver is evidence of circulating vasodilators. I have seen it associated with viral hemorrhagic septicemia virus and *Listonella anguillarum* infection. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. PCR results rule out VHSV and IHNV as potential causes. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).

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

Histopathology

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

Slide 1 (7043-1) - heart, spleen, liver, trunk kidney, head/trunk kidney transition, intestinal ceca, mesenteric adipose tissue

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

Quality control: Liver autolysis: none. Large foci of erythrocytes (e.g., spleen) have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/08/09 @ 9:37 AM

Specimen	ID	Test	Result
Tissue	org	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/08/09 @ 9:37 AM

Specimen	ID	Test	Result
Tissue	org	PCR - VHSV	Negative

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

END OF REPORT

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Final Report AHC Case: 09-109

Last Updated: 01/16/09 1:27 PM
Pathologist: Gary D. Marty
Received Date: 01/14/09
Collected Date: 01/07/09
Client Ref No: 7059

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

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

Case History

Submitted formalized Atlantic Salmon tissue for histopathology.

Fish presented with p.p hem in liver, p.c. Saltwater entry 07 S1. Vaccinated.

Final Diagnosis

1a. Liver: sinusoidal congestion, multifocal, moderate (slides 1, 2)

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

2. Skeletal muscle: myonecrosis, acute, multifocal, mild (slide 1)

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

Final Comment: These fish have lesions that are fairly common in Atlantic salmon that die in marine net pens in British Columbia. Although none of the lesions are of sufficient severity to have caused death, they provide clues about possible causes of death. Comments on specific lesions follow:

Sinusoidal congestion in the liver is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.

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.

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.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program

(42% were mild, 16% were moderate, and 2.6% were severe).

Histopathology

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

Slides 1 (7059-1) and 2 (7059-2) - heart, spleen, brain, liver, trunk kidney, skeletal muscle, intestinal ceca and mesenteric adipose tissue

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

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

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

END OF REPORT

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Final Report AHC Case: 09-110

Last Updated: 01/16/09 1:43 PM
Pathologist: Gary D. Marty
Received Date: 01/14/09
Collected Date: 11/20/08
Client Ref No: 6996

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

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

Case History

Submitted formalized Atlantic Salmon tissue for histopathology.

Gill with hem 2nd same internal hem. Lesions are similar to our case #6995 and 7057. Saltwater entry 06 S0. Vaccinated. Prior submission 08-4813. Fish died Nov 20, 2008.

Final Diagnosis

1. Gill: lamellar telangiectasis, multifocal, mild

Final Comment: Telangiectasis in the gill most commonly results from trauma (e.g., handling, or hitting something or another fish).

Histopathology

Formalin-fixed gill was submitted in 1 cassette for histopathology. The gill was immersed in Protocol B (hydrochloric acid solution) for about 2 hours for decalcification before being processed routinely into paraffin and sectioned onto a single slide: Slide 1 (6996-1).

Quality control: Gill autolysis: moderate. Decalcification is complete and differential staining is good. 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: 09-111

Last Updated: 01/16/09 3:41 PM
Pathologist: Gary D. Marty
Received Date: 01/14/09
Collected Date: 01/08/09
Client Ref No: 7057/6995

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

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

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

Case History

Submitted formalized Atlantic Salmon tissue for histopathology.

Fish 1 - had pp hem in liver, muscle, pc, swimbladder, and lesion. Fish 2 - had pp hem in liver and pc. 6995 Suspect transport damage as cause of hem in liver and pc but may be caused by agents? Saltwater entry 07 S1. Vaccinated. DOD - Jan 8/09

Final Diagnosis

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

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

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

1d. Liver: vasculitis, fibrinoid, multifocal, moderate (slide 2)

2. Mesenteric adipose tissue: capillary congestion, multifocal, mild (slide 2), moderate (slide 1)

3. Trunk kidney: renal tubular epithelial necrosis and regeneration, multifocal, subacute, mild (slide 2)

4. Heart: myocardial karyomegaly, multifocal, mild (slide 1)

Final Comment: Fish 6995-1 probably died of complications related to systemic inflammation; differentials include bacterial or viral infections. Microscopic lesions in fish 7057-1 are consistent with death due to an inflammatory process, but lesions are not as widespread or severe as fish 6995-1. Fish 6995-2 has only one mild lesion (not sufficient to explain morbidity). Comments on specific lesions follow:

In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria (e.g., *Listonella anguillarum*), and infection with VHSV, but often the cause remains unknown. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions might be remnants of

ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

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.

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

The walls of several medium -sized vessels in the liver of fish 6995-1 (slide 2) are distended by a homogenous pale eosinophilic material (protein). Thickened vessel walls might be a reaction to inflammatory mediators in the circulation. Fibrinoid vasculitis is an unusual lesion in the liver of pen-reared Atlantic salmon. Differentials include a bacterial or viral infection; the lesion is commonly associated with *Piscirickettsia salmonis* infection, but I don't see any organisms in the section.

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.

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

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.4% of the 1609 Atlantic salmon hearts examined as part of the province's Fish Health Auditing and Surveillance Program form 2006 through 2008). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Histopathology

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

Slide 1 (7057-1) - heart, brain, spleen, liver, head kidney, skeletal muscle, mesenteric adipose tissue

Slide 2 (6995-1) - heart, spleen, liver, trunk kidney, intestine, mesenteric adipose tissue

Slide 3 (6995-2) - spleen, liver, trunk kidney, intestine, mesenteric adipose tissue

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

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

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

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Final Report AHC Case: 09-112

Last Updated: 01/19/09 3:00 PM
Pathologist: Gary D. Marty
Received Date: 01/14/09
Collected Date:
Client Ref No: 7060/7061

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

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

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

Case History

Submitted formalized Atlantic Salmon tissue for histopathology.

Histo collected from recent salt water entry smolts. Mortality is increasing and there are many dark fish hanging along side of pen. Histo was collected over 2 days on 2 separate visits hence the 2 case numbers. Saltwater entry 2009 S0. DOD Jan 9 and Jan 12.

Final Diagnosis

The most significant lesions in these fish:

1. Eye, choroid plexus: vascular mineralization (confirmed with VonKossa stain), regionally diffuse, none (slide 0- 2B), severe (slide 0-1B)

2. Spleen: splenitis, granulomatous, multifocal, with intralesional vacuoles about 50 µm in diameter, moderate (slides 0- 2B, 1-1, 1-2, 1-4)

3. Intestinal ceca and stomach: peritonitis, granulomatous, multifocal, with intralesional vacuoles about 50 µm in diameter, moderate (slides 1-2, 1-4, 1-7, 1-9, 1-10), severe (slide 1-1)

4. Gill: lamellar hyperplasia and fusion, multifocal, moderate (slide 0-1A)

5. Gill lamellae: intravascular erythrophagocytosis and karyorrhexis, multifocal, mild (slide 0-2A), moderate (slide 0- 1A)

6a. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, moderate (slides 0-1B, 1-5)

6b. Trunk kidney: renal tubular mineralization, multifocal, with dilated tubules and tubular epithelial hyperplasia (nephrocalcinosis), moderate (slides 0-2B, 1-1, 1-3, 1-8, 1-9, 1- 10)

Final Comment: The most significant lesions in these fish include visceral granulomatous inflammation (probably a vaccine reaction) and renal mineralization (with mineralization affecting the choroid plexus in one fish). Renal tubular epithelial necrosis might also be significant, but it seems to be a secondary lesion associated with renal mineralization. Consider PCR for VHSV, if not already done. After fish are transferred to the marine environment, the severity of vaccine reactions and renal mineralization tends to lessen over time.

Lesion score details for this case are in an Excel spreadsheet (2009-0112.xls) that is not included with the official final report generated by the Animal Health Centre's VADDS database (the database cannot handle spreadsheets). Most of the lesions scored on the spreadsheet are the same as those included in the histopathology part of the BC Fish Health Auditing and Surveillance Program. Therefore, the spreadsheet includes a few lesions not present in this case. Specific comments on significant lesions in these fish follow:

I don't recall having ever seen a case of choroid plexus mineralization as severe as in fish 7060-1. It is probably a case of metastatic mineralization, resulting from abnormal calcium levels in the blood. It might have the same pathogenesis as nephrocalcinosis; lack of known correlation with choroid plexus mineralization might simply be a result of eyes not being routinely examined in nephrocalcinosis cases.

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).

Gill lamellar intravascular erythrophagocytosis and karyorrhexis is an unusual lesion in Atlantic salmon. It might be part of a aberrant reaction to a vaccine.

Splenitis and peritonitis is consistent with a reaction to foreign material. Splenic peritonitis is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe). Vacuoles are probably a result of vaccine material lost during tissue processing.

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 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).

For comments on other lesions, see the "Abbreviations" worksheet in the spreadsheet.

Histopathology

Formalin-fixed tissues from 12 fish were submitted in 14 cassettes for histopathology.

Slide #s 0-1A (7060-1) and 0-2A (7060-2) - gill

Slide #s 0-1B (7060-1) and 0-2B (7060-2) - eye, skin/skeletal muscle, trunk kidney, head kidney, heart, liver, spleen, intestinal ceca, and mesenteric adipose tissue

Slide #s 1-1 through 1-10 are labeled in the same order as client #s 7061-1 through 7061-10; organs included on most slides - trunk kidney, heart, liver, spleen, brain, stomach, intestinal ceca, and mesenteric adipose tissue

All organs were examined. A Von Kossa mineral stain was done on a section from the same block as slide 0-1B. Organs not listed elsewhere (e.g., brain) have no significant lesions.

Quality control: Details are included on the spreadsheet (2008-3592.xls). Autolysis varies from none to severe. Tissues have no postfixation dehydration and no acid hematin deposits. Mild artifact is normal for paraffin-embedded sections.

History of Communication

Date	To	Description
01/22/09 9:49 AM	Marine Harvest Canada - fax	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: 09-114

Last Updated: 02/05/09 2:06 PM
Pathologist: Gary D. Marty
Received Date: 01/14/09
Collected Date: 01/12/09
Client Ref No:

Veterinarian: **Dr. Peter McKenzie**
Clinic: **Mainstream Canada**
Phone: (250) 286-0022
Fax: (250) 286-0042

Submitter: **Phil Wiper - Mainstream**
Phone:
Fax:
Owner: **Cliff Bay**
Phone:
Fax:

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

Case History

Submitted Atlantic Salmon for bacteriology, virology, and PCR.

Fish have some visual problems as case 08-4481. Site was treated with Trib to which mortality dropped, but it has since increased again. One fish submitted from pens 1, 8, 9, 10, 11, 12, 14. Saltwater. Vaccinated. Prior submission 08-4481. DOD Jan 12/09

Necropsy

Seven Atlantic salmon were received in individual zip- seal bags in a Styrofoam cooler with two packs of gel ice and two packs of water ice (still mostly frozen). From each fish, the trunk kidney was aseptically sampled with a sterile plastic loop for bacterial culture. Samples from the trunk kidney and spleen were harvested from each fish for viral culture and PCR for VHSV and IHNV. Gross lesions for each fish:

Cliffe 1 - superficial ulcer on left flank, 2 × 5 cm

Cliffe 8 - both eyes are ruptured; yellow liver, pale gills

Cliffe 9 - deep ulcer on left caudal peduncle, 2 × 5 cm

Cliffe 10 - deep ulcer on right caudal peduncle, 2 × 7 cm

Cliffe 11 - superficial ulcer near left pectoral fin, 3 × 3 cm; tissues autolyzed

Cliffe 12 - multiple superficial ulcers on left and right sides, 1.5 × 1.5 to 2 × 3 cm

Cliffe 14 - both eyes are ruptured, pale gills; tissues autolyzed

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Erin Zabek on 01/21/09 @ 3:10 PM

Specimen	ID	Isolate	Result	Level
Kidney	#1		No Bacteria Isolated	
Kidney	#8	Yersinia ruckeri	Positive	few
Kidney	#9		No Bacteria Isolated	
Kidney	#10		No Bacteria Isolated	
Kidney	#11	Aliivibrio wodanis	Positive	few
**: Sensitivities are not available due to poor growth				
Kidney	#12	Aliivibrio wodanis	Positive	few
Kidney	#14	Yersinia ruckeri	Positive	few

Fish Resulted by: Jaime Osei-Appiah Verified by: Erin Zabek on 01/21/09 @ 3:10 PM

Organism	ID	e	ffc	sor	s3	sxt	ot
Yersinia ruckeri	#8	r	s	s	r	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline							

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/16/09 @ 1:38 PM

Specimen	ID	Test	Result
Kidney	#1	PCR - IHNV	Negative
Kidney	#8	PCR - IHNV	Negative
Kidney	#9	PCR - IHNV	Negative
Kidney	#10	PCR - IHNV	Negative
Kidney	#11	PCR - IHNV	Negative
Kidney	#12	PCR - IHNV	Negative
Kidney	#14	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/16/09 @ 1:38 PM

Specimen	ID	Test	Result
Kidney	#1	PCR - VHSV	Negative
Kidney	#8	PCR - VHSV	Negative
Kidney	#9	PCR - VHSV	Negative
Kidney	#10	PCR - VHSV	Negative
Kidney	#11	PCR - VHSV	Negative
Kidney	#12	PCR - VHSV	Negative
Kidney	#14	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 02/05/09 @ 2:06 PM

Specimen	ID	Isolate	Result	Level
Kidney	#1		No viruses isolated	
Kidney	#8		No viruses isolated	
Kidney	#9		No viruses isolated	

Kidney	#10	No viruses isolated
Kidney	#11	No viruses isolated
Kidney	#12	No viruses isolated
Kidney	#14	No viruses isolated

History of Communication

Date	To	Description
01/21/09 4:22 PM	-	bc report generated
01/22/09 8:49 AM	-	bc report generated
01/29/09 1:17 PM	-	bc report generated
01/30/09 12:00 PM	-	bc report generated
02/05/09 3:36 PM	Mainstream Canada - e-mail	bc report generated
02/10/09 1:21 PM	Mainstream Canada - e-mail	Case Invoiced

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

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

Final Report AHC Case: 09-149

Last Updated: 01/19/09 9:33 AM
Pathologist: Gary D. Marty
Received Date: 01/16/09
Collected Date: 01/14/09
Client Ref No: BI150109

Veterinarian: **Dr. Peter McKenzie**
Clinic: **Mainstream Canada**
Phone: (250) 286-0022
Fax: (250) 286-0042

Submitter: **Nathan Cassan - Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted one Atlantic Salmon plate for bacteriology.

Saltwater. Vaccinated. Lesions located near mid lateral line at or near caudal peduncle. 1 fish plated above tissues noted (brain and head kidney).

Farm "Burdwood" BI150109

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 01/19/09 @ 9:33 AM

Specimen	ID	Isolate	Result	Level
Isolate	Kidney		No Bacteria Isolated	
Isolate	Brain		No Bacteria Isolated	

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Final Report AHC Case: 09-269

Last Updated: 02/03/09 9:44 AM
Pathologist: Gary D. Marty
Received Date: 01/26/09
Collected Date: 01/23/09
Client Ref No: VP220109

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Nathan Cassan**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 3 fish bacti plates for bacteriology.

Saltwater. Vaccinated. Dead. Euthanized - "stunned". Plates derived from 3 fish for bacti. 1 plate w/3 lesions directly sampled each. 1 plate 2/fish #2&3 brain. 1 plate w/fish #1 brain and kidney.

Farm location - Venture Point.

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 02/03/09 @ 9:43 AM

Specimen	ID	Isolate	Result	Level
Isolate	Fish #1 Kidney and Brain		No Bacteria Isolated	
Isolate	Fish #2 Brain		No Bacteria Isolated	
Isolate	Fish #3 Brain		No Bacteria Isolated	
Isolate	Fish #1 Lesion	Vibrio tapetis	Positive	
Isolate	Fish #2 Lesion	Vibrio tapetis	Positive	
Isolate	Fish #2 Lesion	Pseudoalteromonas sp.	Positive	
Isolate	Fish #3 Lesion	Vibrio tapetis	Positive	

Fish Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 02/03/09 @ 9:44 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Pseudoalteromonas sp.	Fish #2 Lesion	s	s	s	s	s	s

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

Staff Comments:
No sensitivities on V.tapetis due to its slow growth.

History of Communication

Date	To	Description
02/03/09 9:44 AM	-	bc report generated
02/03/09 9:46 AM	-	bc report generated
02/03/09 9:58 AM	-	bc report generated
02/03/09 10:01 AM	Mainstream Canada - e-mail	bc report generated
02/03/09 10:01 AM	-	bc report generated
02/05/09 9:41 AM	Mainstream Canada - e-mail	Case Invoiced

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Final Report AHC Case: 09-270

Last Updated: 02/16/09 4:50 PM
Pathologist: Gary D. Marty
Received Date: 01/26/09
Collected Date: 01/23/09
Client Ref No: BI220109

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Nathan Cassan**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 1 fish bacti plates for bacteriology and fresh tissue (heart, spleen, and head kidney) for viral culture and PCR for VHS and IHN.

Saltwater. Vaccinated. Euthanized - "stunned". Follow up sampling to BI251108. 1 plate w/2 fish kidney for bacteriology. 2 whirls w/ above tissues noted for virology and PCR.

Farm location - Brent Island.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 02/12/09 @ 9:30 AM

Specimen	ID	Isolate	Result	Level
Isolate	Fish #1 Kidney	Psychrobacter sp.	Positive	
Isolate	Fish #2 Kidney	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. Due to poor growth antibiotic sensitivites were unable to be performed.

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 01/29/09 @ 9:55 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Psychrobacter sp.	Fish #1 Kidney	s	s	r	r	r	s

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

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/28/09 @ 4:24 PM

Specimen	ID	Test	Result
Tissue	Fish #1	PCR - IHNV	Negative
Tissue	Fish #2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/28/09 @ 4:24 PM

Specimen	ID	Test	Result
Tissue	Fish #1	PCR - VHSV	Negative
Tissue	Fish #2	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 02/16/09 @ 4:50 PM

Specimen	ID	Isolate	Result	Level
Tissue	Fish #1		No viruses isolated	
Tissue	Fish #2		No viruses isolated	

History of Communication

Date	To	Description
01/29/09 1:21 PM	-	bc report generated
01/30/09 2:05 PM	-	bc report generated
02/10/09 12:27 PM	Mainstream Canada - e-mail	bc report generated
02/17/09 1:16 PM	Mainstream Canada-T - fax	bc report generated
02/17/09 1:22 PM	Mainstream Canada - e-mail	bc report generated
02/18/09 9:10 AM	Mainstream Canada - e-mail	Case Invoiced

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Final Report AHC Case: 09-296

Last Updated: 01/31/09 12:20 PM
Pathologist: Gary D. Marty
Received Date: 01/28/09
Collected Date: 01/15/09
Client Ref No: 7074

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

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

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

Case History

Submitted fresh Atlantic Salmon kidney tissue for PCR for IHN and VHS.

Saltwater entry - 07S0. Vaccinated. Dead. Kidney slightly swollen with hem around sides.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/31/09 @ 12:19 PM

Specimen	ID	Test	Result
Tissue	#7074	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/31/09 @ 12:19 PM

Specimen	ID	Test	Result
Tissue	#7074	PCR - VHSV	Negative

History of Communication

Date	To	Description
01/31/09 12:46 PM	-	bc report generated
01/31/09 12:47 PM	-	bc report generated
01/31/09 12:51 PM	Marine Harvest Canada - fax	bc report generated
01/31/09 12:51 PM	-	bc report generated
02/02/09 9:19 AM	-	bc report generated
02/05/09 10:00 AM	Marine Harvest Canada - fax	Case Invoiced

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Final Report AHC Case: 09-297

Last Updated: 01/31/09 12:20 PM
Pathologist: Gary D. Marty
Received Date: 01/28/09
Collected Date: 01/19/09
Client Ref No: 7087

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

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

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

Case History

Submitted one fresh Atlantic Salmon tissue for IHN and VHS by PCR.

Saltwater entry 2008S1. Vaccinated. Fresh dead. Routine site visit during mort retrieval. Virology taken from one fish with hem in muscle 2nd reddening of swim bladder and edge of kidney.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/31/09 @ 12:20 PM

Specimen	ID	Test	Result
Tissue	#7087	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/31/09 @ 12:20 PM

Specimen	ID	Test	Result
Tissue	#7087	PCR - VHSV	Negative

History of Communication

Date	To	Description
01/31/09 12:48 PM	-	bc report generated
01/31/09 12:57 PM	Marine Harvest Canada - fax	bc report generated
02/02/09 9:26 AM	-	bc report generated
02/05/09 10:02 AM	Marine Harvest Canada - fax	Case Invoiced

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Final Report AHC Case: 09-298

Last Updated: 01/31/09 12:20 PM
Pathologist: Gary D. Marty
Received Date: 01/28/09
Collected Date: 01/15/09
Client Ref No: 7075

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

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

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

Case History

Submitted one formalized Atlantic Salmon tissue for histology, and one fresh tissue for IHNV and VHSV PCR.

Saltwater entry 2007S0. Vaccinated. Blood in swim bladder, stomach, with pale gills.

Final Diagnosis

1a. Liver: hepatic necrosis, acute, multifocal, moderate

1b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate

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

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

Final Comment: This fish probably died of complications related to hepatic necrosis. Hepatocellular necrosis can be caused by inadequate vascular perfusion (e.g., as occurs with harmful algal blooms or hypoxia) or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Renibacterium salmoninarum*, or *Piscirickettsia salmonis*); the cause is not determined in most cases. This case has no obvious organisms. Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Hepatic necrosis is somewhat common in salmon that die in marine net pens, 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.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). In Atlantic salmon livers sampled as part of the Province's Fish Health Auditing and Surveillance Program, prevalence of these vacuoles steadily increased from 42% in 2006 to 50% in 2007 and 55% in 2008; vacuoles 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.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460

Atlantic salmon fresh mortalities ("silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Histopathology

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/31/09 @ 12:20 PM

Specimen	ID	Test	Result
Tissue	#7075	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/31/09 @ 12:20 PM

Specimen	ID	Test	Result
Tissue	#7075	PCR - VHSV	Negative

History of Communication

Date	To	Description
01/28/09 2:18 PM	-	bc report generated
01/30/09 2:10 PM	-	bc report generated
01/31/09 12:49 PM	-	bc report generated
01/31/09 12:58 PM	Marine Harvest Canada - fax	bc report generated
02/02/09 9:09 AM	-	bc report generated
02/05/09 10:03 AM	Marine Harvest Canada - fax	Case Invoiced

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Final Report AHC Case: 09-299

Last Updated: 01/31/09 12:20 PM
Pathologist: Gary D. Marty
Received Date: 01/28/09
Collected Date: 01/27/09
Client Ref No: 7059

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

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

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

Case History

Submitted one fresh Atlantic Salmon tissue for PCR for IHN and VHS.

Saltwater entry 2007S1. Vaccinated. Fresh dead Fish with ham on liver and pyloric caeca.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/31/09 @ 12:20 PM

Specimen	ID	Test	Result
Tissue	#7059	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/31/09 @ 12:20 PM

Specimen	ID	Test	Result
Tissue	#7059	PCR - VHSV	Negative

History of Communication

Date	To	Description
01/29/09 10:04 AM	-	bc report generated
01/31/09 12:50 PM	-	bc report generated
01/31/09 12:58 PM	Marine Harvest Canada - fax	bc report generated
02/02/09 9:14 AM	-	bc report generated
02/05/09 10:04 AM	Marine Harvest Canada - fax	Case Invoiced

Gary D. Marty
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Final Report AHC Case: 09-329

Last Updated: 01/31/09 12:19 PM
Pathologist: Gary D. Marty
Received Date: 01/29/09
Collected Date: 01/27/09
Client Ref No: 12654

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted 2 fresh Salmon tissues (head kidney, liver, and spleen) for PCR.

125g. Saltwater. Vaccinated. 37000 in group.

Animal ID "Millar Channel"

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/31/09 @ 12:19 PM

Specimen	ID	Test	Result
Tissue	Pen#103	PCR - IHNV	Negative
Tissue	Pen#108	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 01/31/09 @ 12:19 PM

Specimen	ID	Test	Result
Tissue	Pen#103	PCR - VHSV	Negative
Tissue	Pen#108	PCR - VHSV	Positive

History of Communication

Date	To	Description
02/02/09 9:54 AM	Mainstream Canada-T - fax	bc report generated
02/02/09 9:58 AM	Mainstream Canada-T - fax	bc report generated
02/05/09 10:12 AM	Mainstream Canada-T - fax	Case Invoiced

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Final Report AHC Case: 09-342

Last Updated: 02/04/09 11:21 AM
Pathologist: Gary D. Marty
Received Date: 01/30/09
Collected Date:
Client Ref No: CH280109

Veterinarian: **Dr. Peter McKenzie**
Clinic: **Mainstream Canada**
Phone: (250) 286-0022
Fax: (250) 286-0042

Submitter: **Nathan Cassan**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 2 fish plates for bacteriology.

Saltwater. Vaccinated. 4 fish sampled, 4 lesions for bacteriology. 1 plate with fish #1-2 directly sampled fresh lesions and 2 plate with fish #3-4 directly sampled fresh lesions.

Cypress Harbour location.

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 02/04/09 @ 11:20 AM

Specimen	ID	Isolate	Result	Level
Isolate	Lesion Fish 1	Vibrio tapetis	Positive	
Isolate	Lesion Fish 2	Vibrio tapetis	Positive	
Isolate	Lesion Fish 3	Vibrio tapetis	Positive	
Isolate	Lesion Fish 4	Vibrio tapetis	Positive	

Fish Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 02/04/09 @ 11:21 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Vibrio tapetis	Lesion Fish 3	r	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
02/01/09 9:37 AM	-	bc report generated
02/06/09 10:38 AM	Mainstream Canada - e-mail	bc report generated

02/06/09 10:38 AM	-	bc report generated
02/10/09 1:44 PM	Mainstream Canada - e-mail	Case Invoiced

Gary D. Marty
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Final Report AHC Case: 09-362

Last Updated: 02/11/09 1:03 PM
Pathologist: Gary D. Marty
Received Date: 02/02/09
Collected Date: 01/29/09
Client Ref No: 12662

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted 5 Salmon plates for bacteriology.

65g - 85g. Saltwater. Vaccinated. Confirm or rule out Aeromonas salmonicida.

Animal location - Bawden Pt.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 02/11/09 @ 1:02 PM

Specimen	ID	Isolate	Result	Level
Isolate	Fish 1/2	Aliivibrio wodanis	Positive	
Isolate	Fish 3/4	Aliivibrio wodanis	Positive	
**: Due to poor growth antibiotic sensitivities were unable to be performed on this isolate.				
Isolate	Fish 7/8	Aliivibrio wodanis	Positive	
**: Due to poor growth antibiotic sensitivities were unable to be performed on this isolate.				
Isolate	Fish 11/12/13/14	Photobacterium sp.	Positive	
**: Due to poor growth antibiotic sensitivities were unable to be performed on this isolate.				
Isolate	Pen 103	Aeromonas salmonicida	Positive	

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 02/11/09 @ 1:03 PM

Organism	ID	e	ffc	sor	s3	sxt	ot
Aeromonas salmonicida	Pen 103	s	s	s	s	s	s
Aliivibrio wodanis	Fish 1/2	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
02/11/09 3:48 PM	Mainstream Canada-T - fax	bc report generated
02/11/09 3:49 PM	-	bc report generated
02/12/09 1:49 PM	Dr. Peter McKenzie - In Person	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: 09-363

Last Updated: 02/24/09 10:27 AM
Pathologist: Gary D. Marty
Received Date: 02/02/09
Collected Date: 01/29/09
Client Ref No: 12663

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted 8 Salmon for histopathology and viral culture.

1 kg. Saltwater. Vaccinated. Flesh missing to spine. Would like to rule out disease.

Animal location - Fortune Ch.

Final Diagnosis

Gross Diagnoses:

1. Caudal peduncle and caudal fin: dermatitis, cellulitis, myositis, and osteitis, necrotizing, severe (fish #s 1 ,2 ,3, 4)

2. Skin: dermatitis, ulcerative, with cellulitis and myositis, necrotizing, severe (fish #s 4, 5, 6, 7, 8, 9)

Comment: These fish probably died from complications related to filamentous bacteria infection of the skin and caudal fin. Underlying infection with other common fish pathogens is ruled out by lack of bacteria in the kidney and negative PCR results. Filamentous bacteria commonly invade skin ulcers. Once filamentous bacteria become established, the ulcers often get larger (as in this case). Enlargement of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection). Identification of the bacteria requires culture or PCR, but the Animal Health Centre does not maintain the specialized media needed to culture most filamentous bacteria. In saltwater, *Tenacibaculum maritimum* is likely.

Hemorrhage in the brain of two fish is evidence of trauma.

Necropsy

Nine dead Atlantic were received on ice in two plastic bags. One bag had 5 fish (arbitrarily numbered 1 - 5); the other had 4 fish (numbered 6 - 9). Measurements and observations on fish #s 1 - 5:

Total length - 42 - 47 mm

Body weight - ~1.0 - 1.5 Kg/fish

Caudal fin - almost completely eroded in fish #s 1 - 4; moderate fraying in #5

Other fin fraying - moderate, all fish

Fin base reddening - mild, all fish

Focal Skin Reddening - none, fish #s 1 - 3; severe, fish #s 4 (1-cm -diameter deep ulcers on left commissure of the mouth and just ventral to the left pectoral fin) and # 5 (0.5 × 1.0 shallow ulcers along left flank)

Brain reddening - none, fish #s 1, 3, 4; mild, fish #s 2 and 5

Mesenteric adipose tissue - none (fish #2), small amounts (fish #1), moderate amounts (fish # 3 and 5), and abundant (fish #4)

Other findings and tests:

Fish #s 6 - 9 - caudal fins intact, but each fish had deep ulcers varying from 3 to 6 cm in diameter.

All 9 fish - no ascites, no visceral petechiae, uniform hepatic colouration

Bacteriology - trunk kidney, fish #s 4, 5, 8, 9

Virology - pooled trunk kidney and spleen from fish #s 1 - 5

Histopathology - not done (due to 96-h postmortem interval)

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 02/04/09 @ 11:11 AM

Specimen	ID	Isolate	Result	Level
Kidney	4		No Bacteria Isolated	
Kidney	5		No Bacteria Isolated	
Kidney	8		No Bacteria Isolated	
Kidney	9		No Bacteria Isolated	

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/04/09 @ 11:20 AM

Specimen	ID	Test	Result
Tissue	sp,kd Fish 1-5	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/04/09 @ 11:20 AM

Specimen	ID	Test	Result
Tissue	sp,kd Fish 1-5	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 02/24/09 @ 10:27 AM

Specimen	ID	Isolate	Result	Level
Tissue	sp,kd Fish 1-5		No viruses isolated	

Staff Comments:
Interim report with final bacteriology and PCR results e-mailed to Zarah Vansnick and Peter McKenzie via Outlook; Wed. 04-02-2009, 3:03PM.

History of Communication

Date	To	Description
02/24/09 11:43 AM	Mainstream Canada-T - fax	bc report generated
02/26/09 11:55 AM	Mainstream Canada-T - fax	Case Invoiced

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

END OF REPORT

Case: 09-363

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Final Report AHC Case: 09-398

Last Updated: 02/07/09 11:53 AM
Pathologist: Gary D. Marty
Received Date: 02/05/09
Collected Date: 01/15/09
Client Ref No: 7107

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

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

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

Case History

Submitted fresh Atlantic Salmon tissue for PCR for IHN and VHS.

Saltwater entry - 07S0. Vaccinated. Hem in muscle.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/07/09 @ 11:53 AM

Specimen	ID	Test	Result
Tissue	Pen 10	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/07/09 @ 11:53 AM

Specimen	ID	Test	Result
Tissue	Pen 10	PCR - VHSV	Negative

History of Communication

Date	To	Description
02/07/09 1:16 PM	Marine Harvest Canada - fax	bc report generated
02/10/09 1:51 PM	Marine Harvest Canada - fax	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: 09-399

Last Updated: 02/07/09 11:54 AM
Pathologist: Gary D. Marty
Received Date: 02/05/09
Collected Date: 01/15/09
Client Ref No: 7102

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

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

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

Case History

Submitted fresh Atlantic Salmon tissue for PCR for IHN and VHS.

Saltwater entry - 07S1. Fish with signs of furunculosis, but no A. salmonicida isolated. Histo and tissues enclosed for PCR.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/07/09 @ 11:54 AM

Specimen	ID	Test	Result
Tissue	#7102	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/07/09 @ 11:54 AM

Specimen	ID	Test	Result
Tissue	#7102	PCR - VHSV	Negative

History of Communication

Date	To	Description
02/07/09 1:16 PM	Marine Harvest Canada - fax	bc report generated
02/07/09 1:16 PM	-	bc report generated
02/10/09 1:51 PM	Marine Harvest Canada - fax	Case Invoiced

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

Case: 09-399

END OF REPORT

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Toll-Free : 1-800-661-9903

Final Report AHC Case: 09-421

Last Updated: 02/11/09 12:54 PM
Pathologist: Gary D. Marty
Received Date: 02/05/09
Collected Date: 02/03/09
Client Ref No: 12667

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted one salmon plate for bacteriology.

Saltwater. 1300g. Vaccinated. Fish have been recently moved from one site to another using a pump. There have been predators on site.

Location - Fortune Channel.

Bacteriology

Aerobic Culture - Prod Resulted by: Sean Byrne Verified by: Sean Byrne on 02/11/09 @ 12:54 PM

Specimen	ID	Isolate	Result	Level
Isolate	1	Aliivibrio wodanis	Positive	
Isolate	2	Aliivibrio wodanis	Positive	
Isolate	3	Aliivibrio wodanis	Positive	
Isolate	4	Aliivibrio wodanis	Positive	

**: Due to poor growth antibiotic sensitivities were unable to be performed on this isolate.

History of Communication

Date	To	Description
02/11/09 3:58 PM	Mainstream Canada-T - fax	bc report generated
02/11/09 3:58 PM	-	bc report generated
02/12/09 1:54 PM	Mainstream Canada - 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: 09-425

Last Updated: 02/11/09 12:52 PM
Pathologist: Gary D. Marty
Received Date: 02/05/09
Collected Date: 02/03/09
Client Ref No: 12673

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted two salmon plates for bacteriology, 1 formalized sample for histopathology, and multiple tissues for PCR

Saltwater. 1800g. Vaccinated. Prior case 09-363.

Location - Fortune Channel.

Final Diagnosis

1a. Liver: hepatic necrosis, acute, focal, mild (slide 2)

1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1, 3, 4, 6, 8), moderate (slides 2, 5, 7)

1c. Liver: hepatocellular cytoplasmic lipid, diffuse, small amounts (slides 1, 2, 3, 4, 5, 7, 7)

2a. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slides 2, 6), moderate (slides 1, 5)

2b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 5)

3. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slide 5), moderate (slide 1)

4a. Heart: endocarditis, diffuse, with endothelial cell hypertrophy, mild (slides 2, 8), moderate (slide 4)

4b. Heart: endocarditis, multifocal, lymphoplasmacytic, mild (slide 5)

5. Intestine (surface of absorptive enterocytes): bacterial overgrowth, diffuse, moderate (slide 2)

6. Brain: encephalitis and ependymitis, regionally diffuse, with gliosis and intralesional parasites (uni- or bi- cellular, 2 - 4 µm in diameter; presporogonic myxosporean?), moderate (slide 3)

Final Comment: These fish have lesions in several organs, most of which could be related to external ulcers. Only the brain parasite (one fish only) was associated with significant lesions distinct from 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, but systemic changes associated with ulcers might have led to these lesions. 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.

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.

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.

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. The presence of moderate amounts in fish less than 1.5 Kg in unusual and probably related to the severe tissue damage associated with the large ulcers. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination, handling, or concurrent ulcers) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469).

Endothelial cell hypertrophy in the heart is consistent with a systemic immune stimulation, usually a result of a bacterial or viral infection (e.g., VHSV); the cause is often not determined, but in this case might be related to ulcers. Hypertrophic endothelial cells are basophilic and up to 10 µm thick.

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

The presence of bacteria on the surface of intestinal epithelial cells is evidence of bacterial overgrowth (bacteria are normally not seen on routine preparations of intestine from fish). The bacteria might have been producing toxins that adversely affected fish health. Bacterial overgrowth was probably also related to decreased intestinal motility and poor digestion.

The unclassified parasite in the brain of fish #3, tentatively classified as a presporogonic myxosporean, is associated with meningitis in Atlantic salmon. Organisms are most common in the cytoplasm of glial cells and ependymal cells, but they occasionally occur in the neuropil. In slide 3 they pack and distend the central canal of the proximal spinal cord. Organisms vary from 2.5 × 3 µm to 3 × 4 µm; they seem to be intracytoplasmic and unicellular, but some are either bi- or tri-nucleate or have deeply lobed nuclei. They have a central basophilic core (nucleus?) and thin hyaline capsule surrounded by a clear halo. They do not stain with ZN acid-fast or Twort's Gram stain. Some organisms seem to be surrounded by host nuclei, but I think this is just a function of the thick sections. Inflammation in other cases is common, varying from neutrophilic, to lymphocytic and granulomatous. The parasite is rare in farmed fish in British Columbia; although it kills a few fish at affected farms, it has not been associated with significant increases in mortality.

Histopathology

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

Slides 1 (Feb 3/09 Fortune 1), 2 (Feb 3/09 Fortune 2), 3 (Feb 3/09 Fortune 3), 4 (Feb 3/09 Fortune 4), 5 (Feb 3/09 Fortune 5) and 7 (Feb 3/09 Fortune 7) - brain (and brainstem or proximal spinal cord), heart, spleen, liver, trunk kidney, and intestinal ceca

Slide 6 (Feb 3/09 Fortune 6) - brain, heart, spleen, liver, head kidney, and intestinal ceca

Slide 8 (Feb 3/09 Fortune 8) - brain, heart, liver, trunk kidney, and intestinal ceca

All organs were examined. Organs not listed elsewhere have no significant lesions. Special stains applied to sections from the same block as slide 3 include ZN acid-fast and Twort's Gram stains.

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

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 02/11/09 @ 12:52 PM

Specimen	ID	Isolate	Result	Level
Isolate	Plate 1: #1,2,3		No Bacteria Isolated	
Isolate	Plate 2: #5,6,7,8		No Bacteria Isolated	
Isolate	Plate 1: #4	Aliivibrio wodanis	Positive	
**: Due to poor growth antibiotic sensitivities were unable to be performed on this isolate.				

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/07/09 @ 11:55 AM

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

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/07/09 @ 11:55 AM

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

Staff Comments:
PCR and histopathology results sent to Zarah Vansnick and Peter McKenzie via Outlook Mon. 09-02-2009 4:43PM.

History of Communication

Date	To	Description
02/11/09 4:04 PM	Mainstream Canada-T - fax	bc report generated
02/11/09 4:04 PM	-	bc report generated
02/12/09 1:59 PM	Mainstream Canada - 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: 09-426

Last Updated: 02/07/09 11:57 AM
Pathologist: Gary D. Marty
Received Date: 02/05/09
Collected Date: 02/03/09
Client Ref No: 12667

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted fresh salmon tissue for PCR.

Saltwater. 85g. Vaccinated. These fish are from the site that had wild Herring test positive for VHS. Prior case 09-330.

Location - Bowden Pt.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/07/09 @ 11:57 AM

Specimen	ID	Test	Result
Tissue	Pen 103	PCR - IHNV	Negative
Tissue	Pen 105	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/07/09 @ 11:57 AM

Specimen	ID	Test	Result
Tissue	Pen 103	PCR - VHSV	Negative
Tissue	Pen 105	PCR - VHSV	Negative

History of Communication

Date	To	Description
02/07/09 1:17 PM	-	bc report generated
02/07/09 1:28 PM	Mainstream Canada-T - fax	bc report generated
02/07/09 1:28 PM	-	bc report generated
02/10/09 1:52 PM	Mainstream Canada - e-mail	Case Invoiced

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

END OF REPORT

Case: 09-426

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Final Report AHC Case: 09-442

Last Updated: 03/04/09 4:29 PM
Pathologist: Gary D. Marty
Received Date: 02/06/09
Collected Date:
Client Ref No: WB 040209

Veterinarian: **Dr. Peter McKenzie**
Clinic: **Mainstream Canada**
Phone: (250) 286-0022
Fax: (250) 286-0042

Submitter: **Nathan Cassan**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 3 fresh salmon tissues for viral culture and PCR - VHS & IHN.

15 fish total sampled. 5 fish pooled each whirl. 3 whirls total for viral culture & PCR.

DOD: Feb 4, 09. Saltwater. Vaccinated. Euthanized: "bonked". Farm: Wehlis Bay.

Please expedite is possible as results are urgently required.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/07/09 @ 11:58 AM

Specimen	ID	Test	Result
Tissue	Fish 1-5	PCR - IHNV	Negative
Tissue	Fish 6-10	PCR - IHNV	Negative
Tissue	Fish 11-15	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/07/09 @ 11:58 AM

Specimen	ID	Test	Result
Tissue	Fish 1-5	PCR - VHSV	Negative
Tissue	Fish 6-10	PCR - VHSV	Negative
Tissue	Fish 11-15	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Cheryl Cecconi Verified by: Dr. J. Robinson on 03/04/09 @ 4:29 PM

Specimen	ID	Isolate	Result	Level
Tissue	Fish 1-5		No viruses isolated	
Tissue	Fish 6-10		No viruses isolated	
Tissue	Fish 11-15		No viruses isolated	

History of Communication

Date	To	Description
02/07/09 1:05 PM	Mainstream Canada - e-mail	bc report generated
02/07/09 1:06 PM	-	bc report generated
03/05/09 8:48 AM	Mainstream Canada - e-mail	bc report generated
03/10/09 10:25 AM	Mainstream Canada - 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: 09-455

Last Updated: 02/11/09 1:29 PM
Pathologist: Gary D. Marty
Received Date: 02/09/09
Collected Date:
Client Ref No: 7115

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

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

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

Case History

Submitted 10 formalized for histology and 4 fresh Salmon tissues for PCR (IHN and VHS) .

Saltwater entry Feb 4/09. Euthanized - TMS. Sudden die off at sea site - suspect non- smolt. Histo #6-10 and #16-20 were from affected/slow swimmers from pen #1 and pen #3. 4 samples for PCR to rule out viral agent.

Final Diagnosis

The most significant lesions in these fish:

1a. Eye, choroid plexus: vascular mineralization, diffuse along central margin, with intralesional yellow-brown pigment (probably lipofuscin), moderate (slide 16)

1a. Eye, choroid plexus: vascular eosinophilic material, multifocal along central margin, mild (slide 8)

2. Spleen: peritonitis, granulomatous, multifocal, with intralesional vacuoles about 50 µm in diameter, mild (16, 17), moderate (slides 8, 9), severe (slides 6, 7, 18)

3. Intestinal ceca and stomach: peritonitis, granulomatous, multifocal, with intralesional vacuoles about 50 µm in diameter, mild (slides 7, 20), moderate (slides 6, 10, 16, 17, 18), severe (slides 8, 9)

6a. Trunk kidney: renal tubular mineralization, multifocal, mild (slides 7, 8, 9, 16, 18, 19); with dilated tubules, moderate (slides 10, 20)

6b. Trunk kidney: tubular intracytoplasmic protein droplets , multifocal , mild (slides 6, 19, 17, 18), moderate (slides 7, 8, 9, 16, 19, 20)

Final Comment: The most significant lesions in these fish include visceral granulomatous inflammation (probably a vaccine reaction) and renal mineralization (with mineralization affecting the choroid plexus in two fish). After fish are transferred to the marine environment, the severity of vaccine reactions and renal mineralization tends to lessen over time. A high proportion of fish with moderate renal tubular epithelial protein droplets is consistent with stress associated with transfer from fresh to salt water.

Lesion score details for this case are in an Excel spreadsheet (2009-0455.xls) that is not included with the official final report generated by the Animal Health Centre's VADDS database (the database cannot handle spreadsheets). Most of the lesions scored on the spreadsheet are the same as those included in the histopathology part of the BC Fish Health Auditing and Surveillance Program. Therefore, the spreadsheet

includes a few lesions not present in this case. Specific comments on significant lesions in these fish follow:

Choroid plexus mineralization is similar to MH case 7060-1 (AHC case # 2009-0112). It is probably a case of metastatic mineralization, resulting from abnormal calcium levels in the blood. It might have the same pathogenesis as nephrocalcinosis ; lack of known correlation with choroid plexus mineralization might simply be a result of eyes not being routinely examined in nephrocalcinosis cases. Yellow-brown pigment with the lesion is evidence of chronic tissue damage. Eosinophilic material in fish #8 is in the same location as the yellow-brown pigment of fish #16; the material might be fibrin or necrotic endothelial cells (the Schmorl's stain rules out lipofuscin).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe). Vacuoles are probably a result of vaccine material lost during tissue processing.

Renal mineralization is common in cultured fish species; when moderate to 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, handling, or moving from fresh to salt water). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469) and Pacific salmon (prevalence = 31%; n = 118).

In wild juvenile pink salmon sampled in March 2008, they did not occur in fish sampled from fresh water (n = 20), but they occurred in half the fish sampled from salt water (n = 45): evidence of stress associated with the transition from fresh to salt water.

For comments on other lesions, see the "Abbreviations" worksheet in the spreadsheet.

Histopathology

Formalin-fixed tissues from 10 fish were submitted in 10 cassettes (with eyes separate) for histopathology. Most slides contain eye, brain, skin/skeletal muscle, trunk kidney, heart, liver, spleen, stomach, intestinal ceca, and mesenteric adipose tissue. Slide 17G contains sections of two gill arches from fish 17. A Schmorl's lipofuscin stain was done on a section from the same paraffin block as slide 8. All organs were examined. Organs not listed elsewhere in this report have no significant lesions.

Quality control: Details are included on the spreadsheet (2009-0455.xls). Intestinal autolysis varies from none to moderate, but autolysis in other organs is never more than mild. Large foci of erythrocytes (e.g., spleen in slide 17) 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). Tissues have no postfixation dehydration. Mild artifact is normal for paraffin-embedded sections.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/11/09 @ 12:43 PM

Specimen	ID	Test	Result
Tissue	#1-4	PCR - IHNV	Negative
Tissue	#5-8	PCR - IHNV	Negative
Tissue	#9-12	PCR - IHNV	Negative
Tissue	#13-16	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/11/09 @ 12:43 PM

Specimen	ID	Test	Result
Tissue	#1-4	PCR - VHSV	Negative
Tissue	#5-8	PCR - VHSV	Negative

Tissue	#9-12	PCR - VHSV	Negative
Tissue	#13-16	PCR - VHSV	Negative

History of Communication

Date	To	Description
02/11/09 1:33 PM	Marine Harvest Canada - fax	bc report generated
02/11/09 1:34 PM	-	bc report generated
02/11/09 1:35 PM	-	bc report generated
02/12/09 2:17 PM	Marine Harvest Canada - fax	Case Invoiced

Gary D. Marty
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Final Report AHC Case: 09-534

Last Updated: 02/18/09 10:18 AM
Pathologist: Gary D. Marty
Received Date: 02/16/09
Collected Date:
Client Ref No: 12694

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted one Atlantic Salmon bacti plate for Bacteriology. (confirm Aeromonas salmonicida).

90g. Saltwater. Vaccinated. 37000 in group. Prior submission 09-362. Small amount of hemorrhaging pyloric cecea.

Farm location - Bowden Pt.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Jaime Osei-Appiah on 02/18/09 @ 10:18 AM

Specimen	ID	Isolate	Result	Level
Isolate	Pen 105	Aeromonas salmonicida	Positive	

Fish Resulted by: Erin Zabek Verified by: Jaime Osei-Appiah on 02/18/09 @ 10:18 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Aeromonas salmonicida	Pen 105	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
02/19/09 3:51 PM	Mainstream Canada-T - fax	bc report generated
02/25/09 9:26 AM	Mainstream Canada - e-mail	Case Invoiced

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Final Report AHC Case: 09-535

Last Updated: 02/20/09 1:52 PM
Pathologist: Gary D. Marty
Received Date: 02/16/09
Collected Date:
Client Ref No: 12700

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted Atlantic Salmon tissue for PCR (confirm VHS).

170g. Saltwater. Vaccinated. DOD - Feb 12/09. Hemorrhaging -petechial - liver, cecea, and flesh.

Farm location: Ross Pass

Molecular Diagnostics

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/20/09 @ 1:52 PM

Specimen	ID	Test	Result
Tissue	mixed org	PCR - VHSV	Negative

History of Communication

Date	To	Description
02/23/09 8:51 AM	Mainstream Canada-T - fax	bc report generated
02/25/09 9:32 AM	Mainstream Canada-T - fax	Case Invoiced

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Final Report AHC Case: 09-554

Last Updated: 02/23/09 2:03 PM
Pathologist: Gary D. Marty
Received Date: 02/17/09
Collected Date: 02/11/09
Client Ref No: 7117

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

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

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

Case History

Submitted fresh and formalized salmon tissue for Histology and PCR (IHN and VHS)

Saltwater entry 09 S1. Vaccinated. Prior submission - yes 7115 (2009-0455) and 7114 (2009-0456). DOD - Feb 11/09. Sudden die off, 1 week since entry - suspected non-smolt. Viral sample pool 10 groups of 3. Histo 1-8 were from fresh mort (pen1, 2, 6, 3, 8, and 7).

Final Diagnosis

The most significant lesions in these fish:

1. Liver: hepatocellular hydropic degeneration, disseminated, acute, mild (slides 5, 8), moderate (slide 1)

2. Eye, periocular skin: dermatitis, ulcerative, focal, severe, with moderate numbers of filamentous bacteria (slides 4, 7)

3a. Spleen: splenitis, granulomatous, multifocal, with intralesional vacuoles 40 - 80 µm in diameter, severe (slide 7)

3b. Spleen: peritonitis, granulomatous, multifocal, with fibrocellular fronds, moderate (slide 5), of with intralesional vacuoles ~50 µm in diameter, moderate (slide 6)

4. Intestinal ceca and stomach: peritonitis, granulomatous, multifocal, with intralesional vacuoles about 50 µm in diameter, mild (slides 7, 20), moderate (slides 6, 10, 16, 17, 18), severe (slides 8, 9)

5a. Trunk kidney: renal tubular epithelial necrosis, focal, subacute, mild (slide 3)

5b. Trunk kidney: tubular intracytoplasmic protein droplets, multifocal, mild (slides 2, 3, 7, 8), moderate (slides 1, 5, 6)

Final Comment: Lesions in these fish are consistent with the clinical history of fish that did not transfer well to the marine environment. Significant lesions include periocular ulcers with filamentous bacteria, visceral granulomatous inflammation (probably a vaccine reaction), and degeneration of liver and kidney cells.

Lesion score details for this case are in an Excel spreadsheet (2009-0554.xls) that is not included with the official final report generated by the Animal Health Centre's VADDS database (the database cannot handle spreadsheets). Most of the lesions scored on the spreadsheet are the

same as those included in the histopathology part of the BC Fish Health Auditing and Surveillance Program. Therefore, the spreadsheet includes a few lesions not present in this case. Specific comments on significant lesions in these fish follow:

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 large foamy vacuoles.

Filamentous bacteria commonly invade skin ulcers. Once filamentous bacteria become established, the ulcers often get larger. Enlargement of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection). Identification of the bacteria requires culture or PCR. In saltwater, *Tenacibaculum maritimum* is likely.

Renal 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. PCR results in this case rule out a common differential, viral hemorrhagic septicemia virus (VHSV); other differentials include exposure to toxins (e.g., bacterial toxins, algal toxins, heavy metals, or aminoglycoside antibiotics such as gentamicin).

Granulomatous splenitis with associated with intralesional vacuoles is consistent with a reaction to foreign material; it is most likely a result of vaccination. Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe). Vacuoles are probably a result of vaccine material lost during tissue processing.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination, handling, or moving from fresh to salt water). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469) and Pacific salmon (prevalence = 31%; n = 118). In wild juvenile pink salmon sampled in March 2008, they did not occur in fish sampled from fresh water (n = 20), but they occurred in half the fish sampled from salt water (n = 45): evidence of stress associated with the transition from fresh to salt water.

For comments on other lesions, see the "Abbreviations" worksheet in the spreadsheet.

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 most slides - brain, heart, liver, spleen, trunk kidney, intestinal ceca, mesenteric adipose tissue, skin/skeletal muscle, and eye; some slides include stomach.

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

Quality control: Details are included on the spreadsheet (2009-0554.xls). Tissue preservation is very good (no more than mild hepatic autolysis) for all but one fish, which has severe autolysis. Large foci of erythrocytes (e.g., liver in slide 2) have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Tissues have no postfixation dehydration. Mild artifact is normal for paraffin-embedded sections.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/20/09 @ 1:50 PM

Specimen	ID	Test	Result
Tissue	Pen 4	PCR - IHNV	Negative
Tissue	Pen 3&4	PCR - IHNV	Negative
Tissue	Pen 3	PCR - IHNV	Negative
Tissue	Pen 3&5	PCR - IHNV	Negative

Tissue	Pen 1	PCR - IHNV	Negative
Tissue	Pen 1&2	PCR - IHNV	Negative
Tissue	Pen 2	PCR - IHNV	Negative
Tissue	Pen 2&6	PCR - IHNV	Negative
Tissue	Pen 6&7	PCR - IHNV	Negative
Tissue	Pen 7	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/20/09 @ 1:52 PM

Specimen	ID	Test	Result
Tissue	Pen 4	PCR - VHSV	Negative
Tissue	Pen 3&4	PCR - VHSV	Negative
Tissue	Pen 3	PCR - VHSV	Negative
Tissue	Pen 3&5	PCR - VHSV	Negative
Tissue	Pen 1	PCR - VHSV	Negative
Tissue	Pen 1&2	PCR - VHSV	Negative
Tissue	Pen 2	PCR - VHSV	Negative
Tissue	Pen 2&6	PCR - VHSV	Negative
Tissue	Pen 6&7	PCR - VHSV	Negative
Tissue	Pen 7	PCR - VHSV	Negative

History of Communication

Date	To	Description
02/23/09 2:06 PM	Marine Harvest Canada - fax	bc report generated
02/25/09 9:36 AM	Marine Harvest Canada - fax	Case Invoiced

Gary D. Marty
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Final Report AHC Case: 09-580

Last Updated: 02/23/09 4:20 PM
Pathologist: Gary D. Marty
Received Date: 02/18/09
Collected Date: 02/17/09
Client Ref No:

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

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

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

Case History

Submitted one formalized tissue for histopathology.

Saltwater. Smolt. Vaccinated. Smolts exhibiting severe "head rot". Some fish seen w/ external skin ulcers. No treatments.

Farm location - Concepcion

Final Diagnosis

1a. Trunk kidney: renal tubular epithelial necrosis, diffuse, with tubular dilation and interstitial fibrosis, subacute, severe (slide 4A)

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

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

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

3. Skin: dermatitis, ulcerative, diffuse, severe, with moderate numbers of filamentous bacteria (slides 1A, 4A)

4. Intestinal ceca: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, mild (slides 2A, 4A)

5. Spleen: peritonitis, lymphohistiocytic, focal, with occasional fine fibrocellular fronds, mild (slides 2A, 4A)

6. Gill: lamellar telangiectasis, multifocal, mild (slide 2B)

Final Comment: All fish have microscopic features that are common in fish with mouthrot. Renal tubular epithelial necrosis in slide 4A is more often associated with VHSV than with head ulcers; consider PCR for VHSV, if not already done. 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. Fibrosis is evidence of more extensive damage and efforts at repair. Causes in fish include viral hemorrhagic septicemia virus (VHSV), exposure to toxins (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin), or hypoxia followed by re-oxygenation. I have also seen this change in salmon exposed to high concentrations of chromium (Farag et al. 2006). Consider bacteriology and virology, if

not already done.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469) and Pacific salmon (prevalence = 31%; n = 118). In wild juvenile pink salmon sampled in March 2008, they did not occur in fish sampled from fresh water (n = 20), but they occurred in half the fish sampled from salt water (n = 45): evidence of stress associated with the transition from fresh to salt water.

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.

Filamentous bacteria commonly invade skin ulcers. Once filamentous bacteria become established, the ulcers often get larger. Enlargement of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection). Identification of the bacteria requires culture or PCR. In saltwater, *Tenacibaculum maritimum* is likely.

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

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

Literature cited:

Farag, A. M., T. May, G. D. Marty, M. Easton, D. D. Harper, E. E. Little, and L. Cleveland. 2006. The effect of chronic chromium exposure on the health of juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Aquatic Toxicology 76:246-257.

Histopathology

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

Slide 1A (Concepcion Feb 17/09) - brain (2 pieces), spleen, liver (2 pieces), trunk kidney, skin/skeletal muscle (2 pieces)

Slide 2A (Concepcion Feb 17/09) and 4A (Concepcion Feb 17/09) - brain (2 pieces), spleen (2 pieces), liver (2 pieces), intestine, trunk kidney (2 pieces), skin/skeletal muscle (2 pieces), mesenteric adipose tissue

Slide 3A (Concepcion Feb 17/09) - brain, liver (2 pieces), intestine, trunk kidney (2 pieces), skin/skeletal muscle (2 pieces)

Slides 1B (Concepcion Feb 17/09), 2B (Concepcion Feb 17/09), 3B (Concepcion Feb 17/09) and 4B (Concepcion Feb 17/09) - gill

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

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

History of Communication

Date	To	Description
02/23/09 4:23 PM	Grieg Seafoods BC Ltd. - e-mail	bc report generated
02/25/09 9:39 AM	Grieg Seafoods BC Ltd. - e-mail	Case Invoiced

Case: 09-580

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

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Final Report AHC Case: 09-581

Last Updated: 02/24/09 9:45 AM
Pathologist: Gary D. Marty
Received Date: 02/18/09
Collected Date: 02/13/09
Client Ref No:

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

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

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

Case History

Submitted formalized tissues in 3 cassettes for histopathology.

Saltwater. Smolt. Vaccinated. 1.3 kg (average). Severe skin ulcers - Pens with ulcers graded approx 1 month ago. No treatments.

Farm location - Bennett Pt.

Final Diagnosis

1. Skin: dermatitis, ulcerative, diffuse, severe, with moderate numbers of filamentous bacteria that extend into foci of deep myonecrosis (slides 2A, 3A), and with moderate numbers of superficial protozoa (slide 2A)

2a. Liver: hepatocellular hydropic degeneration, with rare single cell necrosis, disseminated, acute, moderate (slide 3A)

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

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

2d. Liver: hepatitis, pericholangial, perivascular, lymphohistiocytic, multifocal, mild (slide 1A)

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

4. Brain: meningitis, lymphohistiocytic, with fibroplasia, multifocal, moderate (slide 1A)

Final Comment: Most lesions in these fish are consistent with the clinical history of ulcers. Comments on specific lesions follow:

Filamentous bacteria commonly invade skin ulcers. Enlargement of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection). Identification of the bacteria requires culture or PCR, but in saltwater *Tenacibaculum maritimum* is likely. Protozoa on the surface of the ulcer are probably secondary invaders. In slide 1A, the surface of the dermis is only partly covered by epidermis; however, because the denuded surface has no filamentous bacteria, I am not able to say with confidence whether this is an ulcer or an artifact.

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 large foamy vacuoles. After hydropic degeneration can no longer be reversed, the changes are called single cell necrosis.

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

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

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

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469) and Pacific salmon (prevalence = 31%; n = 118). In wild juvenile pink salmon sampled in March 2008, they did not occur in fish sampled from fresh water (n = 20), but they occurred in half the fish sampled from salt water (n = 45): evidence of stress associated with the transition from fresh to salt water.

Cerebral meninges have several foci of diffuse thickening, with plump fibroblasts and scattered lymphocytes and histiocytes. Lack of organisms rules out common infectious causes of meningitis. The reaction might be a result of systemic change from inflammation focused elsewhere in the body.

Histopathology

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

Slide 1A (Bennett Pen 6 Feb 13/09), 2A (Bennett Pen 6 Feb 13/09), and 3A (Bennett Pen 6 Feb 13/09) ¿ brain, spleen, liver, intestinal ceca, trunk kidney, skin/skeletal muscle, mesenteric adipose tissue

Slides 1B (Bennett Pen 6 Feb 13/09), 2B (Bennett Pen 6 Feb 13/09), and 3B (Concepcion Bennett Pen 6 Feb 13/09) - gill

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

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

History of Communication

Date	To	Description
02/24/09 9:46 AM	Grieg Seafoods BC Ltd. - e-mail	bc report generated
02/25/09 9:39 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: 09-582

Last Updated: 02/24/09 11:23 AM
Pathologist: Gary D. Marty
Received Date: 02/18/09
Collected Date: 02/12/09
Client Ref No:

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

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

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

Case History

Submitted formalized tissues in 4 cassettes for histopathology.

Saltwater. Smolt. Vaccinated. Recent submissions from Lutes Cr. = 2009-0499/2009-0498. Severe "head rot" and external lesion. Numerous Tribrissen treatments.

Farm location - Lutes Creek.

Final Diagnosis

1. Skin and underlying skeletal muscle: dermatitis, ulcerative, diffuse, with deep myonecrosis and moderate numbers of superficial filamentous and rod-shaped bacteria, severe (slides 1A, 2A, 3A, 4A)

2a. Liver: hepatocellular hydropic degeneration, with rare single cell necrosis, disseminated, acute, mild (slide 3A)

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

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

3. Brain: meningitis, lymphohistiocytic, with fibroplasia, multifocal, moderate (slide 1A)

4. Intestinal ceca: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles up to 80 µm in diameter, mild (slide 4A), moderate (slide -1A)

5. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles 50 µm in diameter, mild (slide 3A)

6. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slides 1A, 2A, 4A), moderate (slide 3A)

Final Comment: Most of the lesions in these fish are consistent with death due to complications related to deep cutaneous ulcers. Large ulcers that involve skeletal muscle have a poor prognosis, despite treatment. Most fish also have significant peritoneal inflammation consistent with a vaccine reaction. Comments on specific lesions follow:

Filamentous bacteria commonly invade skin ulcers. Enlargement of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection). Identification of the bacteria requires culture or PCR, but in saltwater, *Tenacibaculum maritimum* is likely. The rod-shaped bacteria are probably a *Vibrio* or related species.

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 large foamy vacuoles. After hydropic degeneration can no longer be reversed, the changes are called single cell necrosis.

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

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

Cerebral meninges in slide 1A have several foci of diffuse thickening, with plump fibroblasts and scattered lymphocytes and histiocytes. Lack of organisms rules out common infectious causes of meningitis. The reaction might be a result of systemic change from inflammation focused elsewhere in the body.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting the spleen in 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe). Vacuoles are probably a result of vaccine material lost during tissue processing.

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). In wild juvenile pink salmon sampled in March 2008, protein droplets did not occur in fish sampled from fresh water (n = 20), but they occurred in half the fish sampled from salt water (n = 45): evidence of stress associated with the transition from fresh to salt water.

Histopathology

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

Slide 1A (Lutes Feb 12/09) - brain (2 pieces), spleen, liver, intestinal ceca, trunk kidney (2 pieces), skin/skeletal muscle (2 pieces), mesenteric adipose tissue

Slide 2A (Lutes Feb 12/09) and Slide 3A (Lutes Feb 12/09) - brain (2 pieces), spleen (2 pieces), liver (2 pieces), intestinal ceca, trunk kidney (2 pieces), skin/skeletal muscle (2 pieces), mesenteric adipose tissue

Slide 4A (Lutes Feb 12/09) - brain (2 pieces), spleen (2 pieces), liver (2 pieces), intestinal ceca, trunk kidney, skin/skeletal muscle (2 pieces), mesenteric adipose tissue

Slides 1B (Lutes Feb 12/09), 2B (Lutes Feb 12/09), 3B (Lutes Feb 12/09) and 4B (Lutes Feb 12/09) - gill

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

Quality control: Liver autolysis: mild (slide 4A), moderate (slide 3A), severe (slides 1A, 2A). Large foci of erythrocytes (e.g., liver 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.

History of Communication

Date	To	Description
02/24/09 11:24 AM	Grieg Seafoods BC Ltd. - e-mail	bc report generated
02/26/09 12:06 PM	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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Final Report AHC Case: 09-707

Last Updated: 03/03/09 4:21 PM
Pathologist: Gary D. Marty
Received Date: 02/26/09
Collected Date:
Client Ref No: 7127

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

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

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

Case History

Submitted fresh and formalized fish tissue for histopathology and PCR (IHN and VHS).

Saltwater entry 06 SO. Vaccinated. Fish # 1 septicemia with multiple hepatic cysts, #2 septicemia with one large cyst on heart.

Final Diagnosis

1a. Liver: sinusoidal congestion, diffuse severe, with acid hematin granules and small numbers of intracytoplasmic spherical amphophilic inclusions (slide 2)

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

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

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

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

2. Mesenteric adipose tissue: capillary congestion and hemorrhage, multifocal, moderate (slide 2)

3a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1)

3b. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slide 1)

Final Comment: Both fish have lesions that provide clues to their death, but not a specific cause. Whatever caused preductular biliary hyperplasia (fish 1) and hepatic sinusoidal congestion (fish 2) probably killed these fish. Comments on specific lesions follow:

In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria (e.g., *Listonella anguillarum*), and infection with VHSV, but often the cause remains unknown. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Because severe sinusoidal congestion is rare (affecting only 7 of 1674 Atlantic salmon in the BC Fish Health Auditing and Surveillance Program examined since 2006), consider adding PCR for ISAV. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high

mycotoxin concentrations (unpublished data). The amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin deposits in congested foci are evidence that the congested foci were acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins or inflammatory mediators) or come from outside the fish (e.g., from the water or the feed). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 14% of the 524 Atlantic salmon livers examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2008 was sufficient to identify a trend towards greater prevalence in the winter and spring (21-30%) than in the summer and fall (1.9-4.4%). Biliary preductular cell hyperplasia did not occur in the 118 farmed Pacific salmon examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program.

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.

Pigment in the liver and spleen probably is lipofuscin, and it might also include hemosiderin. Accumulation of lipofuscin is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish.

Foci of capillary distension and hemorrhage in the mesenteric adipose tissue are often part of the inflammatory response to many infectious diseases; hemorrhage sometimes occurs in severe cases. Differentials are the same as for congestion in the liver.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Histopathology

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

Slide 1 (7127-1) - heart, liver, spleen, head kidney, trunk kidney, skin/skeletal muscle, intestinal ceca, and mesenteric adipose tissue

Slide 2 (7127-2) - heart, liver, spleen, head kidney, trunk kidney, skin/skeletal muscle, and mesenteric adipose tissue

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/28/09 @ 1:50 PM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/02/09 @ 1:31 PM

Specimen	ID	Test	Result
Tissue		PCR - ISA	Negative
Paraffin Block	Fish #2	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/03/09 @ 4:21 PM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

History of Communication

Date	To	Description
03/03/09 4:36 PM	Marine Harvest Canada - fax	bc report generated
03/10/09 1:51 PM	Marine Harvest Canada - fax	Case Invoiced

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

END OF REPORT

Case: 09-707

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Final Report AHC Case: 09-708

Last Updated: 03/03/09 4:22 PM
Pathologist: Gary D. Marty
Received Date: 02/26/09
Collected Date: 02/18/09
Client Ref No: 7132

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

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

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

Case History

Submitted fresh and formalized fish tissue for Histopathology and PCR (IHN and VHS).

Vaccinated. Hem. throughout visceral organs and belly wall.

Final Diagnosis

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

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

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

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

3. Head kidney: multinucleate giant cell, focal (1 cell), mild

4a. Spleen: parenchymal golden pigment, scattered, intracellular, mild

4b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild

5. Mesenteric adipose tissue (adjacent to spleen): capillary congestion and hemorrhage, multifocal, mild

6. Heart, stratum compactum: congestion and hemorrhage, multifocal, moderate

Final Comment: Vascular congestion in the heart and single cell hepatocellular necrosis are consistent with septicemia as the cause of death. Comments on specific lesions follow:

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

liver for processing).

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.

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 or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469)

The single multinucleate giant cell in the head kidney has multiple nuclei packed on the margin of the 60-µm-diameter cell. These cells usually form in response to persistent foreign material, which in this case might be vaccine material.

Pigment in the spleen probably 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.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Foci of capillary distension and hemorrhage in the mesenteric adipose tissue are often part of the inflammatory response to many infectious diseases; hemorrhage sometimes occurs in severe cases. In this case, it seems to be related to a vaccine reaction.

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.

Histopathology

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

Slide 1 (Doctor 2/18/9 7132) - heart, liver, spleen, head kidney/trunk kidney transition, brain, skeletal muscle, intestinal ceca, and mesenteric adipose tissue

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/28/09 @ 1:50 PM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/03/09 @ 4:22 PM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

History of Communication

Date	To	Description
03/03/09 4:41 PM	Marine Harvest Canada - fax	bc report generated
03/10/09 1:52 PM	Marine Harvest Canada - fax	Case Invoiced

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

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Final Report AHC Case: 09-709

Last Updated: 03/03/09 4:22 PM
Pathologist: Gary D. Marty
Received Date: 02/26/09
Collected Date: 02/16/09
Client Ref No: 7121

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

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

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

Case History

Submitted fresh and formalized fish tissue for Histopathology and PCR (IHN and VHS).

Saltwater entry 2008 S0. Vaccinated. Fish had pin point hem. in pyloric caeca and along belly wall.

Final Diagnosis

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

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

2. Spleen and intestinal ceca: peritonitis, chronic, multifocal, with fibrocellular fronds, mild

3. Mesenteric adipose tissue: capillary congestion and hemorrhage, focal, mild

Final Comment: None of the microscopic lesions in this fish are of sufficient severity to assign a cause of death. Comments on specific lesions follow:

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

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

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Foci of capillary distension and hemorrhage in the mesenteric adipose tissue are often part of the inflammatory response to many infectious diseases; hemorrhage sometimes occurs in severe cases. It sometimes seems to be related to a vaccine reaction.

Histopathology

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

Slide 1 (7121 2/16/09) - heart, liver, spleen, head kidney, trunk kidney, brain, intestinal ceca, and mesenteric adipose tissue

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 02/28/09 @ 1:50 PM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/03/09 @ 4:22 PM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

History of Communication

Date	To	Description
03/03/09 4:46 PM	Marine Harvest Canada - fax	bc report generated
03/10/09 1:52 PM	Marine Harvest Canada - fax	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: 09-710

Last Updated: 03/04/09 11:48 AM
Pathologist: Gary D. Marty
Received Date: 02/26/09
Collected Date:
Client Ref No: 7126

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

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

Case History

Submitted fresh fish tissue for PCR (IHN and VHS).

Saltwater entry 2008 S1. 2 samples from site with previous VHSV diagnosis. Samples taken from 2 fish.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/04/09 @ 11:48 AM

Specimen	ID	Test	Result
Tissue	organ #1	PCR - IHNV	Negative
Tissue	organ #2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/04/09 @ 11:48 AM

Specimen	ID	Test	Result
Tissue	organ #1	PCR - VHSV	Positive
Tissue	organ #2	PCR - VHSV	Positive

History of Communication

Date	To	Description
03/03/09 4:49 PM	Marine Harvest Canada - fax	bc report generated
03/04/09 11:57 AM	Marine Harvest Canada - fax	bc report generated
03/10/09 1:53 PM	Marine Harvest Canada - fax	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: 09-711

Last Updated: 03/04/09 11:48 AM
Pathologist: Gary D. Marty
Received Date: 02/26/09
Collected Date:
Client Ref No: 7130

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

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

Case History

Submitted fresh and formalized fish tissue for Histopathology and PCR (IHN and VHS).

Vaccinated. Pin point hem on pyloric caeca, hem on liver and belly wall.

Final Diagnosis

1a. Liver: sinusoidal congestion, multifocal, moderate, with acid hematin granules and small numbers of intracytoplasmic spherical amphophilic inclusions
1b. Liver: encysted cestode, 700 × 400 µm, with prominent rostellar hooks and spines on the scolex, focal, moderate
1c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild
2. Heart: endocarditis, diffuse, with endothelial cell hypertrophy, mild
3. Spleen and intestinal ceca: peritonitis, chronic, multifocal, with fibrocellular fronds, mild
4a. Trunk kidney: membranous glomerulonephritis, diffuse, mild to moderate
4b. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, moderate

Final Comment: This fish died of complication related to systemic inflammation, but the specific cause is unknown. Comments on specific lesions follow:

In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria (e.g., *Listonella anguillarum*), and infection with VHSV; as in this case, the cause often remains unknown. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

This is the first case of an encysted cestode that I have seen in farmed Atlantic salmon. Because cestodes have an intermediate host, its presence is evidence that this fish ate something other than processed fish pellets (e.g., an invertebrate from its environment).

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

The pattern of inflammation in the heart is consistent with a systemic immune stimulation, usually a result of a bacterial or viral infection (e.g., VHSV).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Features of glomerulonephritis include thickening of the glomerular basement membranes by homogeneous eosinophilic material. Membranous glomerulonephritis is fairly common in Chinook salmon, but it is rare Atlantic salmon, affecting only 13 of 1663 Atlantic salmon kidneys examined since 2006 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 (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 tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469).

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 a cassette for histopathology.

Slide 1 (Pen 2 2/17/09) - heart, liver, spleen, head kidney, trunk kidney, brain, intestinal ceca, and mesenteric adipose tissue

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

Quality control: Liver autolysis: moderate. Interestingly, hepatocytes in the congested foci are well preserved, whereas those outside the congested foci have severe autolysis. Congested foci in the liver contain acid hematin, which accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/04/09 @ 11:48 AM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/04/09 @ 11:48 AM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

History of Communication

Date	To	Description
03/03/09 5:02 PM	Marine Harvest Canada - fax	bc report generated

03/04/09 12:01 PM	Marine Harvest Canada - fax	bc report generated
03/10/09 1:54 PM	Marine Harvest Canada - fax	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: 09-743

Last Updated: 03/05/09 11:32 AM
Pathologist: Gary D. Marty
Received Date: 03/02/09
Collected Date: 02/23/09
Client Ref No: 12762

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted fresh Atlantic salmon tissue for PCR (to confirm VHS).

Saltwater. Vaccinated. DOD - Feb 23/09.

Animal location - Bedwell

Molecular Diagnostics

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 03/05/09 @ 11:32 AM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Positive

History of Communication

Date	To	Description
03/05/09 2:48 PM	Mainstream Canada-T - fax	bc report generated
03/05/09 2:48 PM	Dr. Peter McKenzie - e-mail	bc report generated
03/12/09 9:07 AM	Mainstream Canada - e-mail	Case Invoiced

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Final Report AHC Case: 09-745

Last Updated: 03/09/09 11:38 AM
Pathologist: Gary D. Marty
Received Date: 03/02/09
Collected Date:
Client Ref No: 12766

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted fresh Atlantic salmon tissue and bacti plate for Bacteriology and PCR (confirm or rule out VHS).

3kg. Saltwater. Vaccinated. DOD - Feb 25/09. Few fish (3) over 2 weeks - hemorrhaged livers/ acites/ Hem. swim bladders and cecea/ Hem flesh.

Animal location - West Side

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 03/09/09 @ 11:38 AM

Specimen	ID	Isolate	Result	Level
Isolate	1	Moritella sp.	Positive	
**: This isolate has not grown on sub-culture and therefore was identified using DNA sequencing from the initial colony on the submission plate.				
Isolate	2		No Bacteria Isolated	
**: No growth on culture plate upon submission or re-incubation				

Molecular Diagnostics

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 03/05/09 @ 11:32 AM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

History of Communication

Date	To	Description
03/09/09 1:28 PM	Mainstream Canada-T - fax	bc report generated

03/09/09 1:29 PM	Dr. Peter McKenzie - e-mail	bc report generated
03/12/09 9:09 AM	Mainstream Canada - e-mail	Case Invoiced

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Final Report AHC Case: 09-782

Last Updated: 03/09/09 11:47 AM
Pathologist: Gary D. Marty
Received Date: 03/05/09
Collected Date:
Client Ref No: 7141

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

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

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

Case History

Submitted 4 fresh tissues for PCR for IHN and VHS.

Hemorrhage in internal organ (liver, p.c.). Some mechanical damage. Blood pooling under skin. No growth on bacterial culture.

Saltwater entry 08SO. Vaccinated. Fish died Feb. 24/09.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/09/09 @ 10:15 AM

Specimen	ID	Test	Result
Tissue	7141-1	PCR - IHNV	Negative
Tissue	7141-2	PCR - IHNV	Negative
Tissue	7141-3	PCR - IHNV	Negative
Tissue	7141-4	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/09/09 @ 11:47 AM

Specimen	ID	Test	Result
Tissue	7141-1	PCR - VHSV	Negative
Tissue	7141-2	PCR - VHSV	Negative
Tissue	7141-3	PCR - VHSV	Negative
Tissue	7141-4	PCR - VHSV	Negative

History of Communication

Date	To	Description
03/09/09 1:38 PM	Marine Harvest Canada - fax	bc report generated
03/12/09 9:24 AM	Marine Harvest Canada - fax	Case Invoiced

Gary D. Marty
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Final Report AHC Case: 09-783

Last Updated: 03/12/09 3:12 PM
Pathologist: Gary D. Marty
Received Date: 03/05/09
Collected Date:
Client Ref No: 7114

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

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

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

Case History

Submitted 2 fish culture plates for bacterial identification.

Fish had petechial hem. in pyloric ceca. Two plates labelled P1 and P2 for identification.

Saltwater entry 2009 S1. Vaccinated. PO #7114.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Jaime Osei-Appiah on 03/12/09 @ 3:12 PM

Specimen	ID	Isolate	Result	Level
Isolate	P1	Bacteria	Positive	
**: Bacteria identified as Obesumbacterium proteus, also known as Flavobacterium proteus.				
Isolate	P2	Bacteria	Positive	
**: Bacteria identified as Obesumbacterium proteus, also known as Flavobacterium proteus.				

History of Communication

Date	To	Description
03/12/09 4:34 PM	Marine Harvest Canada - fax	bc report generated
03/17/09 9:06 AM	Marine Harvest Canada - fax	Case Invoiced

Gary D. Marty

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Final Report AHC Case: 09-784

Last Updated: 03/09/09 11:47 AM
Pathologist: Gary D. Marty
Received Date: 03/05/09
Collected Date:
Client Ref No: 7144

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

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

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

Case History

Submitted 7 fresh tissues for PCR for IHN and VHS.

Routine check of VHS site. Samples labelled: #2, 4, 5, 6, 8, 10, and 11. Please run PCR for IHN and VHS on all samples. Saltwater entry 08 S1. Vaccinated. DOD Feb 25/09.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/09/09 @ 10:15 AM

Specimen	ID	Test	Result
Tissue	7144-2	PCR - IHNV	Negative
Tissue	7144-4	PCR - IHNV	Negative
Tissue	7144-5	PCR - IHNV	Negative
Tissue	7144-6	PCR - IHNV	Negative
Tissue	7144-8	PCR - IHNV	Negative
Tissue	7144-10	PCR - IHNV	Negative
Tissue	7144-11	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/09/09 @ 11:47 AM

Specimen	ID	Test	Result
Tissue	7144-2	PCR - VHSV	Positive
Tissue	7144-4	PCR - VHSV	Positive
Tissue	7144-5	PCR - VHSV	Positive
Tissue	7144-6	PCR - VHSV	Positive
Tissue	7144-8	PCR - VHSV	Positive
Tissue	7144-10	PCR - VHSV	Positive
Tissue	7144-11	PCR - VHSV	Positive

History of Communication

Date	To	Description
03/09/09 1:43 PM	Marine Harvest Canada - fax	bc report generated
03/12/09 9:25 AM	Marine Harvest Canada - fax	Case Invoiced

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Final Report AHC Case: 09-785

Last Updated: 03/09/09 1:51 PM
Pathologist: Gary D. Marty
Received Date: 03/05/09
Collected Date:
Client Ref No: 7138

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

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

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

Case History

Submitted fresh/formalized fish tissues for histopathology and PCR for IHN and VHS.

Saltwater entry 07 S0. Vaccinated. Euthanized by percussion. Dipped 2 moribunds. Both fish had bilateral cataracts developing. They were average size 2-2.3 kg. Both fish had been eating. No internal or external lesions.

Final Diagnosis

1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 1, 2)
1b. Liver: pericholangitis , lymphohistiocytic, multifocal, mild (slide 1)
1c. Liver: yellow-brown to yellow-green pigmented macrophage aggregates and sinusoidal macrophages (lipofuscin and hemosiderin?), disseminated, mild (slide 1)
1d. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate (slide 2)
2. Mesenteric adipose tissue, intestine, and stomach: peritonitis, chronic, multifocal, with fibrocellular fronds, mild (slide 1), moderate, with hemorrhage (slide 2)
3a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1, 2)
3b. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slide 2)
4. Kidney: nephritis, interstitial, granulomatous, multifocal, mild (slide 2)
5. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slide 1)

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

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

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

Pigment in the liver and spleen probably is lipofuscin, and it might also include hemosiderin. Accumulation of lipofuscin is a nonspecific

change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish.

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.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe). Hemorrhage in slide 2 is very fresh, and it might have occurred during the sampling process.

Foci of granulomatous inflammation in the kidney (slide 2) are poorly defined, and the primary differential for this type of inflammation is a vaccine reaction. The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease, and chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469) and Pacific salmon (prevalence = 31%; n = 118).

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology.
Slides 1 (7138-1) and 2 (7138-2) - heart, liver, spleen, head kidney, trunk kidney, stomach, intestinal ceca, and mesenteric adipose tissue
All organs were examined. Organs not listed elsewhere have no significant lesions.

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

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/09/09 @ 10:15 AM

Specimen	ID	Test	Result
Tissue	7138	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/09/09 @ 11:47 AM

Specimen	ID	Test	Result
Tissue	7138	PCR - VHSV	Negative

History of Communication

Date	To	Description
03/09/09 1:47 PM	Marine Harvest Canada - fax	bc report generated
03/12/09 9:26 AM	Marine Harvest Canada - fax	Case Invoiced

Case: 09-785

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Final Report AHC Case: 09-795

Last Updated: 03/09/09 11:47 AM
Pathologist: Gary D. Marty
Received Date: 03/05/09
Collected Date:
Client Ref No: 7140

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

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

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

Case History

Submitted fresh fish tissue for PCR (IHN and VHS).

Increased mortality, externally lesions are present, internally no visible lesions. Saltwater entry 09 S1. Vaccinated. DOD - Feb 25/09.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/09/09 @ 10:16 AM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/09/09 @ 11:47 AM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

History of Communication

Date	To	Description
03/09/09 1:57 PM	Marine Harvest Canada - fax	bc report generated
03/12/09 9:27 AM	Marine Harvest Canada - fax	Case Invoiced

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

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Final Report AHC Case: 09-805

Last Updated: 03/09/09 2:14 PM
Pathologist: Gary D. Marty
Received Date: 03/06/09
Collected Date: 02/12/09
Client Ref No: PO 12778

Veterinarian: **Dr. Peter McKenzie**
Clinic: **Mainstream Canada-T**
Phone: (250) 725-1255
Fax: (250) 725-1250

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

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

Case History

Submitted formalized fish tissues for histopathology.

Saltwater entry. Vaccinated. Prior submission: 2009-0535 (tissues were PCR-negative for VHSV).

Farm name: Ross. PO 12776 revised to PO 12778

Final Diagnosis

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

1b. Liver: sinusoidal congestion and fibrin, with intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate (slide 1)

1c. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slides 1, 2, 3A, 3B)

1d. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 1, 3A)

2. Mesenteric adipose tissue: capillary congestion, diffuse, mild (slide 1)

3. Head kidney: interstitial cell hyperplasia, diffuse, mild (slides 2, 3A, 3B)

Final Comment: These fish have several lesions that are common among young farmed Atlantic salmon in BC. The most significant in this case is hepatic necrosis in slide 1. A finding of concern is the lack of vaccine reaction in these fish. Vaccine- associated peritonitis is best assessed during gross necropsy, but consider whether the lack of reaction in these fish is a result of (or evidence of) incomplete vaccination. 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. 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.

Sinusoidal congestion and fibrin in the liver is evidence of circulating vasodilators; congestion sometimes occurs as a postmortem artifact. In slide 1, all foci of necrosis are associated with sinusoidal congestion, but several foci of congestion are not associated with hepatic necrosis. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).

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.

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.

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 in 4 cassettes for histopathology.

Slides 1 (Ross Feb 12/09 Fish 1), 2 (Ross Feb 12/09 Fish 3), 3A (Ross Feb 12/09 Fish 3) and Slide 3B (Ross Feb 12/09 Fish 4) - brain, heart, spleen, liver, intestinal ceca, head kidney, mesenteric adipose tissue

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

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

History of Communication

Date	To	Description
03/09/09 2:20 PM	Mainstream Canada-T - fax	bc report generated
03/09/09 2:20 PM	Dr. Peter McKenzie - e-mail	bc report generated
03/12/09 9:28 AM	Mainstream Canada-T - fax	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: 09-806

Last Updated: 03/10/09 4:38 PM
Pathologist: Gary D. Marty
Received Date: 03/06/09
Collected Date:
Client Ref No: PO 12778

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted fresh tissues for PCR to confirm/rule out VHS.

Age: 80g. Saltwater entry. Vaccinated. Fish died Mar. 2/09.

Farm name: Bedwell.

PO 12776 has been revised to 12778.

Molecular Diagnostics

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/10/09 @ 4:38 PM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Positive

History of Communication

Date	To	Description
03/11/09 10:56 AM	Mainstream Canada-T - fax	bc report generated
03/11/09 10:56 AM	Dr. Peter McKenzie - e-mail	bc report generated
03/17/09 9:11 AM	Mainstream Canada-T - fax	Case Invoiced

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

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Final Report AHC Case: 09-961

Last Updated: 03/26/09 9:55 AM
Pathologist: Gary D. Marty
Received Date: 03/16/09
Collected Date: 03/12/09
Client Ref No: MI 031209

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Kelly Abel - Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 2 fish cultures for bacteriology.

Saltwater. Vaccinated. Cultured 4 lesions from live fish. Submitted in 2 plates with 2 fish each for bacteriology.

Animal location - Maude Island.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 03/26/09 @ 9:50 AM

Specimen	ID	Isolate	Result	Level
Isolate	Fish 1	Psychrobacter sp.	Positive	
**: Psychrobacter sp. identified as Psychrobacter okhotskensis				
Isolate	Fish 2	Pseudoalteromonas sp.	Positive	
Isolate	Fish 2	Vibrio splendidus	Positive	
Isolate	Fish 3	Pseudoalteromonas sp.	Positive	
Isolate	Fish 3	Psychrobacter sp.	Positive	
**: Psychrobacter sp. identified as Psychrobacter maritimus				
Isolate	Fish 4	Vibrio splendidus	Positive	

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 03/26/09 @ 9:53 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Pseudoalteromonas sp.	Fish 2	s	s		s	s	s
Pseudoalteromonas sp.	Fish 3	s	s		s	s	s
Psychrobacter sp.	Fish 1	s	s		s	s	s
Psychrobacter sp.	Fish 3	s	s		s	s	s

Vibrio splendidus	Fish 2	s	s		s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline Unable to perform Romet sensitivity due to supplier shortage. Testing will resume for Romet as soon as supply becomes available.							
Vibrio splendidus	Fish 4	s	s		s	s	s

History of Communication

Date	To	Description
04/07/09 12:32 PM	Mainstream Canada - 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: 09-962

Last Updated: 03/18/09 3:17 PM
Pathologist: Gary D. Marty
Received Date: 03/16/09
Collected Date: 03/11/09
Client Ref No: CB 031109

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Kelly Abel - Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 3 formalized Salmon samples for histopathology. Prior submission #CB030909 (2009-0910).

Saltwater. Vaccinated. 3 Dead 3 Cassettes containing above noted tissues.

Farm location - Cliff Bay

Final Diagnosis

1a. Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate (slide 1-2)

1b. Liver: hepatic necrosis with sinusoidal fibrin, acute, multifocal, mild (slide 1-2)

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

1d. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 1-1, 2-2)

2. Skeletal muscle and adjacent adipose tissue (subcutaneous fat?): liquefactive necrosis, with moderate numbers of filamentous bacteria, focal, mild (slide 2-2), severe (slide 1-2)

3. Kidney: interstitial cell hyperplasia, diffuse, mild (slides 1-1, 1-2, 2-2)

4. Heart: endocarditis, diffuse, with endothelial cell hypertrophy, mild (slides 1-1, 1-2, 2-2)

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

6. Intestinal ceca and adjacent mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1-1); with intralesional vacuoles up to 100 µm in diameter, moderate (slide 2-2)

Final Comment: These fish probably died of complications related to VHSV infection and ulcers invaded by filamentous bacteria. Comments of specific lesions follow:

Sinusoidal congestion in the liver is evidence of circulating vasodilators. VHSV infection is the most likely cause in this case. Differentials include substances released from inflammatory cells or bacteria. The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).

Hepatocellular 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., VHSV). 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.

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. In this case, hepatocellular nuclei are slightly larger than normal and have prominent basophilic nucleoli, both of which are consistent with increased protein synthesis.

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

Filamentous bacteria commonly invade skin ulcers. Enlargement of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection-- in this case probably VHSV). Identification of the bacteria requires culture or PCR, but in saltwater, *Tenacibaculum maritimum* is likely.

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). Severe cases of VHSV are often associated with interstitial cell atrophy (i.e., decrease in numbers of interstitial cells). The fact that all 3 fish have interstitial cell hyperplasia is evidence that the ulcers and filamentous bacteria are more important than VHSV in these fish.

The pattern of inflammation in the heart is consistent with a systemic immune stimulation, usually a result of a bacterial or viral infection (in this case, consistent with the recent PCR- positive VHSV test result from this farm, case 2009-0910). Hypertrophic endothelial cells are basophilic and up to 6 µm thick.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe). Comments on intestinal and mesenteric peritonitis are similar. Vacuoles are probably a result of vaccine material lost during tissue processing.

Histopathology

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

Slide 1-1 (Pen 11 Fish #1 03/11/09 Cliff Bay) - heart, spleen, liver, head kidney/trunk kidney transition, skeletal muscle, intestinal ceca, and mesenteric adipose tissue

Slide 1-2 (Pen 2 Fish #1 03/11/09 Cliff Bay) - heart, spleen, liver, head kidney, skeletal muscle, intestinal ceca, and mesenteric adipose tissue

Slide 2 (Pen 11 Fish #2 03/11/09 Cliff Bay) - heart, spleen, liver, intestine, trunk kidney/head kidney transition, skeletal muscle, intestinal ceca, and mesenteric adipose tissue

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

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

Note on sampling for histopathology: histocassettes are an excellent tool for submission of samples for histopathology. When the tissues are processed to a slide, the face of the tissue at the bottom of the cassette is the face that will appear on the slide. Orientation is especially critical for skin biopsies, where the best samples include a cross section of epidermis, dermis, and underlying skeletal muscle; when ulcers are sampled, the section should include the intact skin at the margin of the ulcer (the center of the ulcer is less critical for histopathology). By comparison, shallow skin biopsies that are placed in the cassette with the muscle at the bottom of the cassette will yield only a section of muscle (this might have happened in slide 1-1).

History of Communication

Date	To	Description
03/18/09 3:20 PM	Mainstream Canada - e-mail	bc report generated
03/18/09 3:20 PM	Dr. Peter McKenzie - e-mail	bc report generated
03/31/09 9:06 AM	Mainstream Canada - 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: 09-981

Last Updated: 03/19/09 2:03 PM
Pathologist: Gary D. Marty
Received Date: 03/17/09
Collected Date:
Client Ref No: 7161

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

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

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

Case History

Submitted one fresh fish sample for PCR (IHNV and VHSV).

Saltwater entry 08S0. Vaccinated. 3-fish pool; all 3 had petechial hem in viscera.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 03/19/09 @ 2:03 PM

Specimen	ID	Test	Result
Tissue	7161	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 03/19/09 @ 2:03 PM

Specimen	ID	Test	Result
Tissue	7161	PCR - VHSV	Negative

History of Communication

Date	To	Description
03/19/09 4:42 PM	Marine Harvest Canada - fax	bc report generated
03/31/09 9:20 AM	Marine Harvest Canada - fax	Case Invoiced

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

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Final Report AHC Case: 09-1027

Last Updated: 03/27/09 11:35 AM
Pathologist: Gary D. Marty
Received Date: 03/19/09
Collected Date: 03/17/09
Client Ref No: 12816

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted fresh fish tissue and bacti plate for bacteriology (rule out Aeromonas salminicda) and PCR (confirm/ rule out VHSV - IHNV) .

Saltwater. 150 g. Vaccinated. Prior submission 2009-0534. Internal hemorrhaging - cecea - swim bladder - muscle flesh. Ascites. Pale liver. Enlarged spleen. Please see attached sheet.

Farm location - Bawden Point

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 03/26/09 @ 10:30 AM

Specimen	ID	Isolate	Result	Level
Isolate	1		No Bacteria Isolated	
Isolate	2		No Bacteria Isolated	
Isolate	3	Vibrio sp.	Positive	
**: Verified Results Changed: 03/27/09 10:44 AM by bez Identified as either Aliivibrio wodanis or Aliivibrio logei via DNA sequencing				
Isolate	4		No Bacteria Isolated	

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 03/26/09 @ 10:30 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Vibrio sp.	3	s	s		s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline Unable to perform Romet sensitivity due to supplier shortage. Testing will resume for Romet as soon as supply becomes available.							

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/23/09 @ 3:31 PM

Specimen	ID	Test	Result
Tissue	organ	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/23/09 @ 3:31 PM

Specimen	ID	Test	Result
Tissue	organ	PCR - VHSV	Positive

Staff Comments:
Reoport of DNA sequencing results (Alivibrio wodanis or Alivibrio logei) sent to Dr. McKenzie and Zarah Vasnick via Outlook by GDMarty 2009-03-27 2:03PM.

History of Communication

Date	To	Description
03/23/09 5:33 PM	Mainstream Canada-T - fax	bc report generated
03/23/09 5:33 PM	Dr. Peter McKenzie - e-mail	bc report generated
04/10/09 3:29 PM	Mainstream Canada-T - fax	bc report generated
04/10/09 3:29 PM	Dr. Peter McKenzie - e-mail	bc report generated
04/15/09 2:18 PM	Mainstream Canada - 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: 09-1134

Last Updated: 03/30/09 12:43 PM
Pathologist: Gary D. Marty
Received Date: 03/26/09
Collected Date: 03/18/09
Client Ref No: 7181

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

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

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

Case History

Submitted fresh Salmon tissue for PCR (IHN and VHS).

Saltwater entry (Brood stock). Vaccinated. Increase in mortality, post handling. Sample sent in for routine screening.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/30/09 @ 12:43 PM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/30/09 @ 12:43 PM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

History of Communication

Date	To	Description
04/06/09 1:56 PM	Marine Harvest Canada - fax	bc report generated
04/15/09 2:46 PM	Marine Harvest Canada - fax	Case Invoiced

Gary D. Marty

Case: 09-1134

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Toll-Free : 1-800-661-9903

Final Report AHC Case: 09-1135

Last Updated: 03/30/09 12:43 PM
Pathologist: Gary D. Marty
Received Date: 03/26/09
Collected Date: 03/09/09
Client Ref No: 7162

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

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

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

Case History

Submitted fresh Salmon tissue for PCR (IHN and VHS).

Saltwater entry 2007 S0. 1 sample for routine PCR.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/30/09 @ 12:43 PM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/30/09 @ 12:43 PM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

History of Communication

Date	To	Description
04/06/09 2:00 PM	Marine Harvest Canada - fax	bc report generated
04/15/09 2:47 PM	Marine Harvest Canada - fax	Case Invoiced

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

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Final Report AHC Case: 09-1175

Last Updated: 04/06/09 10:14 AM
Pathologist: Gary D. Marty
Received Date: 03/30/09
Collected Date: 03/26/09
Client Ref No: 12845

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted one fish fresh tissue for bacteriology and PCR (confirm rule out VHS) Bacterial kidney disease/ Piscirickettsia salmonis.

Granulomas livers - spleens - enlarged. Swollen kidneys. Hemorrhages in flesh, swimbladders.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 04/06/09 @ 10:13 AM

Specimen	ID	Isolate	Result	Level
Kidney		Photobacterium iliopiscarium	Positive	4+
Kidney		Vibrio sp.	Positive	4+
**: Vibrio sp. Unable to differentiated between Aliivibrio wodanis, logei or salmonicida via DNA sequencing and biochemical methods.				
Kidney		Pseudoalteromonas sp.	Positive	4+

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 04/06/09 @ 10:13 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Photobacterium iliopiscarium		s	s		s	s	s
Pseudoalteromonas sp.		r	r		r	r	r
Vibrio sp.		s	s		s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline Unable to perform Romet sensitivity due to supplier shortage. Testing will resume for Romet as soon as supply becomes available.							

Molecular Diagnostics

PCR-Renibacterium salmoni Resulted by: A Scouras Verified by: Dr. J. Robinson on 04/02/09 @ 11:07 AM

Specimen	ID	Test	Result
Kidney		PCR-Renibacterium salmoninaru	Positive

PCR-Piscirickettsia salmo Resulted by: A Scouras Verified by: Dr. J. Robinson on 04/02/09 @ 11:07 AM

Specimen	ID	Test	Result
Kidney		PCR - Piscirickettsia salmonis	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 03/31/09 @ 4:38 PM

Specimen	ID	Test	Result
Kidney		PCR - VHSV	Negative

History of Communication

Date	To	Description
04/02/09 5:10 PM	Mainstream Canada-T - fax	bc report generated
04/06/09 2:06 PM	Mainstream Canada-T - fax	bc report generated
04/06/09 2:06 PM	Dr. Peter McKenzie - e-mail	bc report generated
04/15/09 2:55 PM	Mainstream Canada - 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: 09-1215

Last Updated: 04/07/09 2:28 PM
Pathologist: Gary D. Marty
Received Date: 04/01/09
Collected Date: 03/26/09
Client Ref No: 12854

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted formalized Atlantic Salmon tissue for histopathology. (confirm/rule out bacterial kidney disease, Piscirickettsia salmonis)

Saltwater. 2.8 kg. Vaccinated

Final Diagnosis

1a. Trunk kidney: nephritis, granulomatous, multifocal, coalescing, severe, with very small numbers (slide 4) or abundant (slide 1) intralesional short Gram -positive bacterial rods (*Renibacterium salmoninarum*)

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

2a. Liver: hepatitis, focal, granulomatous (probable *Renibacterium salmoninarum*), mild (slide 1)

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

2c. Liver: hepatocellular cytoplasmic lipid, diffuse, small amounts (slide 1)

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

3a. Heart: epicarditis and endocarditis, granulomatous, multifocal, coalescing, moderate, with intralesional short bacterial rods consistent with *Renibacterium salmoninarum* (slide 1)

3b. Heart: endocarditis, diffuse, with endothelial cell hypertrophy and very small numbers of short Gram -positive bacterial rods (*Renibacterium salmoninarum*), moderate (slide 4)

4a. Spleen: splenitis, granulomatous, multifocal, coalescing, severe, with intralesional short Gram -positive bacterial rods (*Renibacterium salmoninarum*) (slides 1, 4)

4b. Spleen: parenchymal golden pigment, scattered, intracellular, mild (slides 2, 3)

4c. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 3)

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

Final Comment: The most common organism associated with disseminated granulomas in farmed BC salmon is *Renibacterium salmoninarum* , the cause of bacterial kidney disease. Lesions are most severe in the trunk kidney of slide 1, where inflammation is associated with renal tubular necrosis, and large foci of granulomatous inflammation are themselves necrotic. The reaction in the kidney of slide 4 includes multinucleate giant cells, and the reaction in the spleen of slide 4 includes central necrosis of granulomatous foci. Tissues have no evidence of *Piscirickettsia salmonis*.

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.

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.

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.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469) and Pacific salmon (prevalence = 31%; n = 118).

The pattern of inflammation in the heart (slide 4) is consistent with systemic immune stimulation, usually a result of a bacterial (e.g. *Renibacterium salmoninarum*) or viral infection (e.g., VHSV); the cause is often not determined. Hypertrophic endothelial cells are basophilic and up to 10 µm thick.

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%). 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 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology. A Twort's Gram stain was done on a section from the same paraffin block as slide 4.

Slide 1 (Mar 26/09 Rant Pt 1) - brain, heart, spleen, liver, intestine, trunk kidney, mesenteric adipose tissue

Slide 2 (Mar 26/09 Rant Pt 2) - brain, heart, spleen, liver, intestinal ceca, head kidney/trunk kidney transition

Slide 3 (Mar 26/09 Rant Pt 3) and 4 (Mar 26/09 Rant Pt 4) - brain, heart, spleen, liver, intestine, head kidney

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

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

History of Communication

Date	To	Description
04/07/09 2:29 PM	Mainstream Canada-T - fax	bc report generated

04/07/09 2:29 PM	Dr. Peter McKenzie - e-mail	bc report generated
04/15/09 3:35 PM	Mainstream Canada - 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: 09-1216

Last Updated: 04/16/09 9:57 AM
Pathologist: Gary D. Marty
Received Date: 04/01/09
Collected Date: 03/30/09
Client Ref No: 12854

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted fresh Atlantic Salmon tissue and 3 culture plates for bacteriology and PCR (confirm/rule out VHS, IHN by PCR and confirm/rule out Aeromonas salmoncida).

Saltwater. Vaccinated. 100g. Prior submission - 09-1027.

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 04/16/09 @ 9:57 AM

Specimen	ID	Isolate	Result	Level
Tissue	miscelaneous, pooled	Vibrio sp.	Positive	3+
**: Cannot distinguish between Aliiv brio wodanis, logei, and salmonicida				
Isolate	Fish 1/2/3/4	Vibrio sp.	Positive	
**: Cannot distinguish between Aliiv brio wodanis, logei and salmonicida.				
Isolate	Fish 4 skin Lesion	Vibrio splendidus	Positive	
Isolate	Fish 5/6/7/8		No Bacteria Isolated	

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 04/16/09 @ 9:57 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Vibrio sp.	Fish 1/2/3/4	s	s		s	s	s
Vibrio splendidus	Fish 4 skin Lesion	s	s		s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline Unable to perform Romet sensitivity due to supplier shortage. Testing will resume for Romet as soon as supply becomes available.							

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/03/09 @ 1:58 PM

Specimen	ID	Test	Result
Tissue	miscelaneous, pooled	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/03/09 @ 1:58 PM

Specimen	ID	Test	Result
Tissue	miscelaneous, pooled	PCR - VHSV	Positive

History of Communication

Date	To	Description
04/06/09 2:10 PM	Mainstream Canada-T - fax	bc report generated
04/06/09 2:10 PM	Dr. Peter McKenzie - e-mail	bc report generated
04/16/09 10:09 AM	Mainstream Canada-T - fax	bc report generated
04/16/09 10:09 AM	Dr. Peter McKenzie - e-mail	bc report generated
04/17/09 8:52 AM	Mainstream Canada - 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: 09-1316

Last Updated: 04/10/09 2:42 PM
Pathologist: Gary D. Marty
Received Date: 04/07/09
Collected Date: 04/06/09
Client Ref No: 7188

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

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

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

Case History

Submitted fresh and formalized fish tissue for histopathology and PCR (IHN and VHS).

Saltwater entry 07 SO. Euthanized - TMS overdose. Pen 4 fish sampled 2-3 hours after death. Fish was part of routine health check . Hem in all p.caeca and liver appeared a bit small with a greenish tinge. Fish was on feed.

Final Diagnosis

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

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

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

2. Heart: endocarditis, focal, lymphohistiocytic, mild

3a. Spleen: parenchymal golden pigment (lipofuscin?), scattered, intracellular, mild

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

4. Intestinal ceca: mucosal cysts, multifocal (4 in the section), 300 - 500 µm in diameter, with intralesional golden pigment (lipofuscin?) (moderate)

Final Comment: The most significant lesion in this fish is in the liver, probably accounting for the gross appearance (small with greenish tinge). 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). Moderate biliary preductular cell hyperplasia is uncommon in Atlantic salmon "fresh silvers" that die in marine net pens, affecting only 5 of 468 (1.1%) of the Atlantic salmon livers examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program. Other lesions probably were not as significant for fish health; specific comments 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 fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

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

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish. 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%).

Mucosal cysts occur occasionally in farmed Atlantic salmon in BC. The golden pigment in the lumen of the cyst is probably a result of degenerating mucus and cellular debris. Lack of inflammation is evidence that the cysts are of minimal significance for fish health. The section included no foci of vascular congestion or hemorrhage.

Histopathology

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

Slide 1 (4188-1) - heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/10/09 @ 2:42 PM

Specimen	ID	Test	Result
Tissue	org(7188-#88)	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/10/09 @ 2:42 PM

Specimen	ID	Test	Result
Tissue	org(7188-#88)	PCR - VHSV	Negative

History of Communication

Date	To	Description
04/10/09 3:40 PM	Marine Harvest Canada - fax	bc report generated
04/16/09 9:44 AM	Marine Harvest Canada - fax	Case Invoiced

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

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Final Report AHC Case: 09-1320

Last Updated: 04/10/09 2:42 PM
Pathologist: Gary D. Marty
Received Date: 04/07/09
Collected Date:
Client Ref No: 7202

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

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

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

Case History

Submitted fresh fish tissue for PCR (IHN and VHS).

Saltwater entry 09S1. Vaccinated. One fresh mort with VHS like symptoms.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/10/09 @ 2:42 PM

Specimen	ID	Test	Result
Tissue	org (#7202)	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/10/09 @ 2:42 PM

Specimen	ID	Test	Result
Tissue	org (#7202)	PCR - VHSV	Positive

History of Communication

Date	To	Description
04/10/09 3:44 PM	Marine Harvest Canada - fax	bc report generated
04/16/09 9:45 AM	Marine Harvest Canada - fax	Case Invoiced

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

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Final Report AHC Case: 09-1321

Last Updated: 04/10/09 2:42 PM
Pathologist: Gary D. Marty
Received Date: 04/07/09
Collected Date:
Client Ref No: 7197

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

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

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

Case History

Submitted one fresh fish tissue for PCR (IHN and VHS)

Saltwater entry 2007 S1. Vaccinated. 1 fish with hem of all internal organs and muscle. No growth on bacteriology.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/10/09 @ 2:42 PM

Specimen	ID	Test	Result
Tissue	org (#7197)	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/10/09 @ 2:42 PM

Specimen	ID	Test	Result
Tissue	org (#7197)	PCR - VHSV	Negative

History of Communication

Date	To	Description
04/10/09 3:48 PM	Marine Harvest Canada - fax	bc report generated
04/16/09 9:45 AM	Marine Harvest Canada - fax	Case Invoiced

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

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Final Report AHC Case: 09-1322

Last Updated: 04/10/09 2:43 PM
Pathologist: Gary D. Marty
Received Date: 04/07/09
Collected Date:
Client Ref No: 7199

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

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

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

Case History

Submitted one fresh fish tissue for PCR (IHN and VHS).

Saltwater entry 08 S1. Fresh mort - visceral hem, pop eye, muscle hem.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/10/09 @ 2:43 PM

Specimen	ID	Test	Result
Tissue	org (#7199)	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 04/10/09 @ 2:43 PM

Specimen	ID	Test	Result
Tissue	org (#7199)	PCR - VHSV	Negative

History of Communication

Date	To	Description
04/10/09 3:50 PM	Marine Harvest Canada - fax	bc report generated
04/16/09 9:46 AM	Marine Harvest Canada - fax	Case Invoiced

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

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Final Report AHC Case: 09-1369

Last Updated: 04/24/09 10:12 AM
Pathologist: Gary D. Marty
Received Date: 04/09/09
Collected Date:
Client Ref No: CH040709

Veterinarian: **Dr. Peter McKenzie**
Clinic: **Mainstream Canada**
Phone: (250) 286-0022
Fax: (250) 286-0042

Submitter: **Kelly Abel**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted fresh Atlantic salmon tissues for PCR for BKD and 2 plates for bacteriology.

Saltwater. Vaccinated. Euthanized: no.

Cultured 2 kidneys and 2 livers, submitting 2 plates w/1 fish each; 1 kidney/ 1 liver per plate and 2 tissues (kidney).

Farm name: Cypress Harbour.

Ref. Number: CH040709

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 04/15/09 @ 11:55 AM

Specimen	ID	Isolate	Result	Level
Isolate	Pen 12 Kidney		No Bacteria Isolated	
Isolate	Pen 12 Liver		No Bacteria Isolated	
Isolate	Pen 13 Kidney		No Bacteria Isolated	
Isolate	Pen 13 Liver	Flavobacterium frigidarium	Positive	
**: Sensitivities unavailable due to poor organism growth				
Isolate	Pen 13 Liver	Psychrobacter sp.	Positive	
**: Psychrobacter sp. identified as Psychrobacter okhotskensis.				

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 04/20/09 @ 9:10 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Psychrobacter sp.	Pen 13 Liver	s	s		r	r	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt =							

Sulfamethoxazole/Trimethropri ot = Oxytetracycline Unable to perform Romet sensitivity due to supplier shortage. Testing will resume for Romet as soon as supply becomes available.

Molecular Diagnostics

PCR-Renibacterium salmoni Resulted by: Ken Sojonky Verified by: R. Richardson on 04/16/09 @ 3:42 PM

Specimen	ID	Test	Result
Kidney	Pen 13 Fish#1	PCR-Renibacterium salmoninaru	Negative
Kidney	Pen 12 Fish#1	PCR-Renibacterium salmoninaru	Negative

**: Test validation in progress.

History of Communication

Date	To	Description
04/17/09 9:07 AM	Mainstream Canada - e-mail	bc report generated
04/17/09 9:07 AM	Dr. Peter McKenzie - e-mail	bc report generated
04/24/09 3:49 PM	Mainstream Canada - e-mail	bc report generated
04/24/09 3:50 PM	Dr. Peter McKenzie - e-mail	bc report generated
04/27/09 4:04 PM	Mainstream Canada - 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: 09-1483

Last Updated: 04/23/09 12:43 PM
Pathologist: Gary D. Marty
Received Date: 04/21/09
Collected Date: 04/15/09
Client Ref No: 12897

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted 2 Salmon culture plates for bacteriology (confirm R/O Aeromonas salmonicida).

Saltwater. Vaccinated. 80g. Gross signs - Furuncles dorsal surface. Petechical hemorrhageing cecea. Hemorrhageal swim bladders.

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 04/23/09 @ 12:41 PM

Specimen	ID	Isolate	Result	Level
Isolate	bare 1	Aeromonas salmonicida	Positive	
Isolate	bare 2	Aeromonas salmonicida	Positive	
Isolate	bare 3		No Bacteria Isolated	
Isolate	bare 4	Aeromonas salmonicida	Positive	
Isolate	bare bluff 1	Aeromonas salmonicida	Positive	
Isolate	bare bluff 2		No Bacteria Isolated	
Isolate	bare bluff 3	Aeromonas salmonicida	Positive	
Isolate	bare bluff 4		No Bacteria Isolated	

Fish Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 04/23/09 @ 12:42 PM

Organism	ID	e	ffc	sor	s3	sxt	ot
Aeromonas salmonicida	bare 1	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
04/23/09 3:56 PM	Mainstream Canada-T - fax	bc report generated
04/23/09 3:56 PM	Dr. Peter McKenzie - e-mail	bc report generated
04/28/09 9:11 AM	Mainstream Canada - e-mail	Case Invoiced

Gary D. Marty
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Final Report AHC Case: 09-1617

Last Updated: 05/01/09 2:15 PM
Pathologist: Gary D. Marty
Received Date: 04/29/09
Collected Date: 04/28/09
Client Ref No: 7228

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

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

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

Case History

Submitted 2 fresh tissue for PCR (IHN and VHS) and one formalized tissue for histopathology.

Saltwater entry - 07 S1. 2 fresh morts sampled. Fish #1 (histo) was septicemic, also thick white lining on liver and kidney.

Final Diagnosis

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

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

1c. Liver: sinusoidal congestion, multifocal, mild

2. Heart, compact cortical layer: congestion and hemorrhage, multifocal, moderate

Final Comment: This fish has several lesions that provide clues to the cause of morbidity, but none of the lesions are of sufficient severity for me to assign a cause of death. Comments on specific lesions follow:

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

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

Sinusoidal congestion in the liver is evidence of circulating vasodilators; sometimes it occurs as a postmortem artifact. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. Consider bacteriology and PCR for VHSV and IHNV.

Congestion and hemorrhage in the stratum compactum of the heart (i.e., the peripheral layer of dense cardiac muscle) is a distinctive lesion that I started seeing in 2008 in clinical submissions from both Atlantic and Pacific salmon; however, I have not seen this change in any of the

2154 hearts I have examined since 2006 from the BC Audit and Surveillance Program. This might be a congenital malformation. Alternatively, it might be a result of endothelial damage, with bacterial and viral infections as possible differentials.

Histopathology

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

Slide 1 (7228-1) - heart, spleen, liver, head kidney, trunk kidney, skin/skeletal muscle, mesenteric adipose tissue

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 05/01/09 @ 10:05 AM

Specimen	ID	Test	Result
Tissue	A)7228-1	PCR - IHNV	Negative
Tissue	B)7228-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 05/01/09 @ 10:04 AM

Specimen	ID	Test	Result
Tissue	A)7228-1	PCR - VHSV	Negative
Tissue	B)7228-2	PCR - VHSV	Negative

History of Communication

Date	To	Description
05/01/09 2:17 PM	Marine Harvest Canada - fax	bc report generated
05/07/09 1:36 PM	Marine Harvest Canada - fax	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: 09-1647

Last Updated: 05/05/09 10:58 AM
Pathologist: Gary D. Marty
Received Date: 05/01/09
Collected Date: 04/27/09
Client Ref No: PO 12920

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted fish tissues (head kidney, liver and spleen) for PCR. Confirm R/O VHS, IHN.

Fish #1 - liquid muscle flesh on flank at ceca. Large hemorrhage in heart, liver, ceca.

Fish #2 - petechae ceca, muscle walls, ascites, hemorrhaged swimbladder.

Farm name: Bare Bluff. Atlantic salmon. Age: 100g. Saltwater. Vaccinated: yes. Prior submission: 09-1483. Fish died: Apr. 27/09.

Molecular Diagnostics

PCR - IHNV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 05/05/09 @ 10:58 AM

Specimen	ID	Test	Result
Tissue	fish x2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 05/05/09 @ 10:58 AM

Specimen	ID	Test	Result
Tissue	fish x2	PCR - VHSV	Negative

History of Communication

Date	To	Description
05/07/09 10:32 AM	Mainstream Canada-T - fax	bc report generated
05/07/09 10:32 AM	Dr. Peter McKenzie - e-mail	bc report generated
05/11/09 1:28 PM	Mainstream Canada - 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: 09-1648

Last Updated: 05/05/09 12:39 PM
Pathologist: Gary D. Marty
Received Date: 05/01/09
Collected Date: 04/27/09
Client Ref No: PO 12920

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted 1 bacterial culture to confirm Aeromonas salmonicida.

Furuncles on dorsal surface. Systemic. Speckled gills.

Species: Atlantic salmon. Age: 70g. Saltwater. Vaccinated: yes. Prior submission: no.

Farm name: Saranac.

PO #12920.

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 05/05/09 @ 12:39 PM

Specimen	ID	Isolate	Result	Level
Isolate	2/3	Aeromonas salmonicida	Positive	
Isolate	1/4		No Bacteria Isolated	

Fish Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 05/05/09 @ 12:39 PM

Organism	ID	e	ffc	sor	s3	sxt	ot
Aeromonas salmonicida	2/3	s	s		s	s	s

**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline Unable to perform Romet sensitivity due to supplier shortage. Testing will resume for Romet as soon as supply becomes available.

History of Communication

Date	To	Description

05/07/09 10:04 AM	Mainstream Canada-T - fax	bc report generated
05/07/09 10:04 AM	Dr. Peter McKenzie - e-mail	bc report generated
05/11/09 1:29 PM	Mainstream Canada - 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: 09-1713

Last Updated: 05/08/09 11:46 AM
Pathologist: Gary D. Marty
Received Date: 05/05/09
Collected Date: 05/04/09
Client Ref No: 7234

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

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

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

Case History

Submitted fresh and formalized fish tissue for histopathology and PCR (IHN and VHS).

Saltwater entry 08 S1. Vaccinated. Euthanized - percussion.6 random fish sampled during brood sort. #1-5 appeared normal except one of these had skin lesion. #19 had granuloma's in liver. Viral was from fresh mort Pen 8 - hem on swimbladder.

Final Diagnosis

1a. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 4A)

1b. Liver: hepatocellular cytoplasmic lipid, diffuse, small amounts (slides 2A, 3A, 4A, 5A)

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

1d. Liver: hepatitis, granulomatous, multifocal, with fibrin and multinucleate giant cells; consistent with vaccine material, moderate (slide 19)

2a. Heart: endocarditis, multifocal, lymphohistiocytic, mild (slides 1A, 2A, 3A, 4A, 5A, 19)

2b. Heart: myocardial karyomegaly, multifocal, mild (slide 3A)

2c. Heart: epicarditis, multifocal, lymphocytic, with eosinophilic granular cells, mild (slide 19)

3a. Spleen: parenchymal golden pigment (lipofuscin?), scattered, intracellular, mild (slides 1A, 2A, 3A, 4A, 5A), moderate (slide 19)

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

4. Intestinal ceca: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 2A), moderate (slide 3A)

5. Skin: dermatitis, ulcerative, granulomatous, fibrinous, regionally diffuse, with re- epithelialisation, severe (slide 4A)

6a. Gil: interfilament metazoan organism (monogenean parasite?), focal (one organism), mild (slide 4B)

6b. Gil: interfilament metazoan organism (copepod?), focal (one organism), mild (slide 1B)

7. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slide 19)

Final Comment: These fish have several lesions that are common among farmed Atlantic salmon in British Columbia, but none are of sufficient severity to be associated with severe morbidity. Comments on specific lesions follow:

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

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.

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.

Granulomas in the liver on slide 19 provide evidence of chronic immune stimulation, and vaccine material is the most likely cause. Vaccine might have been injected directly into the liver of this fish instead of the peritoneal cavity. Lack of organisms on the Gram stain rules out the primary differential: infection with *Renibacterium salmoninarum* .

Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 18% of the 467 Atlantic salmon hearts examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program.

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.4% of the 1609 Atlantic salmon hearts examined as part of the province's Fish Health Auditing and Surveillance Program from 2006 through 2008). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. Lymphocytic epicarditis is common in farmed salmon, but eosinophilic granular cells are an unusual component to the epicarditis. They might be related to the granulomas in the liver.

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.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Fibrinous dermatitis is slide 4A is focused in the scale slots: the scales are replaced by fibrin and neutrophils, and the overlying epidermis is thin and poorly differentiated. This probably was a focus of scale loss that is now resolving. Granulomatous dermatitis is focused on the deep margin of the dermis; it might be a result of ulcers adjacent to the plane of section. If this is a focal lesion, it seems to be resolving with significant inflammation. If this is one of many foci, fish health is likely to be compromised.

Monogeneans are common external parasites in fish. Monogeneans attach to the skin and gills using prominent hooklets that can cause considerable damage to the fish when parasites are in high numbers. Some types are oviparous (produce eggs), whereas others are viviparous (give birth to individuals which already contain well-developed embryos). Because they have a direct life cycle (no intermediate hosts), parasite numbers can increase rapidly when fish are crowded. Damage to the host by the hooklets can lead to secondary bacterial infections. Treatment can be effective if the underlying cause of stress and/or suboptimal water quality is addressed. [Source: PTK Woo. 1995. Fish Diseases and Disorders. Volume 1. Protozoan and Metazoan Infections.] Severity of infection is best assessed by gill wet mounts, but the single organism in slide 4B is probably not a major pathogen. The putative copepod in slide 1B is about 500 µm in diameter.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469).

Histopathology

Formalin-fixed tissues were submitted in 11 cassettes for histopathology. The B cassettes (gills) were decalcified in Protocol 1 (8% formic acid solution) for 30 minutes before being processed routinely into paraffin.

Slides 1A (7234-1 4/30/09), 2A (7234-2 4/30/09), 3A (7234-3 4/30/09), 5A (7234-5 4/30/09) and 19 (7234-19 4/30/09) - heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, and mesenteric adipose tissue

Slide 4A (7234-4 4/30/09) - heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue, skin/skeletal muscle

Slides 1B (7234-1 4/30/09), 2B (7234-2 4/30/09), 3B (7234-3 4/30/09), 4B (7234-4 4/30/09) and 5B (7234-5 4/30/09) - gill

A Twort's Gram stain was done on a section from the same block as slide 19. All organs on all slides were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Liver autolysis: none (slides 1A, 2A, 3A, 4A, 5A, 19). Large foci of erythrocytes (e.g., gill 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, most likely in this case, with acid decalcification). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 05/07/09 @ 3:20 PM

Specimen	ID	Test	Result
Tissue	organ 7234	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 05/07/09 @ 3:20 PM

Specimen	ID	Test	Result
Tissue	organ 7234	PCR - VHSV	Negative

History of Communication

Date	To	Description
05/08/09 11:47 AM	Marine Harvest Canada - fax	bc report generated
05/13/09 3:11 PM	Marine Harvest Canada - fax	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: 09-1714

Last Updated: 05/08/09 1:53 PM
Pathologist: Gary D. Marty
Received Date: 05/05/09
Collected Date: 05/04/09
Client Ref No: 7235

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

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

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

Case History

Submitted fresh and formalized fish tissue for histopathology and PCR (IHN and VHS).

Saltwater entry 07 S0. Vaccinated. 2 fresh morts - hem in peritoneum and liver.

Final Diagnosis

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

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

1c. Liver: hepatocellular cytoplasmic lipid, diffuse, small amounts (slides 2A)

3a. Spleen: parenchymal golden pigment (lipofuscin?), scattered, intracellular, mild (slide 1A), moderate (slide 2A)

3b. Spleen: peritonitis, chronic, fibrinous, diffuse, moderate (slide 1A)

3c. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1A, 2A)

4. Kidney: glomerular capillary congestion, generalized, diffuse, moderate (slides 1A, 2A)

5. Mesenteric adipose tissue: capillary congestion, diffuse, moderate (slide 2A)

Final Comment: Lesions in the liver and spleen (both fish), and spleen (fish 2) are consistent with death due to some type of septic change. Comments on specific lesions follow:

In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria (e.g., *Listonella anguillarum*), and infection with VHSV; as in this case, the cause often remains unknown. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte

nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

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.

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.

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 .

Chronic peritonitis with fibrin (slide 2A) is an unusual presentation for vaccine-related peritonitis in Atlantic salmon, particularly in older fish; lack of organisms on the Gram stain rules out *Renibacterium salmoninarum* as a differential. Chronic peritonitis without fibrin (slide 1A) is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Glomerular capillary congestion is a distinctive lesion that occurs occasionally in Atlantic salmon. The change is not associated with hemorrhage or mesangial changes. It might be a result of passive postmortem congestion of the glomerular capillary supply. It is not described in major fish pathology textbooks and, presumably, is not significant for fish health.

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

Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology.

Slides 1A (7235-1) and 2A (7235-2) - brain, heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slides 1B (7235-1) and 2B (7235-2) - gill

A Twort's Gram stain was done on a section from the same block as slide 2A. All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Liver autolysis: mild (slide 2A), severe (slide 1A). 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). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 05/07/09 @ 3:20 PM

Specimen	ID	Test	Result
Tissue	organ 7235-1	PCR - IHNV	Negative
Tissue	organ 7235-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 05/07/09 @ 3:20 PM

Specimen	ID	Test	Result

Tissue	organ 7235-1	PCR - VHSV	Negative
Tissue	organ 7235-2	PCR - VHSV	Negative

History of Communication

Date	To	Description
05/08/09 1:54 PM	Marine Harvest Canada - fax	bc report generated
05/13/09 3:12 PM	Marine Harvest Canada - fax	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: 09-1766

Last Updated: 05/13/09 3:51 PM
Pathologist: Gary D. Marty
Received Date: 05/08/09
Collected Date: 05/06/09
Client Ref No: 12942

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted fish fresh and formalized tissue for bacteriology and histology and swab for PCR.

300g. Saltwater. Vaccinated. Duration of illness - 7 days. Very few gross signs. some hemorrhaging cecea. some acites. Mucus in gills. Confirm/Rule out disease.

Final Diagnosis

1. Gill: interlamellar filamentous bacteria, multifocal, mild (slide 2B)

2a. Liver: sinusoidal congestion, multifocal, moderate (slide 3A)

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

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

Final Comment: Fish 2 has bacteria in the gills that might have contributed to its death, but the other fish have no lesions of sufficient severity to explain their death. This might indicate that a small but significant proportion of the fish at the farm are dying of filamentous bacterial infection. Comments on specific lesions follow:

Thin rod-shaped to filamentous bacteria are common on the gills of debilitated juvenile salmonids. Although bacterial culture or PCR is required for a definitive diagnosis, the common species in the gill include *Flavobacterium columnare* (the cause of columnaris disease), *F. psychrophilum* (the cause of coldwater disease), *F. branchiophilum* (the cause of bacterial gill disease), and in marine waters *Tenacibaculum maritimum* (one cause of necrotizing branchitis). Infections are usually associated with crowding, poor water quality, or stress. Small numbers of organisms on histology sections are often associated with significant clinical disease (bacterial numbers might decrease during processing).

In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria (e.g., *Listonella anguillarum*), and infection with VHSV; as in this case, the cause often remains unknown. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin

concentrations (unpublished data).

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

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology (Bawden 1 through 4, May 6/09). After processing into paraffin, the gill was separated into a second cassette so that for each fish the 'A' cassette contains multiple organs (liver, head kidney, heart, brain, intestinal ceca; slide 2A also has trunk kidney and mesenteric adipose tissue; slide 4A lack intestinal ceca), and the 'B' cassette contains gill. Fish numbers on submitted cassettes correspond to slide numbers in this report. All organs were examined. Organs not listed elsewhere have no significant lesions.

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

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 05/11/09 @ 11:35 AM

Specimen	ID	Isolate	Result	Level
Isolate	1		No Bacteria Isolated	
Isolate	2	Vibrio tubiashii	Positive	
Isolate	3		No Bacteria Isolated	
Isolate	4		No Bacteria Isolated	

Fish Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 05/11/09 @ 11:35 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Vibrio tubiashii	2	r	s		r	s	s

**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline Unable to perform Romet sensitivity due to supplier shortage. Testing will resume for Romet as soon as supply becomes available.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/13/09 @ 3:51 PM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/13/09 @ 3:51 PM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

History of Communication

Date	To	Description
05/20/09 5:07 PM	Mainstream Canada-T - fax	bc report generated
05/20/09 5:07 PM	Dr. Peter McKenzie - e-mail	bc report generated
05/26/09 8:47 AM	Dr. Peter McKenzie - e-mail	Case Invoiced
05/29/09 11:48 AM	Dr. Peter McKenzie - e-mail	bc report generated
05/29/09 11:48 AM	Mainstream Canada-T - fax	bc report generated

Gary D. Marty
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Final Report AHC Case: 09-1774

Last Updated: 05/13/09 3:51 PM
Pathologist: Gary D. Marty
Received Date: 05/08/09
Collected Date: 05/07/09
Client Ref No: VP060509

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Nathan Cassan - Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 3 fish tissues for viral culture.

Saltwater. Vaccinated. 3 dead. Smolts just entered into SW and pen showing signs of petechial hemorrhage on pyloric caeca. 3 very fresh fish frozen for shipment and delayed viral culture.

Animal Location - Venture Point.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/13/09 @ 3:51 PM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/13/09 @ 3:51 PM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Negative

Staff Comments:
Note: Isolating viruses using virus culture depends on the presence of viable virus particles in the submitted tissues. Because freezing renders most viral hemorrhagic septicemia virus (VHSV) nonviable, many significant cases of VHSV are missed if only virus culture is done. However, freezing does little harm to RNA (the molecule tested by PCR). Therefore, on tissues that are frozen before submission, we do either PCR only or PCR followed by viral culture. For this case, we substituted PCR for the requested viral culture. Tissues for viral culture need to be submitted fresh (on ice) without previous freezing.

History of Communication

Date	To	Description
05/20/09 5:12 PM	Mainstream Canada - e-mail	bc report generated
05/20/09 5:12 PM	Dr. Peter McKenzie - e-mail	bc report generated
05/26/09 8:48 AM	Dr. Peter McKenzie - 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: 09-1825

Last Updated: 05/14/09 9:43 AM
Pathologist: Gary D. Marty
Received Date: 05/12/09
Collected Date:
Client Ref No: 7233

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

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

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

Case History

Submitted fresh fish tissue for PCR (IHN and VHS).

Saltwater entry 2009 S1. Vaccinated. Two samples labeled #1 and #2 for PCR for IHN and VHS. Fresh morts with suspect VHS.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 05/14/09 @ 9:43 AM

Specimen	ID	Test	Result
Tissue	1) 7233-1	PCR - IHNV	Negative
Tissue	2) 7233-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Ken Sojonky on 05/14/09 @ 9:43 AM

Specimen	ID	Test	Result
Tissue	1) 7233-1	PCR - VHSV	Negative
Tissue	2) 7233-2	PCR - VHSV	Negative

History of Communication

Date	To	Description
05/14/09 2:22 PM	Marine Harvest Canada - fax	bc report generated
05/21/09 2:27 PM	Marine Harvest Canada - fax	Case Invoiced

END OF REPORT

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Final Report AHC Case: 09-1826

Last Updated: 05/15/09 1:55 PM
Pathologist: Gary D. Marty
Received Date: 05/12/09
Collected Date: 05/03/09
Client Ref No: 7247

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

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

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

Case History

Submitted formalized fish tissue for histopathology.

Saltwater entry - 08 S1. Vaccinated. Euthanized - TMS. Routine screening of broodstock. Histo samples 1-10. #1-5 (pen 12) #6-10 (pen 11).

Final Diagnosis

Most significant lesions in these fish:

1. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slide 8), moderate (slides 1, 7, 9, 10), severe (slides, 2, 3, 4, 5, 6)

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

3. Intestine: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1, 10), moderate (slides 6, 7, 8, 9)

4. Brain: meningitis, lymphocytic, multifocal, mild (slide 3)

5. Head kidney: nephritis, interstitial, granulomatous-histiocytic, focal, mild (slide 9)

6. Head kidney: interstitial cell hyperplasia, multifocal, mild (slide 10)

Final Comment: All fish have one or more lesions, but none of the lesions are severe enough that they would have caused significant morbidity. Details for this case are included on an Excel spreadsheet (2009-1826.xls) that is not included with the official final report generated by the Animal Health Centre's VADDS database (the database cannot handle spreadsheets). Lesions scored on the spreadsheet are the same as those included in the histopathology part of the BC Fish Health Auditing and Surveillance Program. Therefore, the spreadsheet includes many lesions not present in this case. Specific comments on significant lesions in these fish follow:

Hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition. In fish with severe lipidosis in this case, some lipid vacuoles are larger than normal hepatocytes; this is evidence that the accumulations are abnormal and not just physiologic lipid storage.

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

inflammatory mediators) or come from outside the fish (e.g., from the water or the feed). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 14% of the 468 Atlantic salmon livers examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2008 was sufficient to identify a trend towards greater prevalence in the winter and spring (21-30%) than in the summer and fall (1.9-4.4%).

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

Meningitis is evidence of immune stimulation; differentials include viruses, bacteria, or parasites. In the single affected fish (#3), the section contains no obvious organisms.

Two fish have lesions in the head kidney that involve macrophages (histiocytes), and the total cross sectional area of abnormal tissue is less that 0.5 mm in diameter (i.e., not very large). In fish #9, the macrophages are limited to a single focus diagnosed as granulomatous inflammation. The focus is evidence of chronic immune stimulation; differentials include a vaccine reaction and *Renibacterium salmoninarum* . In fish #9, the macrophages are limited to 3 small foci, each less than 150 µm in diameter. Again this is evidence of chronic immune stimulation, but *Renibacterium salmoninarum* seems unlikely in these foci.

For comments on other lesions, see the "Abbreviations" worksheet in the spreadsheet.

Histopathology

Formalin-fixed tissues were submitted in 10 cassettes for histopathology. All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Details are included on the spreadsheet (2009-1826.xls). Mild artifact is normal for paraffin-embedded sections. Tissues have no postfixation dehydration and no acid hematin deposits. Many of the sections of intestine have greater autolysis that I would expect for euthanized fish. Some of this might be a result of these being healthy fish, and digestive enzymes are more active than the sick/dead fish I normally examine. Also, it is helpful to split organs from larger fish into two cassettes (e.g. one cassette for liver, head kidney, trunk kidney, heart, and spleen; a second cassette for brain and intestinal ceca). The sampling protocol for intestinal ceca used for the BC Fish Health Auditing and Surveillance Program yields consistently good results:

"...because this organ autolyzes rapidly, two ends need to opened for fixation: first make a transverse cut through the distal tip of the ceca (this opens one end); then make another transverse cut 5-10 mm proximal to the first cut (this opens the second end). Histotechnicians at the Animal Health Centre can trim the ceca further so they can be embedded and sectioned transversely. Include mesenteric fat (which will have exocrine pancreas) in your sample."

History of Communication

Date	To	Description
05/15/09 1:55 PM	Marine Harvest Canada - fax	bc report generated
05/26/09 8:56 AM	Marine Harvest Canada - fax	Case Invoiced

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

END OF REPORT

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Animal Health Centre

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1767 Angus Campbell Road
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Facsimile: (604) 556-3010
Toll-Free : 1-800-661-9903

Final Report AHC Case: 09-1876

Last Updated: 05/20/09 1:20 PM
Pathologist: Gary D. Marty
Received Date: 05/14/09
Collected Date: 05/13/09
Client Ref No: PO 13209

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted formalized tissues and one slide. Request histo and PCR. Confirm, rule out VHS, IHN, Bacterial kidney disease, Piscirickettsia.

Farm name: Millar Channel.

Atlantic salmon. Age: 800g. Saltwater. Vaccinated. Prior submission: No.

Massive hemorrhaging - flesh, liver, cecae fat, swim bladder. Pale gills, liver. Swollen spleen. Bloody ascites. Mucus in digestive tract.

Final Diagnosis

1a. Liver: peritonitis and hepatitis, granulomatous, with fibrosis and multinucleate giant cells which sometimes contains golden-brown pigmented fungal hyphae, severe (slide 1)

1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 5), moderate (slides 1, 2, 3, 4)

1c. Liver: hepatocellular fatty change (lipidosis), diffuse, mild (slides 3, 5)

2. Mesenteric adipose tissue: capillary congestion, multifocal, mild (slide 5), moderate (slides 1, 3)

3a. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, mild (slide 3), moderate (slide 4)

3b. Trunk kidney: interstitial vascular congestion, diffuse, mild (slide 4)

3c. Trunk kidney: interstitial edema, diffuse, moderate (slide 5)

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

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

5. Intestinal ceca: peritonitis, chronic, focal to diffuse, with fibrocellular fronds, mild (slides 2, 4), moderate (slide 3)

6. Heart: endocarditis, diffuse, with endothelial cell hypertrophy, mild (slide 2)

Final Comment:

The fish in slide #1 died of complications related to severe peritonitis and hepatitis. Pigmented fungi are common in the environment, and some can be facultative pathogens. Conditions that contribute to these types of infections include immunosuppression (common with stress) and a penetrating foreign body. The nature of the inflammation is evidence that the process has been going on for at least a few days.

The cause of death for fish #2 cannot be determined from the organs examined. Fish #s 3 and 4 died from complications related to renal tubular necrosis and--in fish #3 only--vascular congestion in the mesenteric adipose tissue; lesions are consistent with the PCR-positive result for VHSV. Tissues from fish #5 did not include trunk kidney, but this fish also has lesions consistent with death due to VHSV: interstitial edema in the head kidney and vascular congestion in the mesenteric adipose tissue. Comments on specific lesions follow:

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

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

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.

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).

Renal congestion is evidence of circulating vasodilators. In this case (fish #4), it is probably a result of infection with VHSV; differentials include substances released from inflammatory cells or bacteria.

Interstitial cells in the kidney of fish #5 are separated by clear space characteristic of edema. Edema is evidence of increased vascular leakage that can result from exposure to vasodilators. Common sources of vasodilators include bacterial toxins and inflammatory mediators (as might be released in a viral infection; in this case, probably VHSV). I have associated *Yersinia ruckeri* infection with interstitial renal edema, but this case has no obvious bacteria.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29% ; n = 469) and Pacific salmon (prevalence = 31% ; n = 118).

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

The pattern of inflammation in the heart of fish #2 is consistent with a systemic immune stimulation, usually a result of a bacterial or viral infection (e.g., VHSV); the cause is often not determined. Hypertrophic endothelial cells are basophilic and up to 10 µm thick.

Histopathology

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

Slide 1 (Bawden 2, May 12/09) - brain, heart, spleen, liver, intestinal ceca, mesenteric adipose tissue

Slide 2 (Millar 3) - brain, heart, spleen, liver, intestinal ceca, head kidney

Slide 3 (Millar 1, May 12/09) - brain, gill, heart, liver, intestinal ceca, head kidney, trunk kidney, mesenteric adipose tissue

Slide 4 (Bawden May 12/09) - brain, gill, heart, spleen, liver, intestinal ceca, trunk kidney, mesenteric adipose tissue

Slide 5 (5 Bawden May 12/09) - brain, gill, heart, spleen, liver, intestinal ceca, head kidney

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

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

Molecular Diagnostics

PCR-Renibacterium salmoni Resulted by: A Scouras Verified by: Dr. J. Robinson on 05/20/09 @ 1:20 PM

Specimen	ID	Test	Result
Tissue		PCR-Renibacterium salmoninaru	Negative

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/19/09 @ 1:15 PM

Specimen	ID	Test	Result
Tissue		PCR - IHNV	Negative

PCR-Piscirickettsia salmo Resulted by: A Scouras Verified by: Dr. J. Robinson on 05/20/09 @ 1:20 PM

Specimen	ID	Test	Result
Tissue		PCR - Piscirickettsia salmonis	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/19/09 @ 1:15 PM

Specimen	ID	Test	Result
Tissue		PCR - VHSV	Positive

History of Communication

Date	To	Description
05/19/09 3:19 PM	Mainstream Canada-T - fax	bc report generated
05/19/09 3:19 PM	Dr. Peter McKenzie - e-mail	bc report generated
05/20/09 5:16 PM	Mainstream Canada-T - fax	bc report generated
05/20/09 5:16 PM	Dr. Peter McKenzie - e-mail	bc report generated
05/26/09 10:06 AM	Mainstream Canada - 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: 09-1914

Last Updated: 05/20/09 1:55 PM
Pathologist: Gary D. Marty
Received Date: 05/19/09
Collected Date: 05/14/09
Client Ref No: MI051409

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Kelly Abel - Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted formalized Atlantic Salmon tissue for histopathology.

Saltwater. Vaccinated. 1 dead. Submitted 1 cassette containing above noted tissues. Vet suspects bacterial kidney diseases.

Farm location - Maude Island.

Final Diagnosis

1. Trunk kidney: moderate numbers of interstitial intracytoplasmic eosinophilic granules, diffuse

2. Spleen: parenchymal golden pigment (lipofuscin?), scattered, intracellular, moderate

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

Final Comment: These tissues have no lesions clearly associated with *Renibacterium salmoninarum* infection. Although eosinophilic granules in the renal interstitium (one of the features of this case) sometimes occur in cases of cerebral *R. salmoninarum* infection, the brain was not included for analysis.

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

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%). Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

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

Histopathology

Formalin-fixed tissues were submitted in 1 cassette for histopathology. After processing into paraffin, gills were removed from the original (A) cassette and placed in a separate (B) cassette.

Slide 1A (Pen 3 05/14/09 Maude Island) - heart, spleen, liver, intestine, intestinal cecum, trunk kidney (2 pieces)

Slide 1B (Pen 3 05/14/09 Maude Island) - gill

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

Quality control: Liver autolysis: none. Large foci of erythrocytes (e.g., liver) have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have no postfixation dehydration.

History of Communication

Date	To	Description
05/20/09 1:58 PM	Mainstream Canada - e-mail	bc report generated
05/20/09 1:58 PM	Dr. Peter McKenzie - e-mail	bc report generated
05/21/09 2:40 PM	Dr. Peter McKenzie - 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: 09-1915

Last Updated: 05/28/09 3:12 PM
Pathologist: Gary D. Marty
Received Date: 05/19/09
Collected Date: 05/13/09
Client Ref No: VP130509

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Nathan Cassan - Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 4 Atlantic Salmon bacti plates for bacteriology (bacti ident).

Saltwater. Vaccinated. 8 fish with hem's on pyloric and swim bladder. Suspect ERM. Looking for ident on bacti present. Plated kidney and brain with loop.

Farm Id - Venture Point

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 05/28/09 @ 3:11 PM

Specimen	ID	Isolate	Result	Level
Kidney	Fish 1	Aliivibrio wodanis	Positive	
Kidney	Fish 1	Vibrio pomeroyi	Positive	
Brain	Fish 1	Aliivibrio wodanis	Positive	
Brain	Fish 1	Vibrio pomeroyi	Positive	
Kidney	Fish 2	Aliivibrio wodanis	Positive	
Kidney	Fish 2	Vibrio pomeroyi	Positive	
Brain	Fish 2	Aliivibrio wodanis	Positive	
Kidney	Fish 3		No Bacteria Isolated	
Brain	Fish 3	Aliivibrio wodanis	Positive	
Brain	Fish 3	Vibrio pomeroyi	Positive	
Kidney	Fish 4	Aliivibrio wodanis	Positive	
Kidney	Fish 4	Vibrio pomeroyi	Positive	
Brain	Fish 4	Aliivibrio wodanis	Positive	
Brain	Fish 4	Vibrio pomeroyi	Positive	
Kidney	Fish 5	Aliivibrio wodanis	Positive	

Kidney	Fish 5	Vibrio pomeroyi	Positive
Brain	Fish 5	Aliivibrio wodanis	Positive
Brain	Fish 5	Vibrio pomeroyi	Positive
Kidney	Fish 6	Aliivibrio wodanis	Positive
Kidney	Fish 6	Vibrio pomeroyi	Positive
Brain	Fish 6	Aliivibrio wodanis	Positive
Brain	Fish 6	Vibrio pomeroyi	Positive
Kidney	Fish 7	Aliivibrio wodanis	Positive
Brain	Fish 7	Aliivibrio wodanis	Positive
Brain	Fish 7	Vibrio pomeroyi	Positive
Kidney	Fish 8		No Bacteria Isolated
Brain	Fish 8	Aliivibrio wodanis	Positive
Brain	Fish 8	Vibrio pomeroyi	Positive

Fish Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 05/28/09 @ 3:11 PM

Organism	ID	e	ffc	sor	s3	sxt	ot
Vibrio pomeroyi	Fish 1	s	s		s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline Unable to perform Romet sensitivity due to supplier shortage. Testing will resume for Romet as soon as supply becomes available.							
Aliivibrio wodanis	Fish 1	s	s		s	s	s

Staff Comments:
Preliminary bacteriology results (Alivibrio species) sent to Peter McKenzie and Nathan Cassan via Outlook (2009-05-25 1:26 PM) by GDMarty.

History of Communication

Date	To	Description
05/29/09 11:53 AM	Mainstream Canada - e-mail	bc report generated
05/29/09 11:53 AM	Dr. Peter McKenzie - e-mail	bc report generated
06/04/09 8:35 AM	Mainstream Canada - e-mail	Case Invoiced

Gary D. Marty
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Final Report AHC Case: 09-1932

Last Updated: 05/22/09 10:50 AM
Pathologist: Gary D. Marty
Received Date: 05/20/09
Collected Date:
Client Ref No: 7257

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

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

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

Case History

Submitted fresh and formalized fish tissue for histopathology and PCR (IHN and VHS).

Saltwater entry 07 S0. Vaccinated. Euthanized - percussion. Moribund - petechial hem in liver/swim bladder. Blood in pyloric caeca - fish had been eating.

Final Diagnosis

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

1b. Liver: hepatocellular cytoplasmic lipid, diffuse, moderate amounts

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

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

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

4. Head kidney: nephritis, interstitial, granulomatous, multifocal, mild

Final Comment: This fish has several changes that are common in cultured Atlantic salmon in BC. Although none are of sufficient severity to have killed the fish, sinusoidal congestion in the liver is probably the most significant. Hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).

I suspect that the uniform lipid accumulation in the cytoplasm of hepatocytes in this fish is normal. As a differential, abnormal accumulation of hepatocellular lipid (lipidosis) occurs when fish are not feeding and in cases of inadequate nutrition.

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.

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 28% of the 467 Atlantic salmon hearts examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

The most common organism associated with granulomatous nephritis in salmon is *Renibacterium salmoninarum*, the cause of bacterial kidney disease. However, chronic infections with *Yersinia ruckeri* have also been associated with granulomatous inflammation. The primary differential is a vaccine reaction. The extent of related lesions in this fish is too small to have caused significant morbidity; however, older fish with mild or no lesions in other organs often have severe infection with *Renibacterium salmoninarum* in the brain (not sampled in this fish).

Histopathology

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

Slide 1 (7257-1) - heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, and mesenteric adipose tissue

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/22/09 @ 10:50 AM

Specimen	ID	Test	Result
Tissue	organ #7257	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/22/09 @ 10:50 AM

Specimen	ID	Test	Result
Tissue	organ #7257	PCR - VHSV	Negative

History of Communication

Date	To	Description
05/22/09 1:03 PM	Marine Harvest Canada - fax	bc report generated
05/27/09 2:52 PM	Marine Harvest Canada - fax	Case Invoiced

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

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Final Report AHC Case: 09-1933

Last Updated: 05/22/09 10:51 AM
Pathologist: Gary D. Marty
Received Date: 05/20/09
Collected Date: 05/14/09
Client Ref No: PO #7256

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

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

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

Case History

Submitted fresh Atlantic salmon tissues for PCR - IHN and VHS.

Saltwater entry: 07 SO. Vaccinated. Euthanized: no. Prior submission: no.

Fresh mort with septicemia.

PO #7256.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/22/09 @ 10:50 AM

Specimen	ID	Test	Result
Tissue	organ #7256	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/22/09 @ 10:50 AM

Specimen	ID	Test	Result
Tissue	organ #7256	PCR - VHSV	Negative

History of Communication

Date	To	Description
05/22/09 1:24 PM	Marine Harvest Canada - fax	bc report generated
05/27/09 2:52 PM	Marine Harvest Canada - fax	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: 09-1934

Last Updated: 05/22/09 10:51 AM
Pathologist: Gary D. Marty
Received Date: 05/20/09
Collected Date: 05/19/09
Client Ref No: PO #7240

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

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

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

Case History

Submitted fresh Atlantic Salmon tissues for PCR: IHN and VHS.

Saltwater entry: 07 SO. Vaccinated. Euthanized: no. Prior submission: no.

Fresh mort with septicemia.

PO #7240.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/22/09 @ 10:51 AM

Specimen	ID	Test	Result
Tissue	organ #7240	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 05/22/09 @ 10:51 AM

Specimen	ID	Test	Result
Tissue	organ #7240	PCR - VHSV	Negative

History of Communication

Date	To	Description
05/22/09 1:28 PM	Marine Harvest Canada - fax	bc report generated
05/27/09 2:53 PM	Marine Harvest Canada - fax	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: 09-1966

Last Updated: 06/10/09 10:46 AM
Pathologist: Gary D. Marty
Received Date: 05/22/09
Collected Date: 05/21/09
Client Ref No:

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Nathan Cassan - Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 3 fish bacti plates for bacteriology and formalized tissues from 9 fish for histopathology and PCR (IHN and VHS).

Saltwater. Vaccinated. 9 Dead. Prior submission - VP130509. Original submission was bacti (2009-1915) and now followed by cassettes for histo 4 fish (pen 2/4). Hems present on pyloric ceca and swimbladder. Also 3 plates and 5 cassettes from a pen of different origin (pen 8) displaying furuncles and hem for bacti ident and histo. PCR requested for both sets of cassettes.

Final Diagnosis

Most significant lesions in these fish:

1. Liver: hydropic degeneration and single cell necrosis, scattered, mild (slides 2, 3, 8), moderate (slide 1)

2. Spleen: parenchymal fibrin, multifocal, acute, mild (slide 4), moderate (slide 3)

3. Mesenteric adipose tissue: capillary congestion, multifocal, mild (slides 1, 2, 3), moderate (slide 4)

4. Trunk kidney, liver, spleen: intravascular and interstitial colonies of bacterial rods consistent with *Aeromonas salmonicida*, multifocal, small numbers (slides 6, 8, 9), abundant (slides 5, 7)

Final Comment: Fish from pens 2/4 have lesions consistent with morbidity from exposure to toxins, possibly of bacterial origin. Fish from pen 8 all had intravascular and interstitial bacteria consistent with *Aeromonas salmonicida* . (the cause of furunculosis). Details for this case are included on an Excel spreadsheet (2009-1966.xls) that is not included with the official final report generated by the Animal Health Centre's VADDS database (the database cannot handle spreadsheets). Lesions scored on the spreadsheet are the same as those included in the histopathology part of the BC Fish Health Auditing and Surveillance Program. Therefore, the spreadsheet includes many lesions not present in this case. Specific comments on significant lesions in these fish follow:

Hydropic degeneration among small to moderate numbers of hepatocytes provides evidence that affected livers were being exposed to toxins. Potential sources of the inciting toxins include the water (e.g., algal toxins), 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.

Multifocal fibrin deposits in the spleen (e.g., fish #3) are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin (e.g., the *Alivibrio* sp. cultured from case 2009-1915) or inflammatory cell origin. I have also seen this response in salmon that are PCR positive for VHSV. Lack of remodelling of the fibrin is consistent with these deposits forming just before death.

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

Aeromonas salmonicida is the cause of furunculosis in salmonids. It is most common in freshwater-reared salmonids, but also causes problems in saltwater-reared salmonids (especially Atlantic salmon) and other marine fish species. Infected fish are considered the primary reservoir of the bacterium, but bacteria can survive in fresh and salt water. [Source: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.]

For comments on other lesions, see the "Abbreviations" worksheet in the spreadsheet. Formalin fixation renders PCR ineffective for diagnosis of RNA viruses like VHSV and IHNV; therefore, PCR was not done on these samples. For VHSV or IHNV PCR, submit unfixed tissues (they can be previously frozen with little loss of PCR reactivity).

Histopathology

Formalin-fixed tissues were submitted in 9 cassettes for histopathology.

Slides 1 (VP200509-1), 2 (VP200509-2), 5 (VP200509-1), 6 (VP200509-2) and 7 (VP200509-3) - brain, spleen, liver, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide 3 (VP200509-3) - brain, spleen, liver, intestine, trunk kidney, mesenteric adipose tissue

Slide 4 (VP200509-4) - brain, spleen, liver, intestine, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide 8 (VP200509-4) - brain, spleen, liver, stomach, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide 9 (VP200509-5) - eye, brain, liver, stomach, trunk kidney, intestinal ceca, mesenteric adipose tissue

All organs were examined. Organs not listed elsewhere (e.g., the eye in slide 9) have no significant lesions.

Quality control: Details are included on the spreadsheet (2009-1966.xls). Mild artifact is normal for paraffin-embedded sections. Large foci of erythrocytes (e.g., liver in slide 6) have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue). Tissues have no postfixation dehydration.

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 06/10/09 @ 10:46 AM

Specimen	ID	Isolate	Result	Level
Isolate	#1-kidney	Aeromonas sp.	Positive	
**: Aeromonas sp. is not Aeromonas salmonicida				
Isolate	#1-brain	Aeromonas sp.	Positive	
Isolate	#2-kidney	Aeromonas sp.	Positive	
Isolate	#2-brain	Aeromonas sp.	Positive	
Isolate	#3-kidney	Aeromonas sp.	Positive	
Isolate	#3-brain	Aeromonas sp.	Positive	
Isolate	#3-brain	Aliivibrio wodanis	Positive	
Isolate	#4-kidney	Aeromonas sp.	Positive	

Isolate	#4-brain	Aeromonas sp.	Positive
Isolate	#4-brain	Aliivibrio wodanis	Positive
Isolate	#5-kidney	Aeromonas sp.	Positive
Isolate	#5-brain	Aeromonas sp.	Positive

Fish Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 05/29/09 @ 12:57 PM

Organism	ID	e	ffc	sor	s3	sxt	ot
Aeromonas sp.	#1-kidney	s	s		s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline Unable to perform Romet sensitivity due to supplier shortage. Testing will resume for Romet as soon as supply becomes available.							
Aliivibrio wodanis	#3-brain	s	s		s	s	s

Staff Comments:
Excel spreadsheet with histopathology results e-mailed to Nathan Cassan and Dr. Mckenzie via Outlook 2009-05-25 4:09 PM (by GDMarty).

History of Communication

Date	To	Description
05/25/09 4:04 PM	Mainstream Canada - e-mail	bc report generated
05/25/09 4:04 PM	Dr. Peter McKenzie - e-mail	bc report generated
06/11/09 12:16 PM	Mainstream Canada - e-mail	bc report generated
06/11/09 12:17 PM	Dr. Peter McKenzie - e-mail	bc report generated
06/15/09 10:14 AM	Mainstream Canada - e-mail	Case Invoiced

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

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Telephone : (604) 556-3003
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Toll-Free : 1-800-661-9903

Final Report AHC Case: 09-1989

Last Updated: 05/26/09 2:45 PM
Pathologist: Gary D. Marty
Received Date: 05/25/09
Collected Date: 05/22/09
Client Ref No: PO 13234

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted formalized tissue from 5 fish for histopathology.

Saltwater. Vaccinated. 350g. Duration of illness - 2 weeks. Prior submission - PO 12942 (2009-1766). Confirm/rule out disease. Look for plankton in gills. Scale loss. Some petechial hemorrhaging in cecea fat. Some acites. Mucus in gills.

Farm Location - Bawden Point

Final Diagnosis

1. Gill: interfilament diatom spines/setae (Chaetoceros sp.), focal, mild (slides 1 , 4)

2. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 5), moderate (slide 4)

Final Comment: None of these fish have lesions of sufficient severity to explain their death. However, two of the fish have diatoms in the gills that might have stressed the affected fish. The diatoms *Chaetoceros concavicornis*, *C. convolutus*, and a *Corethron sp*. have been associated with mortality of salmon reared in seawater netpens at numbers as low as 5/mL (Taylor and Harrison 2002). The gills in slide #s 1 and 4 each have a single focus of individual spines/setae that are about 3.5 µm in diameter. The gills have no associated inflammatory response; however, severe autolysis in these specimens might mask subtle inflammation.

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.

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

Histopathology

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

Slide 1 (Bawden May 21/09) - brain, gill, heart, spleen, liver, intestine, head kidney, mesenteric adipose tissue (a 600 × 300 µm piece of mammalian brain is a "float on" artifact from another case)

Slide 2 (Bawden May 21/09) and 4 (Bawden May 21/09) - brain, gill, heart, spleen, liver, intestine, head kidney

Slide 3 (Bawden May 21/09) and 5 (Bawden May 21/09) - brain, gill, heart, spleen, liver, head kidney

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

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

History of Communication

Date	To	Description
05/26/09 2:46 PM	Mainstream Canada-T - fax	bc report generated
05/26/09 2:46 PM	Dr. Peter McKenzie - e-mail	bc report generated
05/27/09 3:03 PM	Mainstream Canada - e-mail	Case Invoiced

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

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Animal Health Centre

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Toll-Free : 1-800-661-9903

Final Report AHC Case: 09-1999

Last Updated: 06/01/09 9:19 AM
Pathologist: Gary D. Marty
Received Date: 05/26/09
Collected Date: 05/25/09
Client Ref No: SP220509

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Nathan Cassen/Kelly Abel-Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted formalized fish tissue for histopathology and bacti plates for bacteriology. **(PCR- no fresh tissue received).**

Saltwater. Vaccinated. 2 in group. Swollen kidneys and hem present. Please provide bacti identification and PCR for IHN and VHS.

Farm Location - Simmonds Point

Final Diagnosis

1. Liver: sinusoidal congestion, multifocal, moderate (slides 1A, 2A)

2. Mesenteric adipose tissue: capillary congestion, diffuse, moderate (slide 1A)

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1A), and with intralesional vacuoles, moderate (slide 2A)

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

5. Mesenteric adipose tissue: steatitis, focal, histiocytic, with intralesional radiating pale eosinophilic material, mild (slide 2A)

6. Brain: capillary (vascular) congestion, diffuse, mild (slide 2A)

Final Comment: Although none of the lesions in these fish are of sufficient severity to explain their death, several lesions provide clues to possible causes of death.

Sinusoidal congestion in the liver is evidence of circulating vasodilators; sometimes it occurs as a postmortem artifact. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. Consider submission of unfixed tissues that can be subjected to PCR for VHSV and IHNV.

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.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe). Vacuoles are probably a result of vaccine material lost during tissue processing.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29% ; n = 469) and Pacific salmon (prevalence = 31% ; n = 118).

Steatitis is an uncommon lesion in salmonids. The radiating pale eosinophilic material might be vaccine material or the remains of a necrotic adipocyte. The single focus in the mesenteric adipose tissue of fish #2 was probably of little consequence for fish health.

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.] Capillary congestion sometimes occurs as part of postmortem change.

Histopathology

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

Slide 1A (Simmonds Fish 2 Pen 6 May ?/09) - brain, heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide 2A (Simmonds Fish 2 Pen 5 May 20/09) - brain, heart, spleen, liver, intestine, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slides 1B (Simmonds Fish 2 Pen 6 May ?/09) and 2B (Simmonds Fish 2 Pen 5 May 20/09) - gill

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

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

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 06/01/09 @ 9:19 AM

Specimen	ID	Isolate	Result	Level
Isolate	Fish 1 Pen 5	Psychrobacter sp.	Positive	
**: Please Note: Plate was overgrown with fungi upon arrival Psychrobacter sp. identified as P.okhotskensis				
Isolate	Fish 1 Pen 6	Psychrobacter sp.	Positive	
**: Please Note: Plate was overgrown with fungi upon arrival Psychrobacter sp. identified as P.okhotskensis				

Fish Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 06/01/09 @ 9:19 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Psychrobacter sp.	Fish 1 Pen 5	s	s		s	r	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline Unable to perform Romet sensitivity due to supplier shortage. Testing will resume for Romet as soon as supply becomes available.							

History of Communication

Date	To	Description
05/27/09 11:49 AM	Mainstream Canada - e-mail	bc report generated
05/27/09 11:49 AM	Dr. Peter McKenzie - e-mail	bc report generated
06/02/09 8:43 AM	Mainstream Canada - e-mail	bc report generated
06/02/09 8:43 AM	Dr. Peter McKenzie - e-mail	bc report generated
06/04/09 12:01 PM	Mainstream Canada - 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: 09-2001

Last Updated: 06/01/09 9:21 AM
Pathologist: Gary D. Marty
Received Date: 05/26/09
Collected Date:
Client Ref No: MI220509

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Nathan Cassan/Kelly Abel-Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 2 fish bacti plates for bacteriology.

Saltwater. Vaccinated. 2 dead. Swollen kidneys, livers, and spleens observed. Please perform bacteriology identification.

Farm Location - Maude Island

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 06/01/09 @ 9:20 AM

Specimen	ID	Isolate	Result	Level
Isolate	Fish 1 Pen 1	Photobacterium phosphoreum	Positive	
**: Please Note: Plate was overgrown with fungi upon arrival				
Isolate	Fish 1 Pen 1	Stenotrophomonas sp.	Positive	
Isolate	Fish 2 Pen 1	Stenotrophomonas sp.	Positive	
**: Please Note: Plate was overgrown with fungi upon arrival Quality control: Plates were submitted sealed with packing tape; this caused the plate to be crushed in an atempt to remove the tape Please submit plates sealed with parafilm in the future				

Fish Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 06/01/09 @ 9:21 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Photobacterium phosphoreum	Fish 1 Pen 1	s	s		s	s	s
Stenotrophomonas sp.	Fish 1 Pen 1	r	s		s	s	r
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline Unable to perform Romet sensitivity due to supplier shortage. Testing will resume for Romet as soon as supply becomes available.							

History of Communication

Date	To	Description
06/01/09 9:58 AM	Mainstream Canada - e-mail	bc report generated
06/01/09 9:58 AM	Dr. Peter McKenzie - e-mail	bc report generated
06/04/09 9:08 AM	Mainstream Canada - 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: 09-2017

Last Updated: 06/02/09 2:57 PM
Pathologist: Gary D. Marty
Received Date: 05/27/09
Collected Date:
Client Ref No: 7264

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

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

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

Case History

Submitted formalized fish tissue for histopathology.

Saltwater entry 2009 S1. Fresh morts. Histo (gills in separate cassette) was collected from 8 fresh dead fish. Mortality has been low except for some predator action at one end of system. Feed rate has been reduced recently. following recent med treatment. Though no visible lesions, many fish are showing severe adhesions. The site was recently pressure washed and new nets were installed.

Final Diagnosis

Most significant lesions in these fish:

1. Liver: hepatitis, granulomatous, focal, with intralesional vacuoles about 40 µm in diameter, severe (slide 3A)

2. Gill: lamellar telangiectasis, multifocal, acute, moderate (slide 8G), severe (slide 5G)

3. Spleen: splenitis, granulomatous, focal, with central neutrophils, moderate (slide 7A)

4. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 4A, 5A), moderate (slides 2A, 3A), severe (slide 6A)

5. Stomach/Intestine: peritonitis, chronic, focal, with fibrocellular fronds, moderate (slides 1A, 2A, 3A, 4A, 5A, 6A, 7A)

Final Comment: All fish have one or more lesions; those most likely to be associated with mortality are the aneurysms in the gills (fish #s 5 and 8). Consistent with clinical findings, all fish had at least moderate peritonitis. Details of all lesions in this case are included on an Excel spreadsheet (2009-2017.xls) that is not included with the official final report generated by the Animal Health Centre's VADDS database (the database cannot handle spreadsheets). Lesions scored on the spreadsheet are the same as those included in the histopathology part of the BC Fish Health Auditing and Surveillance Program. Therefore, the spreadsheet includes many lesions not present in this case. Specific comments on significant lesions in these fish follow:

The large multilocular focus of granulomatous inflammation in slide 3A contains small foci of 40-µm-diameter vacuoles but no organisms on Twort's Gram stain. This inflammation is probably a reaction to a vaccine material, and vacuoles are probably a result of vaccine material lost during tissue processing. The focus of granulomatous inflammation in the spleen in slide 7A probably also has a similar pathogenesis;

neutrophils at the centre of the inflammation probably surrounded a vacuole that was not included in the plane of section.

Telangiectasis in the gill (lamellar capillary aneurysms or ruptured lamellar capillaries) most commonly results from trauma (e.g., handling). Lack of thrombi in the lesions is evidence that the capillaries ruptured soon before death (and, they might have been the cause of death).

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

For comments on other lesions, see the "Abbreviations" worksheet in the spreadsheet.

Histopathology

Formalin-fixed tissues from 8 Atlantic salmon were submitted in 16 cassettes for histopathology. A Twort's Gram stain was done on sections from the same paraffin block as slide #3A. All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Details are included on the spreadsheet (2009-2017.xls). Mild artifact is normal for paraffin-embedded sections. Large foci of erythrocytes (e.g., spleen in slide 7) 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). Tissues have no postfixation dehydration.

History of Communication

Date	To	Description
06/02/09 2:57 PM	Marine Harvest Canada - fax	bc report generated
06/04/09 12:09 PM	Marine Harvest Canada - fax	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: 09-2039

Last Updated: 06/03/09 1:15 PM
Pathologist: Gary D. Marty
Received Date: 05/28/09
Collected Date: 05/25/09
Client Ref No: BW -052509

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Kelly Abel - Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 7 formalized fish tissues for histopathology (VHS).

Saltwater. Vaccinated. 7 bags of cassettes containing tissues.

Farm location - Burdwood

Final Diagnosis

Most significant lesions in these fish:

1. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slides 4, 5, 6, 7, 8, 9, 12)

2. Brain: meningitis, lymphohistiocytic, focal, mild (slides 8, 12)

3. Stomach/Intestine: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 6, 7, 8), moderate (slides 4, 9), severe (slide 5)

Final Comment: All fish have one or more lesions, but none are of sufficient severity to assign a cause of death. The most intriguing lesion is meningitis in fish #s 8 and 12. The mixture of lymphocytes and histiocytes in the meninges is evidence of chronic immune stimulation. Differentials include viruses, bacteria, or parasites, but the sections contain no obvious organisms. One limitation of the samples is that meningitis is often most severe in the brainstem, but the slides contain very little--if any--brainstem for analysis. The fish do not have microscopic lesions that are common in fish with VHSV (e.g., moderate to severe renal tubular necrosis or hepatocellular necrosis). Note, however, that histopathology is not the best method for diagnosis of VHSV; to rule out the presence of VHSV, please submit unfixed tissues for VHSV. Histopathology is a good method for determining the severity of VHSV that is identified by PCR.

Details of all lesions in this case are included on an Excel spreadsheet (2009-2039.xls) that is not included with the official final report generated by the Animal Health Centre's VADDS database (the database cannot handle spreadsheets). Lesions scored on the spreadsheet are the same as those included in the histopathology part of the BC Fish Health Auditing and Surveillance Program. Therefore, the spreadsheet includes many lesions not present in this case. Specific comments on the most significant lesions in these fish 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.

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

For comments on other lesions, see the "Abbreviations" worksheet in the spreadsheet.

Histopathology

Formalin-fixed tissues from 7 Atlantic salmon were submitted in 7 cassettes for histopathology. Each submitted cassette included the writing, "Burdwood Fish 1 May 25". [Note: Animal Health Centre staff assign all cases a unique #; therefore, all that is needed on the cassette is unique designation (e.g., in this case, the pen number); the 'Burdwood Fish 1 May 25" could simply be included once on the submission form, or on the container in which the cassettes are shipped.] After tissues were processed into paraffin, the gills were removed and embedded into a separate (G) block for sectioning. All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Details are included on the spreadsheet (2009-2039.xls). Mild artifact is normal for paraffin-embedded sections. Large foci of erythrocytes (e.g., spleen in slide 6) 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). Tissues have no postfixation dehydration.

History of Communication

Date	To	Description
06/03/09 1:16 PM	Mainstream Canada - e-mail	bc report generated
06/03/09 1:16 PM	Dr. Peter McKenzie - e-mail	bc report generated
06/04/09 12:10 PM	Mainstream Canada - 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
Telephone : (604) 556-3003
Facsimile: (604) 556-3010
Toll-Free : 1-800-661-9903

Final Report AHC Case: 09-2040

Last Updated: 06/30/09 3:51 PM
Pathologist: Gary D. Marty
Received Date: 05/28/09
Collected Date: 05/25/09
Client Ref No: BW -052509

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Kelly Abel - Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 6 fresh fish tissues for viral culture and PCR (VHS).

6 whirl pak containing tissues.

Farms Location - Bardwood

Molecular Diagnostics

PCR - IPNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/18/09 @ 4:11 PM

Specimen	ID	Test	Result
Fluid	SSN-1A	PCR - IPNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/18/09 @ 4:11 PM

Specimen	ID	Test	Result
Fluid	SSN-1A	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/03/09 @ 2:03 PM

Specimen	ID	Test	Result
Tissue	Fish #1	PCR - VHSV	Positive
Tissue	Fish 1, Pen 6	PCR - VHSV	Negative
Tissue	Fish 1, Pen 1	PCR - VHSV	Negative
Tissue	Fish 2, Pen 4	PCR - VHSV	Positive
Tissue	Fish 2, Pen I	PCR - VHSV	Negative
Tissue	Fish 2, Pen 8	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Cheryl Cecconi on 06/30/09 @ 3:51 PM

Specimen	ID	Isolate	Result	Level
Tissue	Fish #1		No viruses isolated	
Tissue	Fish 1, Pen 6		No viruses isolated	
Tissue	Fish 1, Pen 1		No viruses isolated	
Tissue	Fish 2, Pen 4		No viruses isolated	
Tissue	Fish 2, Pen I		No viruses isolated	
Tissue	Fish 2, Pen 8		No viruses isolated	

History of Communication

Date	To	Description
06/03/09 5:27 PM	Mainstream Canada - e-mail	bc report generated
06/03/09 5:27 PM	Dr. Peter McKenzie - e-mail	bc report generated
06/30/09 5:07 PM	Mainstream Canada - e-mail	bc report generated
06/30/09 5:08 PM	Dr. Peter McKenzie - e-mail	bc report generated
07/08/09 2:51 PM	Mainstream Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-2205

Last Updated: 06/11/09 4:01 PM
Pathologist: Gary D. Marty
Received Date: 06/09/09
Collected Date:
Client Ref No: 7290

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

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

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

Case History

Submitted one fresh Atlantic salmon tissue for PCR IHN/VHS.

Saltwater entry 08 S1. 1 fresh mort with hem in swim bladder/peritoneum and muscle.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/11/09 @ 4:01 PM

Specimen	ID	Test	Result
Tissue	organs	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 06/11/09 @ 4:01 PM

Specimen	ID	Test	Result
Tissue	organs	PCR - VHSV	Negative

History of Communication

Date	To	Description
06/11/09 5:19 PM	Marine Harvest Canada - fax	bc report generated
06/15/09 10:52 AM	Marine Harvest Canada - fax	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: 09-2212

Last Updated: 06/11/09 12:41 PM
Pathologist: Gary D. Marty
Received Date: 06/09/09
Collected Date: 06/05/09
Client Ref No: 13274

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted 5 bacti plates for bacteriology and sensitivities.

Saltwater. Vaccinated. 85g. Hemorrhaging - belly, base of fins, cecea, intestine.

Farm Id - Mussel Rock

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 06/11/09 @ 12:41 PM

Specimen	ID	Isolate	Result	Level
Isolate	1	Aeromonas salmonicida	Positive	
Isolate	2	Aeromonas salmonicida	Positive	
Isolate	3	Aeromonas salmonicida	Positive	
Isolate	4	Aeromonas veronii	Positive	
Isolate	5		No Bacteria Isolated	
Isolate	6	Aeromonas salmonicida	Positive	
Isolate	7		No Bacteria Isolated	
Isolate	8		No Bacteria Isolated	
Isolate	9	Aeromonas salmonicida	Positive	
Isolate	10	Aeromonas salmonicida	Positive	
Isolate	11	Aeromonas salmonicida	Positive	
Isolate	12	Aeromonas salmonicida	Positive	
Isolate	13	Aeromonas salmonicida	Positive	
Isolate	14	Aeromonas salmonicida	Positive	
Intestine	15		No Bacteria Isolated	
Intestine	16	Aeromonas salmonicida	Positive	

Intestine	17	Aeromonas salmonicida	Positive
Isolate	18	Aeromonas salmonicida	Positive
Isolate	19	Aeromonas salmonicida	Positive
Isolate	20	Aeromonas salmonicida	Positive

Fish Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 06/10/09 @ 1:07 PM

Organism	ID	e	ffc	sor	s3	sxt	ot
Aeromonas salmonicida	1	s	s		s	s	s
**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline Unable to perform Romet sensitivity due to supplier shortage. Testing will resume for Romet as soon as supply becomes available.							
Aeromonas veronii	4	s	s		s	s	s

History of Communication

Date	To	Description
06/11/09 2:35 PM	Mainstream Canada-T - fax	bc report generated
06/11/09 2:35 PM	Dr. Peter McKenzie - e-mail	bc report generated
06/15/09 10:53 AM	Mainstream Canada - 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: 09-2353

Last Updated: 06/24/09 10:12 AM
Pathologist: Gary D. Marty
Received Date: 06/19/09
Collected Date: 06/16/09
Client Ref No: SEB-061609

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Kelly Abel - Mainstream Can.**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted fresh and formalized Atlantic salmon tissues for histopathology and PCR (BKD analysis).

4 moribund sampled, Fish 1 and 2 histology tissues and kidney,liver,cecae,brain and gill (in 10% formalin for 24 hrs). Fresh 3 and 4 virology tissues: kidney, liver, brain (into whirl packs frozen).

Fish #1 - Wt 3.455, pale gills; Fish #2 - Wt 2.635, pale gills; Fish #3 - Wt 3.585, pale gills; Fish #4 - Wt 3.325, pale gills, enlarged kidney.

Farm name: Sir Edmund Bay. Species: Atlantic salmon. Saltwater.

Final Diagnosis

1. Brain: meningitis, granulomatous, regionally diffuse, with very small numbers of short Gram -positive rods (*Renibacterium salmoninarum*), severe (slide 2)

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

2b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slide 1)

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

3b. Trunk kidney: renal tubular casts of yellow-brown pigment (lipofuscin?), with tubular epithelial degeneration and regeneration, multifocal, mild (slide 1)

4. Intestinal ceca and mesenteric adipose tissue: peritonitis, chronic, regionally diffuse, with fibrocellular fronds, moderate (slide 1)

Final Comment: Fish #2 died of complications related to severe meningitis caused by the cerebral form of bacterial kidney disease (*Renibacterium salmoninarum*). With the cerebral form of BKD, bacteria often occur sporadically in relation to the severity of the inflammation; in this case, 100× oil immersion was required to confirm the presence of organisms in the Twort's Gram -stained section . In the BC Fish Health Auditing and Surveillance Program from 2006 - 2008, only 4% of the 1113 sampled Atlantic salmon had *Renibacterium salmoninarum* infection in the brain, but in 40% of these cases, *Renibacterium salmoninarum* infection occurred in no organ other than the brain.

Fish #1 has several lesions that provide clues about the cause of death, but none of the lesions are of sufficient severity to have killed the fish. Comments on specific lesions follow:

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

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). In Atlantic salmon livers sampled as part of the Province's Fish Health Auditing and Surveillance Program, prevalence of these vacuoles steadily increased in Atlantic salmon from 42% in 2006 to 50% in 2007 and 55% in 2008; 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.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29% ; n = 469) and Pacific salmon (prevalence = 31% ; n = 118).

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

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology. Both cassettes were subjected to surface decalcification with 8% formic acid prior to sectioning.

Slide 1 (SEB Fish 1 Pen 8 06/16/09) - brain, gill, liver, trunk kidney (2 pieces), intestinal ceca, and mesenteric adipose tissue

Slide 2 (SEB Fish 2 Pen 8 06/16/09) - brain, gill liver, head kidney, trunk kidney, intestinal ceca, and mesenteric adipose tissue; a section from the same block was stained with Twort's Gram stain.

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

Quality control: Liver autolysis: none (slide 2), mild (slide 1). Organs have evidence of mild postfixation dehydration (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cell types stains poorly or not at all). This most commonly results when preserved tissues are removed from liquid for more than a few minutes (e.g., during shipment). Other potential causes include fixation in formalin that is too concentrated (e.g., 100% formalin instead of 10% formalin), or transfer to ethanol that is too concentrated (e.g., >70% ethanol) before processing to paraffin. Organs have no acid hematin deposits.

Molecular Diagnostics

PCR-Renibacterium salmoni Resulted by: A Scouras Verified by: Dr. J. Robinson on 06/23/09 @ 5:51 PM

Specimen	ID	Test	Result
Tissue	#3-lv,kd,br	PCR-Renibacterium salmoninaru	Negative
Tissue	#4-lv,kd,br	PCR-Renibacterium salmoninaru	Positive
**: Test validation in progress.			

History of Communication

Date	To	Description
06/24/09 10:13 AM	Mainstream Canada - e-mail	bc report generated

06/24/09 10:13 AM	Dr. Peter McKenzie - e-mail	bc report generated
07/08/09 12:02 PM	Mainstream Canada - 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: 09-2411

Last Updated: 06/26/09 3:04 PM
Pathologist: Gary D. Marty
Received Date: 06/24/09
Collected Date: 06/22/09
Client Ref No: 13322

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted formalized fish tissue for histopathology (identify pathogen in gills).

Saltwater. Vaccinated. Previous ID 13274. All fish excessive mucus, hemorrhaged and necrotic gills. Successfully treated for A.S. (Areomonis salmoncida).

Farm location - Mussel Rock

Final Diagnosis

1. Gill, tips of filaments: branchial necrosis, focal (involves several filaments), with abundant superficial filamentous bacteria, severe (slide 2, one arch)

Final Comment: One of 15 gill arches examined has a broad focus of filament necrosis covered by filamentous bacteria. Thin rod-shaped to filamentous bacteria are common on the gills of debilitated juvenile salmonids. Although bacterial culture or PCR is required for a definitive diagnosis, the most common form in marine waters is *Tenacibaculum maritimum* (one cause of necrotizing branchitis). Infections are usually associated with crowding, poor water quality, or stress. The other 14 gill arches provide no clues about the cause of death.

Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology. Tissues were immersed in Protocol B (hydrochloric acid solution)

Slides 1 (unreadable cassette label), 2 (Mussel June 22/09) and 3 (Mussel June 22/09) - gills (5 arches per slide)

All gills were examined. Gills not listed elsewhere have no significant lesions.

Quality control: Autolysis: mild (slide 3, 2 arches), moderate (slides 1, 2, and 3 arches on slide 3). Decalcification is complete. Organs have no postfixation dehydration and no acid hematin deposits.

History of Communication

Date	To	Description
06/26/09 3:04 PM	Mainstream Canada-T - fax	bc report generated
06/26/09 3:05 PM	Dr. Peter McKenzie - e-mail	bc report generated
07/08/09 1:26 PM	Mainstream Canada - e-mail	Case Invoiced

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

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Toll-Free : 1-800-661-9903

Final Report AHC Case: 09-2412

Last Updated: 06/26/09 4:00 PM
Pathologist: Gary D. Marty
Received Date: 06/24/09
Collected Date: 06/22/09
Client Ref No: 13322

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted fresh and formalized fish tissue for histopathology and PCR (viral PCR).

300g. Saltwater. Vaccinated. Necrotic gills. Excessive mucus. Hemorrhaged gills.

Farm location - Saranac

Final Diagnosis

1a. Gill, tips of filaments: branchial necrosis, multifocal, with abundant superficial filamentous bacteria, moderate (slide G, one arch), severe (slide G, one arch)

1b. Gill: interfilament diatom spines/setae, focal, mild (slide 4B)

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

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

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

Final Comment: Two of five gill arches in slide G have foci of filament necrosis covered by filamentous bacteria. Thin rod-shaped to filamentous bacteria are common on the gills of debilitated juvenile salmonids. Although bacterial culture or PCR is required for a definitive diagnosis, the most common form in marine waters is *Tenacibaculum maritimum* (one cause of necrotizing branchitis). Infections are usually associated with crowding, poor water quality, or stress. PCR results provide evidence that the fish were not infected with VHSV or IHNV. The other three gill arches in slide G provide no clues about the cause of death.

Other changes in these fish are mostly minor:

The diatoms *Chaetoceros concavicornis*, *C. convolutus*, and a *Corethron sp*. have been associated with mortality of salmon reared in seawater netpens at numbers as low as 5/mL (Taylor and Harrison 2002). The gill in slide 4B has only a single focus of *Chaetoceros*, with no associated inflammation; therefore, the *Chaetoceros* probably did not contribute to mortality.

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

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29% ; n = 469) and Pacific salmon (prevalence = 31% ; n = 118).

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

Histopathology

Formalin-fixed tissues were submitted in 6 cassettes for histopathology. Gills were removed from the original (A) cassettes and placed in separate (B) cassettes for blocks 1 through 5.

Slides 1A (Sar June 22/09), 2A (Sara June 22/09) and 4A (Sara June 22/09) - brain, heart, spleen, liver, head kidney, intestinal ceca, mesenteric adipose tissue

Slide 3A (Sar June 22/09) - brain, heart, liver, head kidney, intestinal ceca, mesenteric adipose tissue

Slide 5A (Sar June 22/09) - heart, spleen, liver, intestinal ceca, mesenteric adipose tissue

Slides 1B (Sar June 22/09), 2B (Sara June 22/09), 3B (Sar June 22/09), 4B, (Sara June 22/09), 5B (Sar June 22/09) and 6 (Sar) - gills (5 arches)

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

Quality control: Liver and gill autolysis: severe (slides 1A, 2A, 3A, 4A, 5A, G). Gill morphology can be improved by preserving gill separately in its own cassette; the Animal Health Centre does not charge extra for the second cassette/fish. More than one arch per cassette works fine as long as the arches do not touch one another. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: A Scouras on 06/26/09 @ 12:54 PM

Specimen	ID	Test	Result
Tissue	organs	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: A Scouras on 06/26/09 @ 12:54 PM

Specimen	ID	Test	Result
Tissue	organs	PCR - VHSV	Negative

History of Communication

Date	To	Description
06/26/09 4:01 PM	Mainstream Canada-T - fax	bc report generated
06/26/09 4:02 PM	Dr. Peter McKenzie - e-mail	bc report generated
07/08/09 1:39 PM	Mainstream Canada - 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: 09-2421

Last Updated: 07/02/09 9:31 AM
Pathologist: Gary D. Marty
Received Date: 06/25/09
Collected Date:
Client Ref No: 9-2696

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

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

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

Case History

Submitted formalized fish for histology.

The samples have been preserved in a buffered formalin solution then transferred to tap water for transport. Please reference case file 9-2696 on all reports and invoices. If you require any further information please contact me at 250-652- 4482 ext 201.

Final Diagnosis

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

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

Final Comment: Although no history was provided with this submission, these fish would have had an enlarged kidney with irregular, coalescing, white nodules that would have been gritty when cut. These gross changes are classic for nephrocalcinosis. 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.

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.

Histopathology

Formalin-fixed tissues were submitted in 2 cassettes for histopathology. Gills were removed from the original (A) cassettes and placed in separate (B) cassettes prior to processing into paraffin. Blocks 1A and 2A were subjected to surface decalcification in 8% formic acid prior to sectioning.

Slides 1A (Fish 1) and 2A (Fish 2) - brain, spleen, liver, head kidney, trunk kidney, intestinal ceca, and mesenteric adipose tissue

Slide 1B and 2B - gill

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

Quality control: Liver autolysis: none (slide 2A), mild (slide 1A). 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). Organs have no postfixation dehydration.

History of Communication

Date	To	Description
07/02/09 10:10 AM	Hewison, Tim - e-mail	bc report generated
07/08/09 2:49 PM	Microtek International In - e-mail	Case Invoiced

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

END OF REPORT

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

Final Report AHC Case: 09-2477

Last Updated: 07/08/09 12:04 AM
Pathologist: Gary D. Marty
Received Date: 06/30/09
Collected Date:
Client Ref No: PO #7312

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

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

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

Case History

Submitted 6 cassettes (2 cass/fish) and 3 fresh tissues for PCR for VHS and IHN.

6 fresh morts sampled - all with similar symptoms. Hind gut was red - inflamed, petechial hem. in liver. Stomach was bloated in most fish with feed mash in stomach. Blood vessels dilated, spleen enlarged.

Viral sample #3 - mort had petechial hem. in swim bladder, liver and ecchymotic hem. along kidney margins.

Species: Atlantic salmon. Saltwater entry: 07 SO. Vaccinated. No prior submissions. Fish died June 24/09.

Final Diagnosis

1. Intestine: mucosal vascular congestion, diffuse, mild (slides 1A, 6A), moderate (slides 1A, 2A)

2a. Liver: pericholangitis, lymphohistiocytic, focal to multifocal, mild (slides 2A, 5A, 6A)

2b. Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, mild (slide 4A)

2c. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slides 3A, 5A), moderate (slide 1A), abundant (slide 6A)

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

3b. Kidney: congestion, diffuse, with hematopoietic cell atrophy, moderate (slide 4A)

3c. Head kidney: multiple foci of pale cells (probably some type of macrophage or supporting cell), moderate (slide 6A)

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

5. Mesenteric adipose tissue: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 5A, 6A)

Final Comment: These fish have several microscopic changes that provide clues to their death (see detailed comments below). Unfortunately, none of the changes are specific for a cause. If ongoing bacteriology and PCR results do not provide a satisfactory answer, consider

submitting freshly harvested tissues for tissue culture (call ahead so we know that the tissues are on their way).

Vascular congestion of the intestinal lamina propria is an uncommon lesion in Atlantic salmon. Vascular congestion is often evidence of circulating vasodilators. Differentials include substances released from inflammatory cells, bacteria, or a viral infection. Alternatively, some of the congestion might be a result of postmortem change (passive congestion). In this case, it is not associated with increased numbers of inflammatory cells. The mucosal epithelium is too autolyzed in the affected fish to determine if its morphology is altered. The intestinal ceca are not involved (consistent with gross findings).

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.

Sinusoidal congestion (sometimes called "peliosis") in the liver is evidence of circulating vasodilators. I have seen it associated with viral hemorrhagic septicemia virus and *Listonella anguillarum* infection. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions might be remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).

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

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 interstitial congestion with hematopoietic cells atrophy is an uncommon manifestation of infection with viral hemorrhagic septicemia virus (VHSV). The age of the fish and season of the year are unusual for VHSV infection. Differentials include other viruses (ISAV, but unlikely in BC) and bacteria.

Multiple foci of pale cells in the kidney are probably some type of macrophage or supporting cell. The foci seem to be a loss of normal deeply basophilic hematopoietic cells rather than hyperplasia of the pale cells. The foci probably are a result of some type of immune reaction, but I cannot be more specific as to the cause.

Splenic and mesenteric peritonitis are consistent with a reaction to foreign material; they are common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Histopathology

Formalin-fixed tissues from 6 fish were submitted in 12 cassettes for histopathology.

Slide #s ¿ 1A and 1B (7312-1), 2A and 2B (7312-1), 3A and 3B (7312-1), 4A and 4B (7312-1), 5A and 5B (7312- 1), 6A and 6B (7312-1)

Organs included on most slides - heart, liver, spleen, head kidney, trunk kidney, intestine, intestinal ceca, mesenteric adipose tissue, brain; slides 2A, 4A, and 5A include stomach

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

Quality control: Liver autolysis: mild (slides 5A, 6A), moderate (slides 1A, 2A, 3A), severe (slide 4A). Intestinal autolysis: moderate (slides 1A, 2 A, 3A, 5A, 6A), severe (slide 4A). Large foci of erythrocytes (e.g., liver 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.

History of Communication

Date	To	Description
07/08/09 12:08 AM	Marine Harvest Canada - fax	bc report generated
07/08/09 12:16 AM	Marine Harvest Canada - e-mail	bc report generated

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: 09-2492

Last Updated: 07/23/09 12:10 PM
Pathologist: Gary D. Marty
Received Date: 07/02/09
Collected Date: 06/29/09
Client Ref No: PO 7313

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

Submitter: **Tiffany MacWilliams - Marine H. Can.**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

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

Case History

Submitted 5 cassettes for histopathology and 10 (3 fish pool) for viral culture.

Atlantic salmon. Saltwater entry 08 SO. Vaccinated.

in group: 30. Euthanized: TMS. Fish died June 25/09.

Cull sampling - routine sample of fish prior to moves. No visible lesions.

Final Diagnosis

1a. Liver: sinusoidal congestion, multifocal, moderate (slide 4)

1b. Liver: hepatocellular cytoplasmic vacuoles, multifocal, mild (slides 3, 4); diffuse, mild (slides 1, 2, 5)

1c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slides 3, 5)

2. Intestinal ceca, mesenteric adipose tissue, and stomach: peritonitis, granulomatous, multifocal, mild (slide 2), with intralesional vacuoles about 50 µm in diameter, moderate (slides 1, 4, 5), severe (slide 3)

3. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1, 2, 3, 5), and with intralesional vacuoles about 50 µm in diameter, moderate (slide 4)

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

Final Comment: These fish have several lesions that are common in farmed Atlantic salmon in British Columbia, but none are of sufficient severity to be lethal. Comments on specific lesions follow:

Sinusoidal congestion in the liver is evidence of circulating vasodilators; sometimes it occurs as a postmortem artifact. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.

Hepatocellular 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.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe). Vacuoles are probably a result of vaccine material lost during tissue processing.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469) and Pacific salmon (prevalence = 31%; n = 118).

Histopathology

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

Slide #s 1-5 are labeled in the same order as client #s 1 - 5

Organs included on most slides: heart, liver, spleen, trunk kidney, intestinal ceca, and mesenteric adipose tissue. Slide 1 also has skin/skeletal muscle; slide 5 also has stomach; slide 4 has stomach instead of intestinal ceca

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

Quality control: Liver autolysis: none (slides 1, 3), mild (slides 2, 4, 5). Organs have no postfixation dehydration and no acid hematin deposits.

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 07/23/09 @ 12:10 PM

Specimen	ID	Isolate	Result	Level
Tissue	7313-1		No viruses isolated	
Tissue	7313-2		No viruses isolated	
Tissue	7313-3		No viruses isolated	
Tissue	7313-4		No viruses isolated	
Tissue	7313-5		No viruses isolated	
Tissue	7313-6		No viruses isolated	
Tissue	7313-7		No viruses isolated	
Tissue	7313-8		No viruses isolated	
Tissue	7313-9		No viruses isolated	
Tissue	7313-10		No viruses isolated	

Staff Comments:
Histopathology results were sent to Marine Harvest fish health staff by Gary Marty via Outlook on 15 July 2009, 1:56 PM.

History of Communication

Date	To	Description

07/24/09 9:38 AM	Morrison, Diane - fax	bc report generated
07/29/09 10:45 AM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-2594

Last Updated: 07/30/09 10:06 AM
Pathologist: Gary D. Marty
Received Date: 07/09/09
Collected Date: 07/08/09
Client Ref No: 7331

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

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

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

Case History

Submitted fresh and formalized fish tissue for Histopathology and Viral Culture (10 pools of 3).

Saltwater entry 08 S0. Vaccinated. 30 in group. Euthanized - TMS. Cull sample - health check prior to splits. No visible lesions.

Final Diagnosis

1a. Liver: sinusoidal congestion, multifocal, mild (slide 5)

1a. Liver: hepatitis, granulomatous, multifocal, mild (slide 2)

1b. Liver: hepatocellular cytoplasmic vacuoles, multifocal, mild (slide 1); diffuse, moderate (slides 2, 3, 5)

2a. Heart: endocarditis, granulomatous, multifocal, mild (slide 5), moderate (slide 4)

2b. Heart: epicarditis, regionally diffuse, lymphohistiocytic, mild (slide 5)

3. Intestinal ceca, mesenteric adipose tissue, and (when present) stomach: peritonitis, granulomatous, multifocal, with intralesional vacuoles about 50 µm in diameter, severe (slides 1, 3, 4, 5)

4. Spleen: peritonitis, peritonitis, granulomatous, with fibrocellular fronds, mild (slide 3), and with intralesional vacuoles about 50 µm in diameter, focal, severe (slides 2, 5)

5a. Trunk kidney: glomerulonephritis, membranous, diffuse, mild (slide 1)

5b. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slide 2), moderate (slides 1, 3. 4, 5)

Final Comment: These fish have several lesions that are common in farmed Atlantic salmon in British Columbia, but none are of sufficient severity to be lethal. Comments on specific lesions follow:

Sinusoidal congestion in the liver is evidence of circulating vasodilators; sometimes it occurs as a postmortem artifact. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Sinusoidal congestion is one of the classic lesions

associated with ISAV infection, but ISAV has never been identified in British Columbia.

Small foci of granulomatous inflammation in the liver (slide 2) and heart (slides 4 and 5) are probably part of a vaccine reaction. The primary differential is infection with *Renibacterium salmoninarum* .

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 28% of the 467 Atlantic salmon hearts examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program.

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.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe). Vacuoles are probably a result of vaccine material lost during tissue processing. This group of fish includes a higher proportion with severe inflammation than most cases I examine; could this have contributed to their selection for culling?

Features of glomerulonephritis include thickening of the glomerular basement membranes by homogeneous eosinophilic material. Membranous glomerulonephritis is fairly common in Chinook salmon, but it is rare Atlantic salmon, affecting only 13 of 1663 Atlantic salmon kidneys examined since 2006 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).

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29% ; n = 469) and Pacific salmon (prevalence = 31% ; n = 118).

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. Slide #s 1-5 are labeled in the same order as client #s 7331-1 through 7331-5.

Organs included on most slides: heart, liver, spleen, trunk kidney, intestinal ceca, and mesenteric adipose tissue. Slides 1 and 3 also have stomach; slide 1 has no spleen. All organs were examined. Organs not listed elsewhere have no significant lesions.

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

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 07/30/09 @ 10:06 AM

Specimen	ID	Isolate	Result	Level
Tissue	7331-1		No viruses isolated	

Tissue	7331-2	No viruses isolated
Tissue	7331-3	No viruses isolated
Tissue	7331-4	No viruses isolated
Tissue	7331-5	No viruses isolated
Tissue	7331-6	No viruses isolated
Tissue	7331-7	No viruses isolated
Tissue	7331-8	No viruses isolated
Tissue	7331-9	No viruses isolated
Tissue	7331-10	No viruses isolated

Staff Comments:
Gary Marty sent the histopathology results to Marine Harvest fish health staff via Outlook on 16 July 2009, 2:32 PM.

History of Communication

Date	To	Description
07/30/09 11:59 AM	Morrison, Diane - fax	bc report generated
08/06/09 1:15 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-2602

Last Updated: 07/20/09 10:16 AM
Pathologist: Gary D. Marty
Received Date: 07/10/09
Collected Date: 07/09/09
Client Ref No: CH070909

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Nathan Cassan - Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 30 Kidney tissues for BKD screening by PCR.

Species: Atlantic salmon. Saltwater entry. Vaccinated. Euthanized: Stun:bleed.

Farm name: Cypress Harbour.

Send copy of report to: Steve.Fukui@mainstreamcanada.com

Molecular Diagnostics

PCR-Renibacterium salmoni Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 07/20/09 @ 10:16 AM

Specimen	ID	Test	Result
Tissue	1	PCR-Renibacterium salmoninaru	Negative
Tissue	2	PCR-Renibacterium salmoninaru	Positive
Tissue	3	PCR-Renibacterium salmoninaru	Negative
Tissue	4	PCR-Renibacterium salmoninaru	Negative
Tissue	5	PCR-Renibacterium salmoninaru	Negative
Tissue	6	PCR-Renibacterium salmoninaru	Negative
Tissue	7	PCR-Renibacterium salmoninaru	Negative
Tissue	8	PCR-Renibacterium salmoninaru	Negative
Tissue	9	PCR-Renibacterium salmoninaru	Negative
Tissue	10	PCR-Renibacterium salmoninaru	Negative
Tissue	11	PCR-Renibacterium salmoninaru	Negative
Tissue	12	PCR-Renibacterium salmoninaru	Negative
Tissue	13	PCR-Renibacterium salmoninaru	Negative
Tissue	14	PCR-Renibacterium salmoninaru	Negative

Tissue	15	PCR-Renibacterium salmoninaru	Negative
Tissue	16	PCR-Renibacterium salmoninaru	Negative
Tissue	17	PCR-Renibacterium salmoninaru	Negative
Tissue	18	PCR-Renibacterium salmoninaru	Negative
Tissue	19	PCR-Renibacterium salmoninaru	Negative
Tissue	20	PCR-Renibacterium salmoninaru	Negative
Tissue	21	PCR-Renibacterium salmoninaru	Negative
Tissue	22	PCR-Renibacterium salmoninaru	Negative
Tissue	23	PCR-Renibacterium salmoninaru	Negative
Tissue	24	PCR-Renibacterium salmoninaru	Positive
Tissue	25	PCR-Renibacterium salmoninaru	Negative
Tissue	26	PCR-Renibacterium salmoninaru	Negative
Tissue	27	PCR-Renibacterium salmoninaru	Negative
Tissue	28	PCR-Renibacterium salmoninaru	Negative
Tissue	29	PCR-Renibacterium salmoninaru	Negative
Tissue	30	PCR-Renibacterium salmoninaru	Negative

History of Communication

Date	To	Description
07/20/09 1:29 PM	Mainstream Canada - e-mail	bc report generated
07/20/09 1:29 PM	Dr. Peter McKenzie - e-mail	bc report generated
07/22/09 8:20 AM	Mainstream Canada - 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: 09-2712

Last Updated: 07/29/09 1:59 PM
Pathologist: Gary D. Marty
Received Date: 07/17/09
Collected Date: 07/13/09
Client Ref No: 13728

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted fresh fish tissue for PCR and 2 bacti plates for bacteriology.

Saltwater. 150g. Vaccinated.Brain and kidney on same plate. Kidney under K and Brain under B.

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Sean Byrne on 07/29/09 @ 1:59 PM

Specimen	ID	Isolate	Result	Level
Isolate	1-4 K&B			
**: Plate submitted was devided up into sections 1 through 4 and in each section K (kidney) and B (brain); 8 streaks on one plate. Plate was not in good condition upon arrival, the agar was dried out and the plate had gotten wet so that isolates were running into each other. Isolates were therefore identified as a conglomerate, and not "assigned" to one section/organ specifically. In the future please limit the amount of streaks per plate to 4 or less.				
Isolate	1-4 K&B	Bacteria	Positive	
**: Bacteria identifieda as Planococcus maritimus				
Isolate	1-4 K&B	Vibrio lentus	Positive	

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/21/09 @ 11:40 AM

Specimen	ID	Test	Result
Tissue	org pool	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/21/09 @ 11:40 AM

Specimen	ID	Test	Result

Tissue	org pool	PCR - VHSV	Negative

History of Communication

Date	To	Description
07/29/09 2:14 PM	Mainstream Canada-T - fax	bc report generated
07/29/09 2:14 PM	Dr. Peter McKenzie - e-mail	bc report generated
08/06/09 1:19 PM	Mainstream Canada - 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: 09-2743

Last Updated: 07/23/09 4:19 PM
Pathologist: Gary D. Marty
Received Date: 07/21/09
Collected Date: 07/20/09
Client Ref No: 7341

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

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

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

Case History

Submitted one fresh fish tissue for PCR (IHN and VHS).

Saltwater entry - 07 S0. Vaccinated. 1 Fresh mort with petechial hem in liver, fat, muscle, sb, and peritoneum.

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 07/23/09 @ 4:19 PM

Specimen	ID	Test	Result
Tissue	7341-1 (org)	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 07/23/09 @ 12:10 PM

Specimen	ID	Test	Result
Tissue	7341-1 (org)	PCR - VHSV	Negative

History of Communication

Date	To	Description
07/24/09 9:43 AM	Morrison, Diane - fax	bc report generated
07/30/09 1:17 PM	Marine Harvest Canada - 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: 09-2849

Last Updated: 07/31/09 7:19 AM
Pathologist: Gary D. Marty
Received Date: 07/28/09
Collected Date:
Client Ref No: 7345

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

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

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

Case History

Submitted fresh and formalized fish tissue for histopathology and PCR (IHN and VHS).

Saltwater entry - 08 S1. Vaccinated. 2 fresh morts sampled. Petechial hem in liver, sb, hind gut was enflamed. Site had noticed moldly feed 8-10 days ago. Mortality is still low.

Final Diagnosis

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

1b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slide 2), moderate (slide 1)

2. Head kidney: small numbers of interstitial intracytoplasmic eosinophilic granules, diffuse (slide 1)

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

Final Comment: In BC Atlantic salmon, hepatic sinusoidal congestion is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria (e.g., *Listonella anguillarum*), and infection with VHSV; as in this case, the cause often remains unknown. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

Hepatocellular 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.

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

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 and bacterial infections. Vessels in the mesenteric fat are not distended.

Literature Cited:

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

Histopathology

Formalin-fixed tissues from two Atlantic salmon were submitted in 2 cassettes for histopathology.

Slide 1 (7345-1) - heart, liver, spleen, head kidney, trunk kidney, stomach, intestine, intestinal ceca, mesenteric adipose tissue

Slide 2 (7345-2) - heart, liver, spleen, head kidney, trunk kidney, stomach, intestine, intestinal ceca, mesenteric adipose tissue

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

Quality control: Liver autolysis: mild (slides 1, 2). Intestinal autolysis: mild (slide 2), moderate (slide 1). Organs have no postfixation dehydration and no acid hematin deposits (outside of congested foci in the liver).

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/30/09 @ 3:16 PM

Specimen	ID	Test	Result
Tissue	#7345-1	PCR - IHNV	Negative
Tissue	#7545-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/30/09 @ 3:16 PM

Specimen	ID	Test	Result
Tissue	#7345-1	PCR - VHSV	Negative
Tissue	#7545-2	PCR - VHSV	Negative

History of Communication

Date	To	Description
07/31/09 7:19 AM	Morrison, Diane - fax	bc report generated
08/06/09 2:48 PM	Marine Harvest Canada - e-mail	Case Invoiced

Case: 09-2849

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

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Final Report AHC Case: 09-2850

Last Updated: 08/04/09 2:06 PM
Pathologist: Gary D. Marty
Received Date: 07/28/09
Collected Date: 07/23/09
Client Ref No: 7354

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

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

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

Case History

Submitted fresh and formalized fish tissue for histopathology and PCR (IHN, ISA and VHS).

Saltwater entry - 2009 S1. Vaccinated. Histology and virology submitted to check on recent mortalities with no visible lesions. Histology collected from 3 fish. 3 Virology samples submitted for PCR/see above)

Final Diagnosis

1a. Liver: sinusoidal congestion, multifocal, mild (slide 1A), moderate (slide 2B)

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

1c. Liver: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 2B)

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

3. Head kidney: vascular congestion, diffuse, mild (slides 1A, 2B, 3A)

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

5. Gill: occlusive thrombus , focal (one lamellar capillary), mild (slide 1A)

6. Mesenteric adipose tissue: capillary congestion, diffuse, mild (slide 2B)

Final Comment: These fish have several lesions that are common in farmed Atlantic salmon in BC, but none are of sufficient severity to explain their death.

Vascular congestion in the liver and head kidney is evidence of circulating vasodilators; sometimes it occurs as a postmortem artifact. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Most commonly, the cause is unknown (as in this case). Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.

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.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29% ; n = 469) and Pacific salmon (prevalence = 31% ; n = 118).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the spleens in 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

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.

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. It is not associated with postmortem change.

Histopathology

Formalin-fixed tissues from 3 Atlantic salmon were submitted in 6 cassettes for histopathology. All cassettes were labeled 7/23/09

Slides 1A (7354 D1-1), 2B (7354 D1-2) - heart, liver, spleen, head kidney, trunk kidney, intestine, intestinal ceca, mesenteric adipose tissue, skin/skeletal muscle, brain

Slide 3A (7354 D1-3) - heart, liver, spleen, head kidney, trunk kidney, intestinal ceca, skin/skeletal muscle, brain

Slides 1B (735? D1-1), 2A (7354 D1-2), and 3B (7354 D1-3) - gill

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/30/09 @ 3:17 PM

Specimen	ID	Test	Result
Tissue	A(#1-3)	PCR - IHNV	Negative
Tissue	B(#4-6)	PCR - IHNV	Negative
Tissue	C(#7-9)	PCR - IHNV	Negative

PCR - ISA Resulted by: R. Richardson Verified by: A Scouras on 08/04/09 @ 2:06 PM

Specimen	ID	Test	Result
Tissue	A(#1-3)	PCR - ISA	Negative
Tissue	B(#4-6)	PCR - ISA	Negative
Tissue	C(#7-9)	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/30/09 @ 3:17 PM

Specimen	ID	Test	Result
Tissue	A(#1-3)	PCR - VHSV	Negative
Tissue	B(#4-6)	PCR - VHSV	Negative
Tissue	C(#7-9)	PCR - VHSV	Negative

Staff Comments:
Gary Marty e-mailed interim results to Marine Harvest Fish Health staff via Outlook on Fri. 2009-07-31 5:35 PM.

History of Communication

Date	To	Description
08/04/09 4:07 PM	Morrison, Diane - fax	bc report generated
08/06/09 2:49 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-2852

Last Updated: 07/31/09 4:22 PM
Pathologist: Gary D. Marty
Received Date: 07/28/09
Collected Date:
Client Ref No: 7346

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

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

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

Case History

Submitted fresh and formalized fish tissue for Histopathology and PCR (IHN/VHS).

Saltwater entry - 09 S1. Vaccinated. Fresh morts. Mortality has been increasing. Sampled 5 fresh morts. 3 for Viral - PCR and 2 for histo - #2 had mouth rot.

Final Diagnosis

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

1b. Liver: sinusoidal congestion, multifocal, mild (slides 1A, 2A)

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

3. Heart: myocardial karyomegaly, multifocal, mild (slide 2A)

4. Head kidney: vascular congestion, diffuse, mild (slide 1A)

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

6. Brain: capillary (vascular) congestion, diffuse, moderate (slide 2A)

Final Comment: These fish have several lesions that are common in farmed Atlantic salmon in BC, but none are of sufficient severity to explain their 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.

Vascular congestion in the liver and head kidney is evidence of circulating vasodilators; sometimes it occurs as a postmortem artifact.

Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Most commonly, the cause is unknown (as in this case). Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29% ; n = 469) and Pacific salmon (prevalence = 31% ; n = 118).

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.4% of the 1609 Atlantic salmon hearts examined as part of the province's Fish Health Auditing and Surveillance Program from 2006 through 2008). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the spleens in 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

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 often occurs as part of postmortem change. 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 2 Atlantic salmon were submitted in 4 cassettes for histopathology.

Slides 1A (7346-1) - heart, liver, spleen, head kidney, trunk kidney, brain

Slides 2A (7346-2) - heart, liver, spleen, intestine, trunk kidney, brain

Slides 1B (7346-1), 2B (7346-2) - gill

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/30/09 @ 3:17 PM

Specimen	ID	Test	Result
Tissue	#7346-1	PCR - IHNV	Negative
Tissue	#7346-2	PCR - IHNV	Negative
Tissue	#7346-3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 07/30/09 @ 3:17 PM

Specimen	ID	Test	Result
Tissue	#7346-1	PCR - VHSV	Negative
Tissue	#7346-2	PCR - VHSV	Negative
Tissue	#7346-3	PCR - VHSV	Negative

History of Communication

Date	To	Description
07/31/09 4:22 PM	Morrison, Diane - fax	bc report generated
08/06/09 2:49 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-2936

Last Updated: 08/27/09 11:05 AM
Pathologist: Gary D. Marty
Received Date: 08/04/09
Collected Date: 07/29/09
Client Ref No: SP-072909

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Kelly Abel - Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted fresh and formalized fish tissue for histopathology, viral culture and PCR (IHN/VHS x PCR).

Saltwater. Vaccinated. 6 submitted dead. 3 fish sampled from pen #2 - Histology 3 cassettes containing Brain, gill, heart, head kidney, trunk kidney, liver, spleen and ceca; into formalin for minimum of 24 hours shipped in water. Virology - 3 whirl bags containing Head kidney and spleen frozen shipped on ice. 3 fish sampled prom pen #4 - Histology 3 cassettes containing Brain, gill heart, head kidney, trunk kidney, liver, spleen and ceca; into formalin for minimum of 24 hours shipped in water. Virology - 3 whirl bags containing head kidney and spleen frozen shipped on ice.

Final Diagnosis

1a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 2-1)

1b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slide 4-1)

1c. Liver: sinusoidal congestion, multifocal, mild (slides 2-2, 2-3), moderate (slides 2-1, 4-1, 4-2)

2. Head and trunk kidney: vascular congestion, diffuse, mild (slide 2-2, 4-3), moderate (slide 2-1)

3. Brain: capillary (vascular) congestion, diffuse, mild (slides 2-2, 2-3), moderate (slides 2-1, 4-2, 4-3)

4. Spleen: peritonitis, granulomatous, multifocal, with fibrocellular fronds, mild (slide 4-2), moderate (slides 2-1, 2-3, 4-3)

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

Final Comment: These fish have several changes that are common among farmed Atlantic salmon that die in BC net pens. However, none of the lesions are of sufficient severity to explain their death. Causes of death with these microscopic features include mouthrot, other cutaneous ulcers, and acute environmental perturbations

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

inflammatory mediators) or come from outside the fish (e.g., from the water or the feed). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 14% of the 468 Atlantic salmon livers examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2008 was sufficient to identify a trend towards greater prevalence in the winter and spring (21-30%) than in the summer and fall (1.9-4.4%).

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.

Vascular congestion in the kidney, liver, and brain might be evidence of circulating vasodilators; sometimes it occurs as a postmortem artifact (especially in the kidney and brain). Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Most commonly, the cause is unknown (as in this case). Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. Consider bacteriology; PCR results rule out VHSV and IHNV.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469).

Histopathology

Formalin-fixed tissues from 6 fish were submitted in 6 cassettes for histopathology; all cassettes include the label "Simmonds Pt.," plus pen # and fish #. Slide #s are the same as pen and fish #s; e.g., slide 2-1 = pen 2 fish 1. The other pen-fish combinations are slides 2-2, 2-3, 4-1, 4-2, and 4-3.

Organs included on most slides - gill, heart, liver, spleen, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue, and brain. All organs were examined. Organs not listed elsewhere have no significant lesions.

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

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Julie Bidulka on 08/06/09 @ 1:46 PM

Specimen	ID	Test	Result
Tissue	A	PCR - IHNV	Negative
Tissue	B	PCR - IHNV	Negative
Tissue	C	PCR - IHNV	Negative
Tissue	D	PCR - IHNV	Negative
Tissue	E	PCR - IHNV	Negative
Tissue	F	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Julie Bidulka on 08/06/09 @ 1:46 PM

Specimen	ID	Test	Result
Tissue	A	PCR - VHSV	Negative

Tissue	B	PCR - VHSV	Negative
Tissue	C	PCR - VHSV	Negative
Tissue	D	PCR - VHSV	Negative
Tissue	E	PCR - VHSV	Negative
Tissue	F	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 08/27/09 @ 11:05 AM

Specimen	ID	Isolate	Result	Level
Tissue	A		No viruses isolated	
Tissue	B		No viruses isolated	
Tissue	C		No viruses isolated	
Tissue	D		No viruses isolated	
Tissue	E		No viruses isolated	
Tissue	F		No viruses isolated	

History of Communication

Date	To	Description
08/07/09 11:00 AM	Mainstream Canada - e-mail	bc report generated
08/07/09 11:00 AM	Dr. Peter McKenzie - e-mail	bc report generated
08/28/09 3:33 PM	Mainstream Canada - e-mail	bc report generated
08/28/09 3:34 PM	Dr. Peter McKenzie - e-mail	bc report generated
09/11/09 10:33 AM	Mainstream Canada - 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: 09-2968

Last Updated: 08/10/09 3:25 PM
Pathologist: Gary D. Marty
Received Date: 08/06/09
Collected Date: 08/04/09
Client Ref No: 7371

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

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

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

Case History

Submitted 2 formalized and 3 fresh fish tissues for histology and PCR (IHN and VHS).

Saltwater entry - 07-S0. Vaccinated. Dead. 2 fresh morts. Sample with inflammation in hind gut, some p.[petechial] hem in liver, feed was present throughout gut. Mortality increased July 31 decreasing since. Viral samples - 3 samples.

Final Diagnosis

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

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

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

3. Heart: myocardial karyomegaly, multifocal, mild (slides 1, 2)

4. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1, 2)

5. Head kidney: vascular congestion, diffuse, mild (slide 2)

Final Comment: These fish have a few microscopic changes that provide clues to their death (see detailed comments below). Unfortunately, none of the changes are of sufficient severity to explain mortality.

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

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). In Atlantic salmon livers sampled as part of the Province's Fish Health Auditing and Surveillance Program, prevalence of these vacuoles steadily increased in Atlantic salmon from 42% in 2006 to 50% in 2007 and 55% in 2008; 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.

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, or passive congestion (a postmortem change).

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.4% of the 1609 Atlantic salmon hearts examined as part of the province's Fish Health Auditing and Surveillance Program from 2006 through 2008). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Histopathology

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

Slide 1 (7371-1) - heart, spleen, liver, trunk kidney, intestine, intestinal ceca, mesenteric adipose tissue

Slide 2 (7371-2) - heart, spleen, liver, trunk kidney, intestine, intestinal ceca, mesenteric adipose tissue

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/10/09 @ 9:39 AM

Specimen	ID	Test	Result
Tissue	7371-1	PCR - IHNV	Negative
Tissue	7371-2	PCR - IHNV	Negative
Tissue	7371-3	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/10/09 @ 9:37 AM

Specimen	ID	Test	Result
Tissue	7371-1	PCR - VHSV	Negative
Tissue	7371-2	PCR - VHSV	Negative
Tissue	7371-3	PCR - VHSV	Negative

History of Communication

Date	To	Description
08/10/09 3:26 PM	Morrison, Diane - fax	bc report generated
08/12/09 10:06 AM	Marine Harvest Canada - e-mail	Case Invoiced

Gary D. Marty

Case: 09-2968

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BRITISH COLUMBIA
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Animal Health Centre

AAVLD - Accredited Laboratory

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Toll-Free : 1-800-661-9903

Final Report AHC Case: 09-2969

Last Updated: 08/11/09 12:06 PM
Pathologist: Gary D. Marty
Received Date: 08/06/09
Collected Date: 08/04/09
Client Ref No: 7372

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

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

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

Case History

Submitted 7 formalized and 2 fresh fish tissues for histopathology and PCR (IHN and VHS). Previous submission = 7312; 2009-2477.

7 fresh morts sample - w/similar symptoms. Hind gut was inflamed, p. hem in liver, feed was present in upper gut in all fish. Some had little feed in lower gut. Mortality increased July 31st, decreasing since. Viral samples 1-3 are pooled 2 fish - 4 single fish.

Final Diagnosis

1. Intestine: mucosal vascular congestion, diffuse, mild (slides 1, 2, 6)

2a. Liver: sinusoidal congestion, with acid hematin granules, acute, multifocal, mild (slides 2, 3, 4), moderate (slide 1)

2b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, small amounts (slide 5), moderate (slide 4)

3. Liver: macrophage aggregates and sinusoidal macrophages with yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slide 6)

4. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 7)

3. Mesenteric adipose tissue: capillary congestion, diffuse, moderate (slide 3)

4a. Trunk kidney: moderate numbers of interstitial intracytoplasmic eosinophilic granules, diffuse (slide 7)

4b. Kidney: vascular congestion, diffuse, moderate (slide 4A)

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

6. Mesenteric adipose tissue: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 3); with foci of granulomatous inflammation, moderate (slide 6)

7. Intestine: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 6, 7), moderate (slide 3)

Final Comment: These fish have several microscopic changes that provide clues to their death (see detailed comments below). None of the changes are of sufficient severity to assign a cause of death .

Vascular congestion of the intestinal lamina propria is an uncommon lesion in Atlantic salmon. Vascular congestion in other organs sometimes results from circulating vasodilators. Differentials include substances released from inflammatory cells, bacteria, or a viral infection. Alternatively, some of the congestion might be a result of postmortem change (passive congestion). In this case, it is not associated with increased numbers of inflammatory cells. The mucosal epithelium in affected fish does not seem to be abnormal, but autolysis is too severe in slide #1 to assess mucosal morphology. Intestinal ceca are not involved (consistent with gross findings).

Because the congestion does not seem to be associated with inflammation or infectious disease, if this is an ongoing problem consider systematically scoring the gross lesion (i.e., none, mild, moderate, or severe congestion). Then, compare the gross lesion score with other gross findings (fish size, whether the intestine contains food, what type of feed is being fed, weather conditions, etc.). With time, trends might emerge in the data that help identify the cause or causes of the congestion.

Sinusoidal congestion in the liver is evidence of circulating vasodilators. I have seen it associated with viral hemorrhagic septicemia virus and *Listonella anguillarum* infection. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data).

Pigment in the liver of slide 6 is probably lipofuscin, and it might also include hemosiderin. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish. Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. Hemosiderin accumulation in the liver might result from increased turnover of red blood cells.

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins or inflammatory mediators) or come from outside the fish (e.g., from the water or the feed). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 14% of the 468 Atlantic salmon livers examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2008 was sufficient to identify a trend towards greater prevalence in the winter and spring (21-30%) than in the summer and fall (1.9-4.4%).

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. It is not associated with postmortem change. 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.

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.

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

Renal vascular congestion is fairly common in autolyzed fish as a postmortem artifact (i.e., passive congestion). It also occurs in response to viral infections (e.g., VHSV or ISAV), but PCR results and lack of ISAV in BC make these viruses unlikely in these fish.

Peritonitis involving the spleen, intestine, and mesenteries is consistent with a reaction to foreign material; peritonitis is common in fish that have been vaccinated, affecting 60% of the 460 spleens from Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Literature Cited:

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

Histopathology

Formalin-fixed tissues from 7 fish were submitted in 7 cassettes for histopathology. Slide #s 1-7 are labeled in the same order as client #s 7372-1 through 7372-7.

Organs included on most slides - heart, liver, spleen, head kidney, trunk kidney, intestine, intestinal ceca, mesenteric adipose tissue; some sections contain stomach. All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Liver autolysis: none (slides 5, 7), mild (slides 2, 3, 6), severe (slides 1, 4). Large foci of erythrocytes (e.g., liver in slide 2) have precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/10/09 @ 9:50 AM

Specimen	ID	Test	Result
Tissue	7372-1	PCR - IHNV	Negative
Tissue	7372-2	PCR - IHNV	Negative
Tissue	7372-3	PCR - IHNV	Negative
Tissue	7372-4	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/10/09 @ 9:50 AM

Specimen	ID	Test	Result
Tissue	7372-1	PCR - VHSV	Negative
Tissue	7372-2	PCR - VHSV	Negative
Tissue	7372-3	PCR - VHSV	Negative
Tissue	7372-4	PCR - VHSV	Negative

History of Communication

Date	To	Description
08/11/09 12:06 PM	Morrison, Diane - fax	bc report generated
08/12/09 10:07 AM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-3042

Last Updated: 08/14/09 4:34 PM
Pathologist: Gary D. Marty
Received Date: 08/12/09
Collected Date: 08/09/09
Client Ref No: 7379

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

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

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

Case History

Submitted formalized tissue from 2 fish for histopathology and fresh tissues from 1 fish for PCR (IHN and VHS).

Saltwater entry - 2008 S1. Vaccinated. 4 fresh morts sampled. Fish full of feed, petechial hem in liver. Inflammation of hind gut, water temp 15-16C and possible low/DO 5- 6mg/L.

Final Diagnosis

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

2. Kidney: vascular congestion, diffuse, moderate (slide 2A)

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

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

5a. Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions, acute, bifocal, mild (slide 1)

5b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate (slide 1)

Final Comment: Clinical and microscopic features of this case are similar to other cases submitted recently from Marine Harvest: abundant food in the gastrointestinal tract (clinical finding), vascular congestion of the intestinal lamina propria, and mortality with few significant lesions. The pathogenesis of this suite of lesions might be multifactorial; potential causes include the feed, water quality, and infectious disease. The slides contain no obvious organisms. Comments on specific lesions follow.

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).

Renal congestion is evidence of circulating vasodilators; in autolyzed fish it is probably a result of postmortem passive congestion. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV.

Peritonitis of the intestine is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting the intestinal ceca of 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).

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Sinusoidal congestion in the liver is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV; as in this case, the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). 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.

Histopathology

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

Slide 1 (7379-1) - heart, spleen, liver, head kidney, trunk kidney, intestine

Slide 2A (7379-2) - heart, spleen, liver, intestine, head kidney, trunk kidney, intestinal cecum and mesenteric adipose tissue

Slide 2B (7379-2) - gill

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

Quality control: Liver autolysis: mild (slides 1, 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). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: A Scouras on 08/14/09 @ 1:45 PM

Specimen	ID	Test	Result
Tissue	organs	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: A Scouras on 08/13/09 @ 4:28 PM

Specimen	ID	Test	Result
Tissue	organs	PCR - VHSV	Negative

History of Communication

Date	To	Description
08/14/09 4:35 PM	Morrison, Diane - fax	bc report generated
08/24/09 3:43 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-3043

Last Updated: 08/14/09 1:45 PM
Pathologist: Gary D. Marty
Received Date: 08/12/09
Collected Date: 08/10/09
Client Ref No: 7381

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

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

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

Case History

Submitted one fresh fish tissue for PCR (IHN and VHS).

Saltwater entry - 09 S1. Vaccinated. Dead. # Submitted dead - 16. Fish health check prior to fish transfer. N.V.L mouth rot occurring on site.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: A Scouras on 08/14/09 @ 1:45 PM

Specimen	ID	Test	Result
Tissue	A)#1	PCR - IHNV	Negative
Tissue	B)#2	PCR - IHNV	Negative
Tissue	C)#3	PCR - IHNV	Negative
Tissue	D)#4	PCR - IHNV	Negative
Tissue	E)#5	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: A Scouras on 08/13/09 @ 4:28 PM

Specimen	ID	Test	Result
Tissue	A)#1	PCR - VHSV	Negative
Tissue	B)#2	PCR - VHSV	Negative
Tissue	C)#3	PCR - VHSV	Negative
Tissue	D)#4	PCR - VHSV	Negative
Tissue	E)#5	PCR - VHSV	Negative

History of Communication

Date	To	Description
08/14/09 3:25 PM	Morrison, Diane - fax	bc report generated

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

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Final Report AHC Case: 09-3044

Last Updated: 08/14/09 1:45 PM
Pathologist: Gary D. Marty
Received Date: 08/12/09
Collected Date: 08/10/09
Client Ref No: 7378

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

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

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

Case History

Submitted one fresh fish sample for PCR (IHN and VHS).

Saltwater entry - 2009 S1. Vaccinated. # Submitted dead - 4 pooled. NVL - viral taken on fresh dead (4).

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: A Scouras on 08/14/09 @ 1:45 PM

Specimen	ID	Test	Result
Tissue	organs	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: A Scouras on 08/13/09 @ 4:28 PM

Specimen	ID	Test	Result
Tissue	organs	PCR - VHSV	Negative

History of Communication

Date	To	Description
08/14/09 3:28 PM	Morrison, Diane - fax	bc report generated
08/18/09 1:25 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-3062

Last Updated: 08/19/09 10:59 AM
Pathologist: Gary D. Marty
Received Date: 08/14/09
Collected Date: 08/12/09
Client Ref No: VP130809

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Cassan, Nathan**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 3 samples for Bacteriology identification.

4 fish with Exophthalmia, both eyes and brain looped onto 3 plates. Labelled fish# and eye# for report purposes. Environment - freshwater, Vaccinated - yes, Euthanized - TMS. Prior submission - # 09 - 2927. DOD - Aug 12, 09.

Farm Id - Venture Point

Final Diagnosis

Some stains of ***Vibrio splendidus*** kill oysters and larval fish under controlled laboratory conditions (Le Roux et al. 2002, Thomson et al. 2005), but *Vibrio splendidus* has not been shown to kill salmonids. *Pseudomonas sp* is a common contaminant in bacterial cultures from fish.

Literature cited:

Le Roux, F., M. Gay, C. Lambert, M. Waechter, S. Poubalanne, B. Chollet, J.L. Nicolas, and F. Berthe. 2002. Comparative analysis of *Vibrio splendidus* -related strains isolated during *Crassostrea gigas* mortality events. Aquat. Living Resour. 15:251¿258.

Thomson, R., Macpherson, H.L., Riaza, A., and T.H. Birkbeck, 2005. *Vibrio splendidus* biotype 1 as a cause of mortalities in hatchery-reared larval turbot, *Scophthalmus maximus* (L.). Journal of Applied Microbiology [J. Appl. Microbiol.] 99(2):243-250.

Bacteriology

Aerobic Culture - Prod Resulted by: Jaime Osei-Appiah Verified by: Erin Zabek on 08/19/09 @ 9:24 AM

Specimen	ID	Isolate	Result	Level
Isolate	Fish 1 Eye 1	Pseudomonas sp.	Positive	
Isolate	Fish 1 Eye 2	Pseudomonas sp.	Positive	
Isolate	Fish 2 Eye 1	Pseudomonas sp.	Positive	

Isolate	Fish 2 Eye 1	Vibrio splendidus	Positive
Isolate	Fish 2 Eye 2	Pseudomonas sp.	Positive
Isolate	Fish 3 Eye 1	Pseudomonas sp.	Positive
Isolate	Fish 3 Eye 2	Pseudomonas sp.	Positive
Isolate	Fish 4 Eye 1	Pseudomonas sp.	Positive
Isolate	Fish 4 Eye 2	Pseudomonas sp.	Positive
Isolate	Fish 1 Brain	Pseudomonas sp.	Positive
Isolate	Fish 2 Brain	Pseudomonas sp.	Positive
Isolate	Fish 3 Brain		No Bacteria Isolated
Isolate	Fish 4 Brain	Pseudomonas sp.	Positive

Fish Resulted by: Jaime Osei-Appiah Verified by: Erin Zabek on 08/19/09 @ 9:24 AM

Organism	ID	e	ffc	sor	s3	sxt	ot
Pseudomonas sp.	Fish 1 Eye 1	r	r		s	s	s
Vibrio splendidus	Fish 2 Eye 1	s	s		s	s	s

**: Antibiotic sensitivity legend: e = Erythromycin, ffc = Florfenicol, sor = Romet 30, s3 = Tri-Sulfas, sxt = Sulfamethoxazole/Trimethropri ot = Oxytetracycline Unable to perform Romet sensitivity due to supplier shortage. Testing will resume for Romet as soon as supply becomes available.

History of Communication

Date	To	Description
08/19/09 11:07 AM	Mainstream Canada - e-mail	bc report generated
08/19/09 11:08 AM	Dr. Peter McKenzie - e-mail	bc report generated
08/24/09 4:03 PM	Mainstream Canada - 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: 09-3187

Last Updated: 08/27/09 11:45 AM
Pathologist: Gary D. Marty
Received Date: 08/21/09
Collected Date:
Client Ref No: 14355

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted one formalized fish tissue for histopathology.

Saltwater. 4 Kg. Vaccinated. Euthanized - yes (Stun Bleed). Fish was observed after bawder - internal organs had been removed. Flesh was black.

Farm Id - West Side

Final Diagnosis

1. Skeletal muscle: Abundant melanocytes between muscle bundles, diffuse (slide 1A)

2. Gill arch: Abundant melanocytes, diffuse (slide 1G)

Final Comment: This fish had black flesh due to abundant melanocytes in its muscle (about 20 melanocytes per 40× objective lens field). Melanocytes were also common in the gills [Were they also black?]. Skeletal muscle is not a usual location for melanocytes (three sections from healthy Atlantic Salmon from another case all contained no melanocytes). However, the abundant melanocytes do not seem to be pathologic because they are not accompanied by other processes (such as inflammation, hemorrhage, necrosis, or organisms). Similar hyperpigmented muscle would not be hazardous for human consumption.

If this colouration has only been observed in one fish, then it might be the result of a mutation unique to this fish. If several other animals are affected, then it could be a genetic trait.

Histopathology

Formalin-fixed tissues were submitted in 1 cassette for histopathology. Prior to processing into paraffin, tissues were split into 2 cassettes (1A and 1G).

Slide 1A (West Side Aug 13/09) - skeletal muscle (3 pieces)

Slide 1G - gill

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

Quality control: Tissue preservation is good in skeletal muscle section; gills have moderate autolysis. Decalcification is 100% complete and differential staining is good. Organs have no postfixation dehydration.

Staff Comments:
Fish Histopathology Fellow: Meritxell Diez Padrisa, D.V.M.

History of Communication

Date	To	Description
08/27/09 11:46 AM	Mainstream Canada-T - fax	bc report generated
08/27/09 11:46 AM	Dr. Peter McKenzie - e-mail	bc report generated
09/11/09 1:13 PM	Mainstream Canada - 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: 09-3267

Last Updated: 09/21/09 12:13 PM
Pathologist: Gary D. Marty
Received Date: 08/25/09
Collected Date:
Client Ref No: 7396

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

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

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

Case History

Submitted 9 fresh fish tissues for viral culture.

Saltwater entry: 07 SO. Insurance: No. Euthanized - No.

Viral culture on 9 samples collected from fresh morts. Cell culture on all 5 fish cell lines.

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 09/21/09 @ 12:13 PM

Specimen	ID	Isolate	Result	Level
Tissue	7396-1		No viruses isolated	
Tissue	7396-2		No viruses isolated	
Tissue	7396-3		No viruses isolated	
Tissue	7396-4		No viruses isolated	
Tissue	7396-5		No viruses isolated	
Tissue	7396-6		No viruses isolated	
Tissue	7396-7		No viruses isolated	
Tissue	7396-8		No viruses isolated	
Tissue	7396-9		No viruses isolated	

History of Communication

Date	To	Description
09/21/09 3:00 PM	Morrison, Diane - fax	bc report generated
09/28/09 11:41 AM	Marine Harvest Canada - e-mail	Case Invoiced

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

END OF REPORT

Case: 09-3267

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Final Report AHC Case: 09-3270

Last Updated: 08/27/09 2:04 PM
Pathologist: Gary D. Marty
Received Date: 08/25/09
Collected Date: 08/20/09
Client Ref No: 7388

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

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

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

Case History

Submitted 3 fresh and formalized fish tissue for Histopathology and PCR for IHN, VHS and ISA.

Saltwater entry: 09-S1. Vaccinated - Yes. Legal: No. Euthanized - No. DOD: Aug. 20/09.

High counts of heterosigma around farm. 3 fish sampled were fresh morts. All 3 had mouthmyxo but gills looked slightly hemorrhaged.

Final Diagnosis

1a. Gill filaments: distal necrosis, acute, focal, mild (one filament, slide 2B); multifocal, moderate (multiple filaments, slide 1B)

1b. Gill: lamellar capillary thrombosis, disseminated, acute, severe (slide 3B)

1c. Gill: lamellar capillary thrombosis and necrosis, multifocal, acute, moderate (slide 1B)

1c. Gill: lamellar telangiectasis, multifocal, moderate (slide 1B)

2a. Liver: hepatocellular karyomegaly and megalocytosis, multifocal, mild (slide 1A)

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

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

2d. Liver: sinusoidal congestion, multifocal, moderate (slide 3A)

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

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

3c. Trunk and head kidney: vascular congestion, multifocal to diffuse, mild (slide 3A)

4. Spleen: peritonitis, granulomatous, regionally diffuse, moderate (slide 2A), with intralesional vacuoles about 50 µm in diameter, moderate (slide 1A)

5. Intestinal ceca: peritonitis, chronic, multifocal, with fibrocellular fronds, mild (slide 3A)

Final Comment: Fish #s 1 and 3 have gill lesions of sufficient severity to explain their death, whereas lesions in fish #2 are not as severe. The histopathology of exposure of Atlantic salmon to *Heterosigma* has not been well described, but gill necrosis is consistent with *Heterosigma* exposure described elsewhere [Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada).] Comments on specific lesions follow:

The distal tips of about 8 gill filaments in slide 1B have a fairly sharp line of demarcation between viable and necrotic tissue. This pattern is usually associated with occlusive thrombosis of the main filament afferent artery. Although no such arteries are included in the section, thrombosis and telangiectasis of nearby lamellar capillaries is evidence of increased coagulability. The most severe lesion in slide 3B is disseminated thrombosis of lamellar capillaries. These lesions can result from endothelial damage related to virus, bacterial, or parasitic infection, or exposure to toxins from harmful algal blooms. Telangiectasis in the gill (lamellar capillary aneurysms or ruptured lamellar capillaries) most commonly results from trauma (e.g., handling, or hitting something in the net pen).

Hepatic megalocytosis and karyomegaly can result from exposure to several types of toxins, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. It is a common feature of net-pen liver disease. Hepatic megalocytosis is rare in farmed fish in BC, occurring in only 4 of 1660 "fresh silvers" sampled as part of the BC Fish Health Auditing and Surveillance Program from 2006 - 2008.

Pigment in the liver is probably lipofuscin, and it might also include hemosiderin. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish. 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.

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

Vascular congestion in the liver and kidney is evidence of circulating vasodilators; sometimes it occurs as a postmortem artifact. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Most commonly, the cause is unknown (as in this case). Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia. PCR results rule out VHSV, IHNV, and ISAV in these fish.

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Renal tubular epithelial necrosis was fairly common among fish sampled in 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). The history in this case is consistent with exposure to algal toxins.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29% ; n = 469) and Pacific salmon (prevalence = 31% ; n = 118).

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe). Vacuoles are probably a result of vaccine material lost during tissue processing.

Peritonitis of the intestinal ceca 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 6 cassettes for histopathology. The gills (B cassettes) were immersed 2 h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

Slide 1A (7388-1) - heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slides 2A (7388-2) and 3A (7388-3) - heart, spleen, liver, head kidney, trunk kidney, intestinal ceca

Slide 1B (7388-1), 2B (7388-2) and 3B (7388-3) - gill

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

Quality control: Liver autolysis: mild (slides 1A, 2A, 3A). Large foci of erythrocytes (e.g., liver 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). Gill decalcification is complete and differential staining is good. Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/27/09 @ 11:04 AM

Specimen	ID	Test	Result
Tissue	org 7388-1	PCR - IHNV	Negative
Tissue	org 7388-2	PCR - IHNV	Negative
Tissue	org 7388-3	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/27/09 @ 1:21 PM

Specimen	ID	Test	Result
Tissue	org 7388-1	PCR - ISA	Negative
Tissue	org 7388-2	PCR - ISA	Negative
Tissue	org 7388-3	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/27/09 @ 11:05 AM

Specimen	ID	Test	Result
Tissue	org 7388-1	PCR - VHSV	Negative
Tissue	org 7388-2	PCR - VHSV	Negative
Tissue	org 7388-3	PCR - VHSV	Negative

History of Communication

Date	To	Description
08/27/09 2:05 PM	Morrison, Diane - fax	bc report generated
09/11/09 2:27 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

END OF REPORT

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Animal Health Centre

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Final Report AHC Case: 09-3272

Last Updated: 08/31/09 4:24 PM
Pathologist: Gary D. Marty
Received Date: 08/25/09
Collected Date: 08/17/09
Client Ref No: 7387

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

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

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

Case History

Submitted 2 formalized fish tissue for histopathology and 6 fresh fish tissue for PCR for IHN, VHS and ISA.

Saltwater entry: 09 - S1. Vaccinated - Yes. Legal - No. Euthanized - No.

Mortality has been increasing. Fresh fish sampled had no visible lesions recent low DO in pens.

Final Diagnosis

1a. Heart: endocarditis, lymphohistiocytic, diffuse, with endothelial cell hypertrophy and myocardial degeneration and regeneration, moderate (slide 2A)

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

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

2b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, abundant (slides 1A, 2A)

2c. Liver: sinusoidal congestion, acute, multifocal, moderate (slide 2A)

2d. Liver: hepatocellular single cell necrosis (apoptosis), disseminated, acute, moderate (slide 2A)

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

3a. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, mild (slides 1A, 2A)

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

4. Head kidney: interstitial cell necrosis, bifocal (each about 200 μm in diameter), mild (slide 1A)

5. Gill: lamellar capillary thrombosis, multifocal, acute, mild (slide 1B)

6. Brain: meningoencephalitis, lymphohistiocytic, multifocal, mild (slide 2A)

7a. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1A, 2A)

7b. Spleen: parenchymal golden pigment (lipofuscin?), scattered, intracellular, mild (slide 2A)

Final Comment: Both fish probably died of systemic disease. In fish #2, heart lesions are probably most significant. By comparison, no organ in fish #1 has lesions of sufficient severity to have killed the fish; however, the sum of necrotizing and vascular lesions involving multiple organs is evidence of systemic organ collapse. Comments on specific lesions follow:

Endothelial cell hypertrophy in the heart is consistent with systemic immune stimulation; differentials include a bacterial or viral infection (e.g., VHSV). Inflammatory cells lining the endocardial surface in foci of inflammation are rarely more than 2 cell layers thick. This pattern of inflammation has also been described with Heart and Skeletal Muscle Inflammation in Atlantic salmon reared in Europe (thought to be related with an alphavirus), but this disease has not been identified in BC salmon.

Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 18% of the 467 Atlantic salmon hearts examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program.

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

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

Multifocal sinusoidal congestion in the liver is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV; as in this case, the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data).

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

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

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). 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).

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29% ; n = 469) and Pacific salmon (prevalence = 31% ; n = 118).

Necrotic hematopoietic cells in the renal interstitium have some form of nuclear degeneration (pyknosis, karyorrhexis, or karyolysis). Differentials include a bacterial infection (e.g., *Yersinia ruckeri*) or circulating toxins.

Thrombosis in the gill is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection, or exposure to toxins from harmful algal blooms.

Meningoencephalitis is evidence of immune stimulation; differentials include viruses, bacteria, or parasites. In this case, the cause is probably the same as the cause of endocarditis.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology. The gills (B cassettes) were immersed 2 h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

Slide 1A (7387-1) - brain, heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide 2A (7387-2) - brain, heart, spleen, liver, head kidney, trunk kidney, intestinal ceca

Slide 1B (7387-1) and 2B (7387-2) - gill

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

Quality control: Liver autolysis: mild (slides 1A, 2A). Gill decalcification is complete and differential staining is good. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR-Salmon Alphavirus Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 08/31/09 @ 4:24 PM

Specimen	ID	Test	Result
Tissue	7387 (1-6) Pool A	PCR-Salmon Alphavirus	Negative
Tissue	7387 (1-6) Pool B	PCR-Salmon Alphavirus	Negative
**: A positive control is currently unava ble for this PCR assay.			

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/27/09 @ 11:06 AM

Specimen	ID	Test	Result
Tissue	org 7387-1 (IHN,VHS)	PCR - IHNV	Negative
Tissue	org 7387-2 (IHN,VHS)	PCR - IHNV	Negative
Tissue	org 7387-3 (IHN,VHS)	PCR - IHNV	Negative
Tissue	org 7387-4 (IHN,VHS)	PCR - IHNV	Negative
Tissue	org 7387-5 (IHN,VHS)	PCR - IHNV	Negative
Tissue	org 7387-6 (IHN,VHS)	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/27/09 @ 1:21 PM

Specimen	ID	Test	Result

Tissue	org 7387-1 (ISA)	PCR - ISA	Negative
Tissue	org 7387-2 (ISA)	PCR - ISA	Negative
Tissue	org 7387-3 (ISA)	PCR - ISA	Negative
Tissue	org 7387-4 (ISA)	PCR - ISA	Negative
Tissue	org 7387-5 (ISA)	PCR - ISA	Negative
Tissue	org 7387-6 (ISA)	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 08/27/09 @ 11:05 AM

Specimen	ID	Test	Result
Tissue	org 7387-1 (IHN,VHS)	PCR - VHSV	Negative
Tissue	org 7387-2 (IHN,VHS)	PCR - VHSV	Negative
Tissue	org 7387-3 (IHN,VHS)	PCR - VHSV	Negative
Tissue	org 7387-4 (IHN,VHS)	PCR - VHSV	Negative
Tissue	org 7387-5 (IHN,VHS)	PCR - VHSV	Negative
Tissue	org 7387-6 (IHN,VHS)	PCR - VHSV	Negative

Staff Comments:
Fish Histopathology Fellow: Meritxell Diez Padrisa, D.V.M.

History of Communication

Date	To	Description
08/31/09 4:30 PM	Morrison, Diane - fax	bc report generated
09/21/09 12:40 PM	Marine Harvest Canada - e-mail	Case Invoiced

Gary D. Marty
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Final Report AHC Case: 09-3322

Last Updated: 08/31/09 4:23 PM
Pathologist: Gary D. Marty
Received Date: 08/27/09
Collected Date:
Client Ref No: 7391

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

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

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

Case History

Submitted fresh Atlantic salmon tissue for PCR (IHN, VHS, and ISA).

Saltwater entry 09-S1. Sample 1 fresh mort petechial hem in pc/fat.

Molecular Diagnostics

PCR - IHNV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 08/31/09 @ 9:55 AM

Specimen	ID	Test	Result
Tissue	organ 7391	PCR - IHNV	Negative

PCR - ISA Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 08/31/09 @ 4:23 PM

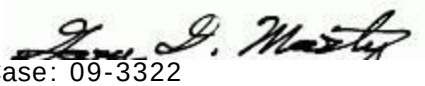
Specimen	ID	Test	Result
Tissue	organ 7391	PCR - ISA	Negative

PCR - VHSV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 08/31/09 @ 9:55 AM

Specimen	ID	Test	Result
Tissue	organ 7391	PCR - VHSV	Negative

History of Communication

Date	To	Description
08/31/09 4:27 PM	Morrison, Diane - fax	bc report generated
09/11/09 3:42 PM	Marine Harvest Canada - 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: 09-3323

Last Updated: 09/01/09 9:54 AM
Pathologist: Gary D. Marty
Received Date: 08/27/09
Collected Date:
Client Ref No: 7397

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

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

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

Case History

Submitted fresh and formalized Atlantic salmon tissue for histopathology and PCR (IHN, VHS, and ISA).

Saltwater entry - 08-S1. Vaccinated. Insurance/Legal - yes. Fish are experiencing hetersigma bloom with high mortality. 2 Fresh floaters sent in for samples. This will be an insurance claim.

Final Diagnosis

1a. Liver: sinusoidal congestion, multifocal, mild (slide 1A), with acid hematin granules, moderate (slide 2A)

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

1c. Liver: biliary preductular cell hyperplasia, multifocal, mild (slide 1A)

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

2b. Head kidney: interstitial intracytoplasmic eosinophilic granules, focal (affected area ~ 200 µm in diameter), mild (slide 1A)

3. Heart: diffuse epicarditis and multifocal endocarditis, lymphohistiocytic, mild (slide 1A)

4. Spleen: parenchymal golden pigment (lipofuscin?), scattered, intracellular, mild (slide 1A)

5. Gill: abundant eosinophilic granular cells in the loose connective tissue of filaments (slides 1B, 2B)

Final Comment: These fish have several lesions that might provide clues to their cause of death, but none are of sufficient severity to have caused mortality. The histopathology of exposure of Atlantic salmon to *Heterosigma* has not been well described. In other cases, *Heterosigma* exposure has been associated with gill necrosis, but this relation has not been confirmed under experimental conditions. In general, *Heterosigma* are not seen in sections of tissues from clinical cases. [Reference: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada).] In this case, gill sections have no *Heterosigma* or necrosis; however, severe gill autolysis limits our ability to detect subtle lesions. Histopathology in this case can still rule out other obvious causes of

death. Several of the lesions in this case could be a result of exposure to toxins; comments on specific lesions follow:

Sinusoidal congestion in the liver is evidence of circulating vasodilators. Differentials include substances released from inflammatory cells or bacteria, infection with VHSV, or exposure to toxins (e.g., from algae). Most commonly, the cause is unknown. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia (and these fish are PCR negative for ISAV). Acid hematin deposits in congested foci (slide 2A), but nowhere else in the section, are evidence that the congested foci were acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

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

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins or inflammatory mediators) or come from outside the fish (e.g., from the water or the feed). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 14% of the 468 Atlantic salmon livers examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2008 was sufficient to identify a trend towards greater prevalence in the winter and spring (21-30%) than in the summer and fall (1.9-4.4%).

Renal congestion is evidence of circulating vasodilators; in autolyzed fish it is probably a result of postmortem passive congestion. Differentials include substances released from inflammatory cells or bacteria, and infection with VHSV. Renal congestion is also one of the classic signs of infectious salmon anemia (ISA), but ISA has never been isolated from fish in BC.

Accumulation of eosinophilic granules in the cytoplasm of cells lining small vessels in the kidney is a distinctive finding in Atlantic salmon. These granules probably accumulate in response to systemic immune stimulation. The granules might be part of eosinophilic granular cells or endothelial cells with eosinophilic granules. These granules are common with *Piscirickettsia* salmonis infection and I have seen them with severe cerebral *Renibacterium salmoninarum* (in at least one case, the affected fish had no granulomatous inflammation in the kidney), but other cases (like this one) have no known cause.

Lymphohistiocytic epicarditis is a commonly related to bacterial and viral infections. Among farmed salmon in British Colombia, related causes include Erythrocytic Inclusion Body Syndrome (EIBS), *Renibacterium salmoninarum*, *Yersinia ruckeri* and viral hemorrhagic septicemia virus. The section from the affected fish has no obvious organisms.

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%).

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

Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology. Prior to processing into paraffin, cassettes containing gills were decalcified in Protocol B (hydrochloric acid solution) for 2 hours.

Slides 1A (7397-1) and 2A (7397-2) - heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, skin/skeletal muscle, mesenteric adipose tissue

Slides 1B (7397-1) and 2B (7397-2) - gill

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

Quality control: Liver autolysis: severe (slides 1A, 2A); gill autolysis: severe (slide 1B, 2B). Some foci of hepatocytes around large vessels (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). Decalcification is 100% complete and differential staining is good. Organs have no postfixation dehydration.

PCR - IHNV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 08/31/09 @ 9:55 AM

Specimen	ID	Test	Result
Tissue	org 7397-1	PCR - IHNV	Negative
Tissue	org 7397-2	PCR - IHNV	Negative

PCR - ISA Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 08/31/09 @ 4:23 PM

Specimen	ID	Test	Result
Tissue	org 7397-1	PCR - ISA	Negative
Tissue	org 7397-2	PCR - ISA	Negative

PCR - VHSV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 08/31/09 @ 9:55 AM

Specimen	ID	Test	Result
Tissue	org 7397-1	PCR - VHSV	Negative
Tissue	org 7397-2	PCR - VHSV	Negative

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

History of Communication

Date	To	Description
09/01/09 9:56 AM	Morrison, Diane - fax	bc report generated
09/11/09 3:43 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Toll-Free : 1-800-661-9903

Final Report AHC Case: 09-3375

Last Updated: 09/03/09 1:48 PM
Pathologist: Gary D. Marty
Received Date: 09/01/09
Collected Date:
Client Ref No: 7399

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

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

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

Case History

Submitted 3 formalized fish tissue for Histopathology and 4 fresh fish tissue for PCR.

Saltwater entry - 08 S1. Vaccinated. Insurance/Legal - yes. 4 fresh morts. Farm has had significant losses due to heterosigma bloom. Will be an insurance claim. (gills may be too old for histology).

Final Diagnosis

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

1b. Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions, acute, focal, mild (slide 2A)

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

1d. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slide 3A)

2. Heart: myocardial karyomegaly, multifocal, mild (slide 2A)

3. Head and trunk kidney: vascular congestion, diffuse, mild (slides 2A, 3A), moderate (slide 1A)

Final Comment: These fish have several lesions that might provide clues to their cause of death, but none are of sufficient severity to have caused mortality. The histopathology of exposure of Atlantic salmon to *Heterosigma* has not been well described. In other cases, *Heterosigma* exposure has been associated with gill necrosis, but this relation has not been confirmed under experimental conditions. In general, *Heterosigma* are not seen in sections of tissues from clinical cases. [Reference: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada).] In this case, gill sections have no *Heterosigma* or necrosis; however, severe gill autolysis limits my ability to detect subtle lesions. Histopathology in this case can still rule out other obvious causes of death. Several of the lesions in this case could be a result of exposure to toxins; 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. 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.

Focal sinusoidal congestion in the liver is a nonspecific vascular lesion. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. PCR results rule out a viral cause in these fish. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). 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.

Pigment in the liver is probably lipofuscin, and it might also include hemosiderin. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish. 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.

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.4% of the 1609 Atlantic salmon hearts examined as part of the province's Fish Health Auditing and Surveillance Program from 2006 through 2008). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Renal congestion in autolyzed fish is probably a result of postmortem passive congestion, but it might be a result of circulating vasodilators. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV.

Histopathology

Formalin-fixed tissues were submitted in 6 cassettes for histopathology. Gills were decalcified in Protocol B (hydrochloric acid solution) for 20 minutes before being rinsed in water and processed routinely with other cassettes into paraffin.

Slides 1A (7399-1) and 3A (7399-3) - heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide 2A (7399-2) - heart, spleen, liver, head kidney, trunk kidney

Slides 1G (7399-1), 2G (7399-2) and 3G (7399-3) - gill

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 09/03/09 @ 12:05 PM

Specimen	ID	Test	Result
Tissue	7399-1	PCR - IHNV	Negative
Tissue	7399-2	PCR - IHNV	Negative
Tissue	7399-3	PCR - IHNV	Negative
Tissue	7399-4	PCR - IHNV	Negative

PCR - ISA Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 09/03/09 @ 1:48 PM

Specimen	ID	Test	Result
Tissue	7399-1	PCR - ISA	Negative
Tissue	7399-2	PCR - ISA	Negative
Tissue	7399-3	PCR - ISA	Negative
Tissue	7399-4	PCR - ISA	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 09/03/09 @ 12:05 PM

Specimen	ID	Test	Result
Tissue	7399-1	PCR - VHSV	Negative
Tissue	7399-2	PCR - VHSV	Negative
Tissue	7399-3	PCR - VHSV	Negative
Tissue	7399-4	PCR - VHSV	Negative

History of Communication

Date	To	Description
09/03/09 4:22 PM	Morrison, Diane - fax	bc report generated
09/17/09 10:10 AM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-3399

Last Updated: 09/25/09 2:34 PM
Pathologist: Gary D. Marty
Received Date: 09/02/09
Collected Date:
Client Ref No: 7403

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

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

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

Case History

Submitted 4 fresh fish tissue for Viral Culture - all 5 cell lines.

Saltwater entry - 07 S0. Sampled 4 fresh morts. #1/#2 with "hot guts".

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 09/25/09 @ 2:34 PM

Specimen	ID	Isolate	Result	Level
Tissue	7403-1		No viruses isolated	
Tissue	7403-2		No viruses isolated	
Tissue	7403-3		No viruses isolated	
Tissue	7403-4		No viruses isolated	

History of Communication

Date	To	Description
09/25/09 4:38 PM	Morrison, Diane - fax	bc report generated
09/30/09 9:24 AM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-3401

Last Updated: 09/08/09 3:45 PM
Pathologist: Gary D. Marty
Received Date: 09/02/09
Collected Date:
Client Ref No: 7402

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

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

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

Case History

Submitted 2 fresh fish tissue for PCR for IHN, VHS, ISA, and Rickettsia.

Saltwater entry:08 - S0. Vaccinated - Yes. 2 fresh morts - both fish with Rickettsia-like lesions in liver; swollen kidney. Both kidney and spleen were friable. Hem in s.b.

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 09/04/09 @ 10:00 AM

Specimen	ID	Test	Result
Tissue	orgA, 7402-1	PCR - IHNV	Negative
Tissue	orgB, 7402-2	PCR - IHNV	Negative

PCR - ISA Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 09/04/09 @ 2:06 PM

Specimen	ID	Test	Result
Tissue	orgA, 7402-1	PCR - ISA	Negative
Tissue	orgB, 7402-2	PCR - ISA	Negative

PCR-Piscirickettsia salmo Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 09/08/09 @ 3:45 PM

Specimen	ID	Test	Result
Tissue	orgA, 7402-1	PCR - Piscirickettsia salmonis	Positive
Tissue	orgB, 7402-2	PCR - Piscirickettsia salmonis	Positive

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 09/04/09 @ 9:59 AM

Specimen	ID	Test	Result
Tissue	orgA, 7402-1	PCR - VHSV	Negative
Tissue	orgB, 7402-2	PCR - VHSV	Negative

History of Communication

Date	To	Description
09/14/09 10:07 AM	Morrison, Diane - fax	bc report generated
09/21/09 1:01 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-3417

Last Updated: 09/08/09 3:45 PM
Pathologist: Gary D. Marty
Received Date: 09/02/09
Collected Date: 08/31/09
Client Ref No: PO14384

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted one fresh fish liver/spleen tissue for PCR. (confirm/rule out Piscirickettsia salmonis)

Saltwater. Vaccinated - Yes. Duration of illness - 7 days. Donut like granulomas liver. Systemic. Swollen spleen.

Molecular Diagnostics

PCR-Piscirickettsia salmo Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 09/08/09 @ 3:45 PM

Specimen	ID	Test	Result
Tissue	lv, spl	PCR - Piscirickettsia salmonis	Positive

History of Communication

Date	To	Description
09/14/09 9:55 AM	Mainstream Canada-T - fax	bc report generated
09/14/09 10:04 AM	Dr. Peter McKenzie - e-mail	bc report generated
09/21/09 1:05 PM	Mainstream Canada - e-mail	Case Invoiced

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

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

Final Report AHC Case: 09-3446

Last Updated: 09/18/09 9:13 AM
Pathologist: Stephen Raverty, DVM
Received Date: 09/04/09
Collected Date:
Client Ref No: CH083109

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Kelly Abel - Mainstream Canada**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted fresh and formalized Atlantic salmon tissues for Histopathology and PCR. (VHS and IHN).

Saltwater. Vaccinated - Yes. Euthanized: No. Internal observation: petechial hemorrhaging of the body walls. External observation: Boil like ulcers on skin. Virology samples frozen shipped on ice. Histology sample in 10% buffered formalin 24 hrs shipped in water.

Final Diagnosis

H ISTOPATHOLOGY:

1). Gills, heart, liver, and spleen: Embolism, septic, moderate, multifocal with occasional coagulative and liquifactive necrosis and hemorrhage

2). Pancreas: Fibrosis, moderate, multifocal with atrophy and steatosis

There are no significant lesions in the brain.

COMMENTS:

Post mortem change hampered microscopic evaluation of the sectioned tissues; nevertheless, the microscopic findings and gross observations are consistent with a bacterial septicemia. PCR results are affixed.

Histopathology

Refer to Morphologic Diagnoses

Molecular Diagnostics

PCR - IHNV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 09/10/09 @ 11:45 AM

Specimen	ID	Test	Result
Tissue	org(Pen8,Fish1)	PCR - IHNV	Negative

PCR - VHSV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 09/09/09 @ 10:02 AM

Specimen	ID	Test	Result
Tissue	org(Pen8,Fish1)	PCR - VHSV	Negative

PCR - Aeromonas salmonici Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/16/09 @ 9:25 AM

Specimen	ID	Test	Result
Tissue	org(Pen8,Fish1)	PCR - Aeromonas salmonicida	Negative
**: Test validation in progress.			

History of Communication

Date	To	Description
09/18/09 9:13 AM	Mainstream Canada - e-mail	bc report generated
09/18/09 9:13 AM	Dr. Peter McKenzie - e-mail	bc report generated
09/21/09 1:08 PM	Mainstream Canada - e-mail	Case Invoiced

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

END OF REPORT

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Final Report AHC Case: 09-3542

Last Updated: 09/16/09 1:38 PM
Pathologist: Gary D. Marty
Received Date: 09/11/09
Collected Date:
Client Ref No: 7405

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

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

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

Case History

Submitted 3 formalized and 3 fresh fish tissues for Histopathology and PCR for IHN, VHS and ISA.

Saltwater entry - 09 - S1. Vaccinated - Yes. Euthanized - No. Mortality increased over last 2 days. Mostly fresh silvers with no visible lesions. Fish were off feed for several days because of plankton but pen has been fed for 3 days before mortality increased.

Final Diagnosis

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

1b. Liver: sinusoidal congestion, multifocal, mild (slides 1A, 2A), moderate (slide 3A)

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

1d. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate (slides 1A, 3A), abundant (slide 2A)

2a. Trunk kidney: renal tubular epithelial necrosis, multifocal, acute, mild (slides 1A, 3A)

2b. Trunk kidney: small numbers of interstitial intracytoplasmic eosinophilic granules, diffuse (slide 1A)

2c. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slides 2A, 3A)

3. Gill: lamellar hyperplasia and fusion, focal, with an intralesional vacuole (200 × 100 µm), mild (slide 1B)

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

5. Intestinal ceca and mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 1A)

Final Comment: These fish have several lesions that provide clues about their cause of death, but none of the lesions are of sufficient severity to fully account for mortality. Consider the potential for interaction between the recent algal bloom and onset of feeding (i.e., some of the

lesions are consistent with toxin exposure). 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. Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Causes of hepatocellular single cell necrosis have not been well defined in fish. Possible differentials include exposure to toxins (endogenous or exogenous), or a viral infection (VHSV, but ruled out in this case by PCR).

Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion; diffuse congestion sometimes occurs as a postmortem artifact. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV (but ruled out in this case by PCR); the cause is usually not determined.

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.

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

Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared (as in this case). Mild cases are fairly common in debilitated fish. Renal tubular epithelial necrosis was fairly common among fish sampled in 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).

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

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29% ; n = 469) and Pacific salmon (prevalence = 31% ; n = 118).

Gill lamellar hyperplasia with fusion is evidence of physical damage from exposure to an irritant. The vacuole in slide 1B is probably the location of that irritant, but the irritating substance or organism was lost during processing (its identity remains unknown).

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe). Vacuoles are probably a result of vaccine material lost during tissue processing.

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

Literature Cited:

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

Histopathology

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

Slides 1A (7405-1) and 2A (7405-2) - heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, skin/skeletal muscle, mesenteric adipose tissue

Slide 3A (7405-3) - heart, spleen, liver, trunk kidney (2 pieces), intestinal ceca, skin/skeletal muscle, mesenteric adipose tissue

Slides 1B (7405-1), 2B (7405-2) and 3B (7405-3) - gill

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/15/09 @ 11:07 AM

Specimen	ID	Test	Result
Tissue	A)7405-1	PCR - IHNV	Negative
Tissue	C)7405-2	PCR - IHNV	Negative
Tissue	E)7405-3	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/15/09 @ 11:07 AM

Specimen	ID	Test	Result
Tissue	B)7405-1	PCR - ISA	Negative
Tissue	D)7405-2	PCR - ISA	Negative
Tissue	F)7405-3	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/15/09 @ 11:07 AM

Specimen	ID	Test	Result
Tissue	A)7405-1	PCR - VHSV	Negative
Tissue	C)7405-2	PCR - VHSV	Negative
Tissue	E)7405-3	PCR - VHSV	Negative

History of Communication

Date	To	Description
09/16/09 1:39 PM	Morrison, Diane - fax	bc report generated
09/21/09 1:27 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

END OF REPORT

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Animal Health Centre

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

Final Report AHC Case: 09-3599

Last Updated: 09/17/09 3:25 PM
Pathologist: Gary D. Marty
Received Date: 09/15/09
Collected Date: 09/09/09
Client Ref No:

Veterinarian: **Dr. Peter McKenzie**
Clinic: **Mainstream Canada-T**
Phone: (250) 725-1255
Fax: (250) 725-1250

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

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

Case History

Submitted 1 formalized atlantic salmon tissue for Histopathology.

Saltwater. Weight - 86g. Vaccinated - Yes. # in group - 50,000. # dead - 12,000. Duration of illness - 24hr. Euthanized - No.

Pale gills - some separation. On feed. Inflated swim bladder.

Final Diagnosis

1. Brain: meningeal and neuropil hemorrhage, multifocal, moderate (slide 6A)

2a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 5A)

2b. Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions, acute, focal, mild (slide 1A)

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

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

4. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slides 5A, 6A)

Final Comment: One fish (#6) probably died of brain hemorrhage, but none of the other fish have lesions of sufficient severity to explain their death. Tissue autolysis decreases the sensitivity of histopathology; for example, subtle changes like gill lamellar epithelial necrosis could not be diagnosed in these tissues. However, these tissues are preserved well enough to rule out pathogens like *Renibacterium salmoninarum* and *Aeromonas salmonicida* as causes of death. The pale appearance of the gills, described grossly, is consistent with postmortem autolysis (i.e., an artifact). Comments on specific lesions follow:

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.

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

Atlantic salmon "fresh silvers" that die in marine net pens, affecting 14% of the 468 Atlantic salmon livers examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2008 was sufficient to identify a trend towards greater prevalence in the winter and spring (21-30%) than in the summer and fall (1.9-4.4%).

Focal sinusoidal congestion in the liver is a nonspecific vascular lesion; diffuse congestion sometimes it occurs as a postmortem artifact. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). 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.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469).

Histopathology

Formalin-fixed tissues from 6 fish were submitted in 6 cassettes for histopathology. Gills were removed from the original (A) cassettes and placed in separate (B) cassettes, yielding 12 cassettes.

Slide #s 1A-6A and 1B - 6B are labeled in the same order as client #s Dixon 1 - Dixon 6.

Organs included on most A slides -heart, liver, spleen, head kidney, trunk kidney, intestinal ceca, and brain.

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

Quality control: Liver autolysis: severe (slides 1A, 2A, 3A, 4A, 5A), or not included for examination (slide 6A). Gill autolysis: severe (slides 1B, 2B, 3B, 4B, 5B, 6B). Organs have no postfixation dehydration and no acid hematin deposits.

Diagnoses:

1. Brain: meningeal and neuropil hemorrhage, multifocal, moderate (slide 6A)

2a. Liver: biliary preductular cell hyperplasia, diffuse, mild (slide 5A)

2b. Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions, acute, focal, mild (slide 1A)

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

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

4. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slides 5A, 6A)

History of Communication

Date	To	Description
09/17/09 3:26 PM	Mainstream Canada-T - fax	bc report generated
09/17/09 3:26 PM	Dr. Peter McKenzie - e-mail	bc report generated
09/21/09 1:53 PM	Mainstream Canada - e-mail	Case Invoiced

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

END OF REPORT

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Animal Health Centre

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Final Report AHC Case: 09-3694

Last Updated: 09/24/09 4:24 PM
Pathologist: Gary D. Marty
Received Date: 09/22/09
Collected Date:
Client Ref No: 7423

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

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

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

Case History

Submitted formalized tissues for Histo and fresh tissues for PCR for IHN, VHS and ISA.

Saltwater entry - 08 S0. Vaccinated- Yes. Insurance - No. Euthanized - No. Prior submission - No.

Sampled 5 fresh morts - routine viral check. Fish sampled for Histology had adhesions and granulomas in the liver but kidney looked okay.

Final Diagnosis

1a. Liver: hepatitis, perivascular, granulomatous and neutrophilic, with intralesional edema, bifocal, moderate

1b. Liver: hepatitis, lymphohistiocytic, multifocal, mild

1c. Liver: hepatocellular cytoplasmic yellow-brown pigment (lipofuscin?), multifocal, mild

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

2a. Heart: epicarditis, lymphohistiocytic, diffuse, moderate

2b. Heart: endocarditis, lymphohistiocytic, multifocal, mild

3. Spleen: parenchymal golden pigment (lipofuscin?), scattered, intracellular, mild

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

Final Comment: This fish has several lesions that might provide clues to its cause of death, but none are of sufficient severity to have caused mortality. Among the lesions, granulomatous and neutrophilic hepatitis is probably the most significant. Other lesions are fairly common in farmed Atlantic salmon. Comments on specific lesions follow:

Inflammation in the liver is evidence of immune stimulation (e.g., from a bacterial infection or reaction to a vaccine). Neutrophilic inflammation around vessels is evidence of an acute process, while lymphohystiocyctic inflammation is evidence of chronic stimulation. The section has no obvious organisms.

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

The 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; it is more common in older fish. Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 18% of the 467 Atlantic salmon hearts examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program.

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 28% of the 467 Atlantic salmon hearts examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469) and Pacific salmon (prevalence = 31%; n = 118).

Histopathology

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

Slide 1 (7423-1) - heart, spleen, liver, head kidney, and trunk kidney

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

Quality control: Liver autolysis: mild. Other organs have a good preservation. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 09/24/09 @ 11:11 AM

Specimen	ID	Test	Result
Tissue	7423-1-IHN/VHS	PCR - IHNV	Negative
Tissue	7423-2-IHN/VHS	PCR - IHNV	Negative
Tissue	7423-3-IHN/VHS	PCR - IHNV	Negative

PCR - ISA Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 09/24/09 @ 2:36 PM

Specimen	ID	Test	Result
Tissue	7423-1-ISA	PCR - ISA	Negative
Tissue	7423-2-ISA	PCR - ISA	Negative
Tissue	7423-3-ISA	PCR - ISA	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 09/24/09 @ 11:11 AM

Specimen	ID	Test	Result
Tissue	7423-1-IHN/VHS	PCR - VHSV	Negative
Tissue	7423-2-IHN/VHS	PCR - VHSV	Negative

Tissue	7423-3-IHN/VHS	PCR - VHSV	Negative

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

History of Communication

Date	To	Description
09/24/09 4:24 PM	Morrison, Diane - fax	bc report generated
09/28/09 3:43 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-3722

Last Updated: 09/30/09 11:48 AM
Pathologist: Gary D. Marty
Received Date: 09/24/09
Collected Date:
Client Ref No:

Veterinarian:
Clinic:
Phone:
Fax:

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

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

Case History

Submitted 4 Atlantic salmon bacterial isolates for identification via PCR sequencing.

All four isolates require saltwater media. If you have any further questions, please give me a call at (250) 652- 4482.

Isolates labelled as: 2735 PL3, CC265, CC266, CC268.

Bacteriology

DNA Sequencing - Bacteria Resulted by: Erin Zabek Verified by: Sean Byrne on 09/28/09 @ 9:17 AM

Specimen	ID	Isolate	Result	Level
Isolate	CC265	Vibrio splendidus	Positive	
Isolate	CC266	Vibrio splendidus	Positive	
Isolate	CC268	Aliivibrio sp.	Positive	
**: Aliiv brio sp. closely matched with A.wodanis. Unable to determine species using DNA J gene.				
Isolate	2735 PL3	Bacteria	Positive	
**: Bacteria identified as Bizionia algoritergicola				

History of Communication

Date	To	Description
09/30/09 5:22 PM	Microtek International In - e-mail	bc report generated
10/05/09 1:30 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: 09-3743

Last Updated: 10/20/09 4:31 PM
Pathologist: Gary D. Marty
Received Date: 09/25/09
Collected Date: 09/23/09
Client Ref No: PO 13752

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

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

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

Case History

Submitted fresh Atlantic salmon tissue for Histo and PCR.

Saltwater. Vaccinated - Yes. Age: 300g.

PCR - Whirl B, PCR & Viral culture Whirl A.

Addendum - Oct 01/09 Received formalized tissue for Histopathology.

Some necrosis in gills, pale liver. Petech hem. Muscle walls, Hem. swim bladder. Bloody ascites.

Final Diagnosis

1. Gill filaments: distal necrotizing branchitis, with superficial filamentous bacteria (*Tenacibaculum maritimum?*), focal, severe (slide 2)

2. Heart, head kidney, spleen: bacteria colonies (coccobacilli), disseminated, moderate numbers (heart and head kidney), abundant (spleen) (slide 1)

3a. Head kidney: interstitial cell atrophy, diffuse, severe (slide 1)

3b. Head kidney: small numbers of interstitial intracytoplasmic eosinophilic granules, diffuse (slide 1)

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

4b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 1)

5. Heart: myocardial karyomegaly, multifocal, mild (slide 1)

Final Comment: This fish probably died as a consequence of necrotizing branchitis with superficial thin rod- shaped to filamentous bacteria. The distal margin of the filaments has a sharply demarcated line of necrosis separating viable from necrotic tissue. These lesions and associated toxins might have caused ionoregulatory disturbance and might have been a source of secondary infections (e.g., the coccobacilli in the spleen, head kidney and heart). Filamentous bacteria are common on the gills of debilitated juvenile salmonids. Affected fish often have

a history of some type of stress (e.g., crowding, suboptimal water quality, or handling). Bacterial culture or PCR is required to identify the bacteria. The most common species of filamentous bacteria in marine waters is *Tenacibaculum maritimum* . This is the first time we have seen disseminated coccobacilli in farm raised salmonids; the lack of immune response is similar to what occurs with *Aeromonas salmonicida* infection, but bacterial morphology is not the same. Organs have no evidence of *Piscirickettsia salmonis*.

Interstitial cell atrophy in the kidney might be a result of decreased production or increased release of hematopoietic cells, or a combination of the two. This could be a result of the bacterial infections.

Accumulation of eosinophilic granules in the cytoplasm of cells lining small vessels in the kidney is a distinctive finding in Atlantic salmon. These granules probably accumulate in response to systemic immune stimulation. The granules might be part of eosinophilic granular cells or endothelial cells with eosinophilic granules. These granules are common with *Piscirickettsia* salmonis infection and I (GDM) have seen them with severe cerebral *Renibacterium salmoninarum* (in at least one case, the affected fish had no granulomatous inflammation in the kidney), but other cases have no known cause.

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 might be related to increased protein needed as part of an inflammatory response.

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.4% of the 1609 Atlantic salmon hearts examined as part of the province's Fish Health Auditing and Surveillance Program from 2006 through 2008). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Histopathology

Formalin-fixed tissues were submitted in 1 cassette for histopathology. The gills were removed, placed into a second cassette (# 2), immersed for 2 h in Protocol B (hydrochloric acid solution) for decalcification, and then rinsed in water before being processed with other cassettes into paraffin.

Slide 1 (Saranac 09/23/09) - brain, heart, spleen, liver, head kidney, intestinal ceca

Slide 2 - gill

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

Quality control: Liver autolysis: severe (slide 1). Gill autolysis: moderate (slide 2). Tissue preservation is good. Decalcification is 100% complete and differential staining is good. Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/28/09 @ 4:40 PM

Specimen	ID	Test	Result
Tissue	organ A	PCR - IHNV	Negative
Tissue	organ B	PCR - IHNV	Negative

PCR-Piscirickettsia salmo Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/01/09 @ 3:29 PM

Specimen	ID	Test	Result
Tissue	organ A	PCR - Piscirickettsia salmonis	Negative
Tissue	organ B	PCR - Piscirickettsia salmonis	Positive

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 09/28/09 @ 4:40 PM

Specimen	ID	Test	Result
Tissue	organ A	PCR - VHSV	Negative
Tissue	organ B	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 10/20/09 @ 4:31 PM

Specimen	ID	Isolate	Result	Level
Tissue	A		No viruses isolated	

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

History of Communication

Date	To	Description
09/29/09 11:30 AM	Mainstream Canada-T - fax	bc report generated
09/29/09 11:30 AM	Dr. Peter McKenzie - e-mail	bc report generated
10/21/09 10:44 AM	Mainstream Canada-T - fax	bc report generated
10/21/09 10:44 AM	Dr. Peter McKenzie - e-mail	bc report generated
10/23/09 1:28 PM	Mainstream Canada - 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: 09-3786

Last Updated: 10/01/09 9:27 AM
Pathologist: Gary D. Marty
Received Date: 09/29/09
Collected Date: 09/23/09
Client Ref No: 7433

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

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

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

Case History

Submitted fresh Atlantic salmon tissues for PCR

Saltwater entry - 09 S1. Vaccinated - Yes. Insurance - No . Prior submission - No.

Hem on swim bladder and blood in p.c tissue.

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 10/01/09 @ 9:27 AM

Specimen	ID	Test	Result
Tissue	Org 7433-1	PCR - IHNV	Negative
Tissue	Org 7433-2	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 10/01/09 @ 9:27 AM

Specimen	ID	Test	Result
Tissue	Org 7433-1	PCR - VHSV	Positive
Tissue	Org 7433-2	PCR - VHSV	Negative

History of Communication

Date	To	Description
10/03/09 8:08 AM	Morrison, Diane - fax	bc report generated
10/08/09 10:20 AM	Marine Harvest Canada - e-mail	Case Invoiced

Gary D. Marty

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

END OF REPORT

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Final Report AHC Case: 09-3819

Last Updated: 10/03/09 1:26 PM
Pathologist: Gary D. Marty
Received Date: 10/01/09
Collected Date: 09/25/09
Client Ref No:

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Kelly Abel - Mainstream Canada**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted formalized fish tissue for Histopathology.

Saltwater. Vaccinated - Yes. Prior submission - No. # Submitted dead - 4.

4 samples taken for Histology. Internal signs Pen 3 fish I had petechial hemorrhages on the visceral organs. External signs all samples had lesions penetrating to the muscle.

Final Diagnosis

1a. Skin: dermatitis, ulcerative, with moderate numbers of filamentous bacteria extending into underlying degenerating skeletal muscle, regionally diffuse, severe (slides 3- 2, 6-1A)

1b. Skin: dermatitis, ulcerative, with scattered superficial filamentous bacteria, focal, moderate (slide 5-1A), diffuse, severe (slides 3-1A)

2. Skeletal muscle: myonecrosis and myositis, lymphohistiocytic, with scattered neutrophils and intralesional filamentous bacteria, multifocal, severe (slide 3-1A)

3a. Liver: hepatocellular hydropic degeneration, disseminated mild (slides 3-2, 5-1A, 6-1A), moderate (slide 3-1A),

3b. Liver: hepatocellular single cell necrosis (apoptosis?), disseminated, mild (slides 3-2, 5-1A, 6-1A), moderate (slide 3-1A)

3c. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slides 3-1A, 3-2, 5-1A, 6-1A)

3d. Liver: sinusoidal congestion, with intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, mild (slide 3-1A)

3e. Liver: perivascular interstitial pigment (lipofuscin and/or hemosiderin ?), multifocal, mild (slide 3-1A)

4. Trunk kidney: renal tubular intraluminal necrotic cells, multifocal, mild (slide 3-1A, 5-1A, 6-1A)

5. Head kidney: small numbers of interstitial intracytoplasmic eosinophilic granules , diffuse (slides 3-2, 5-1A, 6-1A)

6a. Heart: epicarditis, lymphohistiocytic, diffuse, mild (slide 3-1A)

6b. Heart: endothelial cell hypertrophy, diffuse, mild (slides 3-1A, 3-2, 5-1A, 6-1A)

7. Mesenteric adipose tissue: hemorrhage, multifocal, mild (slide 3-1A)

8a. Gill: lamellar epithelial hypertrophy, multifocal, with interlamellar filamentous bacteria and occasional lamellar fusion, moderate (slide 6-1 B)

8b. Gill: interlamellar filamentous bacteria, multifocal, mild (slide 5-1B)

9. Mesenteries: peritonitis, lymphohistiocytic, diffuse, moderate (slide 3-1A, 3-2)

10. Spleen: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate (slide 6-1A)

Final Comment: These fish died from complications related to severe penetrating ulcers in the skin. Fish 6-1A has the best microscopic example of the lesion, with peripheral skin and scales intact, but the central ulcer penetrates through a 7-mm -wide defect in the dermis and penetrates nearly 2 mm into the underlying skeletal muscle. The entire ulcer, including the margin of the ruptured dermis, contains moderate to large numbers of filamentous bacteria. Filamentous bacteria commonly invade skin ulcers. Enlargement of ulcers is enhanced when fish are under some type of stress (e.g., crowding, suboptimal water quality, other infection). Identification of the bacteria requires culture or PCR. In the marine environment, *Tenacibaculum maritimum* is most likely. In other sections, affected skeletal muscle is not directly associated with ulcerated dermis, but bacteria and inflammation in these sections is probably secondary to the ulcers (i.e., the connection was not included in the plane of section). Most of the other changes can be related to these ulcers, but details below illustrate the systemic nature of the disease. Consider sampling internal organs (e.g., kidney) for bacteria and viruses (e.g., VHSV).

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

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

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

Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion; diffuse congestion sometimes occurs as a postmortem artifact. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology and virology and PCR for VHSV, IHNV, and ISAV (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus).

Pigment in the liver is probably lipofuscin, and it might also include hemosiderin. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish. Hemosiderin accumulation in the liver might result from increased turnover of red blood cells.

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

Accumulation of eosinophilic granules in the cytoplasm of cells lining small vessels in the kidney is a distinctive finding in Atlantic salmon. These granules probably accumulate in response to systemic immune stimulation (the ulcers in this csse?). The granules might be part of eosinophilic granular cells or endothelial cells with eosinophilic granules. These granules are common with *Piscirickettsia* salmonis infection and I have seen them with severe cerebral *Renibacterium salmoninarum* (in at least one case, the affected fish had no granulomatous inflammation in the kidney), but other cases have no known cause. Renal eosinophilic granules have also been described in Atlantic salmon naturally infected with chronic pancreas disease in Norway (Salmonid alphavirus subtype 3, SAV3; McLoughlin and Graham 2007), but SAV3

has not been identified in BC salmon.

Lymphohistiocytic epicarditis and endothelial cell hypertrophy is a fairly common combination of lesions related to bacterial and viral infections. Among farmed salmon in British Colombia, related causes include Erythrocytic Inclusion Body Syndrome (EIBS), *Renibacterium salmoninarum* , *Yersinia ruckeri* and viral hemorrhagic septicemia virus. In this case, it might be related to the ulcers.

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.

Lamellar epithelial hypertrophy in the gill is evidence of exposure to toxins. In this case it is probably related to the presence of filamentous bacteria in the interlamellar space (*Tenacibaculum maritimum* or a related organism). As a differential, pen-reared salmon are commonly exposed to algal toxins, but the reaction of the gill to these toxins has not been well characterized.

Peritonitis of the spleen, intestinal ceca, and adjacent mesenteries is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 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). Vacuoles are probably a result of vaccine material lost during tissue processing (Mutoloki et al. 2004).

Literature Cited:

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

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 from 4 fish were submitted in 4 cassettes for histopathology. Cassettes were immersed 2h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed into paraffin. With the exception of cassette 3-2, gills were removed from the original (A) cassettes and placed in separate (B) cassettes.

Slide #s - 3-1A&B (Simmonds Pen 3 Fish 1), 3-2 (Simmonds Pen 3 Fish 2), 5-1A&B (Simmonds Pen 5 Fish 1), 6- 1A&B (Simmonds Pen 6 Fish 1).

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

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

Quality control: Liver autolysis: mild (slides 3-1A, 5-1A, 6-1A), moderate (slide 3-2). Gill autolysis: mild (distal filaments, slides 3-1B, 3- 2, 5-1B, 6-1B), moderate (proximal filaments, slides 3-1B, 3-2, 5- 1B, 6-1B). Decalcification in 80% complete and differential staining is good. Large foci of erythrocytes (e.g., spleen in slide 3-1A) 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.

Staff Comments:
Fish Histopathology Fellow: Meritxell Diez Padrisa, D.V.M.

History of Communication

Date	To	Description
10/03/09 1:26 PM	Mainstream Canada - e-mail	bc report generated
10/03/09 1:26 PM	Dr. Peter McKenzie - e-mail	bc report generated
10/08/09 10:54 AM	Mainstream Canada - e-mail	Case Invoiced

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

END OF REPORT

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Animal Health Centre

AAVLD - Accredited Laboratory

Ministry of Agriculture and Lands
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Abbotsford BC V3G 2M3
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Facsimile: (604) 556-3010
Toll-Free : 1-800-661-9903

Final Report AHC Case: 09-3847

Last Updated: 10/08/09 11:11 AM
Pathologist: Gary D. Marty
Received Date: 10/05/09
Collected Date:
Client Ref No: 09-2764

Veterinarian:
Clinic:
Phone:
Fax:

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

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

Case History

Submitted formalized Atlantic salmon fish tissues for Histopathology.

Please find enclosed 6 cassettes containing tissue samples from atlantic salmon for histology testing. The samples were preserved in formalin then transferred to tap water for transport. The samples have been marked.

Sample Id's: B1, B2, B5, B6, B8a, B8b.

Final Diagnosis

1a. Liver: sinusoidal fibrin, multifocal, mild (slides B1-1, B2-2, B5-2, BG-2), moderate (slide B3-2)

1b. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide B3-2), moderate (slide B2-2, B5-2, B6-1, BG-1)

1c. Liver: interstitial pigment, multifocal, mild (slides B1-2, B2-2, B3-2, B5-2, B6-1, BG-1)

1d. Liver: sinusoidal congestion and hemorrhage, multifocal, mild (slide B3-2, BG-1), moderate (slides B1-2)

1e. Liver: hepatocellular eosinophilic cytoplasmic inclusions, diffuse, mild (slides BG-1), moderate (slide B1-2)

1f. Liver: hepatocellular single cell necrosis (apoptosis?), disseminated, mild (slides B2-2, B6-1)

1g. Liver: hepatocellular hydropic degeneration, disseminated, mild (slide B6-1)

1h. Liver: hepatocellular cytosegresomes, diffuse, mild (slide B2-2, 6B-1, BG-1)

1i. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slides B2-2)

1j. Liver: hepatic coagulative necrosis, bifocal, mild (slide B1-2)

1k. Liver: hepatitis, lymphocytic, focal, mild (slide B3-2)

1l. Liver: hepatitis, lymphohistiocytic, multifocal, mild (slide BG-1)

1m. Liver: fibrinous macrophages aggregates, a few with intralesional bacteria, multifocal, mild (slide B6-2)

2a. Trunk kidney: disseminated intravascular thrombi with scattered bacteria, diffuse, moderate (slide B6-1)

2b. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slide B2-1)

3a. Gill: lamellar hyperplasia and hypertrophy, with fusion of adjacent lamellae and capillary septic thrombosis, diffuse, moderate (slide B6-2)

3b. Gill: lamellar necrosis, diffuse, moderate (slide BG-2)

4. Intestine, lamina propria: vascular congestion, multifocal, mild (slide B1-1)

5. Mesenteric adipose tissue: capillary congestion, diffuse, moderate (slide B1-1)

6. Brain: vascular mineralization, with lymphohistiocytic perivasculitis, bifocal, moderate (slide BG-3)

7. Spleen: peritonitis, lymphohistiocytic, with fibrous fronds, diffuse, mild (slides B1-1, B6-2)

Final Comment: Bacteria, thrombi, and fibrinous macrophage aggregates are evidence that fish B6 died of septicemia. The other fish have several lesions that might provide clues to their cause of death, but none are of sufficient severity to have caused mortality. All fish have hepatic interstitial fibrin, hepatocellular basophilic cytoplasm, and hepatic interstitial pigment; these lesions provide evidence of inflammation or increased cell turnover. Other lesions did not occur in all the fish, but most lesions are consistent with exposure to toxins (e.g., from an algal bloom, or inflammatory mediators associated with bacterial or viral infection).

Foci of distal necrosis (or autolysis?) in gill lamellae (fish BG) is an unusual finding. The sharp line of demarcation separating tissue with abundant karyorrhexis from adjacent tissue with intact nuclei is evidence for necrosis. However, the karyorrhexis is not associated with an obvious cause (e.g.. thrombi). Therefore, these changes might be a result of autolysis.

Comments on each lesion 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. Consider bacteriology and virology, if not already done.

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

Yellow-brown interstitial pigment is a common finding in liver of a mature fish. Pigment might be hemosiderin or lipofuscin. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish. Hemosiderin accumulation in the liver might result from increased turnover of red blood cells.

Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion; diffuse congestion sometimes occurs as a postmortem artifact. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but ISAV has never been identified in British Columbia.

Homogeneous cytoplasmic inclusions in hepatocytes are a distinct change. The inclusions might be phagocytosed cellular debris or plasma protein, or accumulation of protein synthesized in hepatocytes. Features of the inclusions are not consistent with viral inclusions. Transmission electron microscopy might help determine the nature of this change, but TEM is not available at the Animal Health Centre. I have seen this change in fish that have been exposed to toxins, but this change is not described in common fish pathology books.

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

Hydropic degeneration of hepatocytes provides evidence that the liver was being exposed to toxins. After hydropic degeneration can no longer be reversed, the changes are called single cell necrosis.

Cytosegresomes are roughly spherical eosinophilic cytoplasmic foci that occur in liver cells that have been sublethally injured by a variety of insults, ranging from hypoxia through a variety of intoxications to malnutrition, specific deficiencies, and some viral infections. They may be formed when masses of cytoplasmic organelles are gathered and condensed, and are sequestered from remaining cytoplasm by membranes that fuse with lysosomes (autolysosomes).

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.

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

Focal lymphocytic inflammation in the liver is common in wild fish species. It probably is a reaction to a localized infection; when mild, it is of little significance to the health of the fish.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29%; n = 469) and Pacific salmon (prevalence = 31%; n = 118).

Hyperplasia of the epithelium lining gill lamellae is a nonspecific response to irritation. Inciting causes include parasites, bacteria, viruses, and toxins. In this case, it is associated with intralesional bacterial rods. Bacterial culture or PCR is required for a definitive diagnosis; *Yersinia ruckeri* is a primary differential.

Distension of capillaries in the intestinal lamina propria is a distinctive change (called "red gut") that is sometimes attributed to a nutritional (or rather an anti- nutritional) colitis exacerbated by opportunistic colonizing bacteria: mostly vibrios of various types. This type of reaction is recognized in salmon, yellowtail, and *Seriola* (source: Dr. Mark Sheppard, BC-MAL, personal communication).

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.

Vascular mineralization in the brain is a rare finding in fish. Presumably, it results from some type of calcium/phosphorus imbalance.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Histopathology

Formalin-fixed tissues were submitted from 6 Atlantic salmon in 6 cassettes for histopathology. Tissues were split into separate cassettes prior to processing into paraffin, yielding a total of 13 cassettes.

Slide B1-1 (B1) - spleen, intestinal ceca, mesenteric adipose tissue

Slide B1-2 (B1) - gill, liver, trunk kidney

Slide B2-1 (B2) - trunk kidney, intestinal ceca

Slide B2-2 (B2) and B3-2 (B8b) - gill, spleen, liver

Slide B3-1 (B8b) and B5-1 (B5) - trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide B5-2 (B5) - gill, spleen, liver, mesenteric adipose tissue

Slide B6-1 (B6) - liver, trunk kidney, intestinal ceca

Slide B6-2 (B6) - gill, spleen, mesenteric adipose tissue

Slide BG-1 (B8a) - spleen, liver, skeletal muscle

Slide BG-2 (B8a) - gill

Slide BG-3 (B8a) - brain, trunk kidney

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

Quality control: Liver autolysis: mild (slide B2-2, B6-1), severe (slides B1-1, B3-1, B5-2, BG-1). Tissues were submitted packed in biopsy cassettes; this is not acceptable for tissue processing. Biopsy cassettes have small holes designed to keep small tissues (e.g., alevin gills) from exiting the cassette during processing. For the larger tissues submitted in this case, standard histology cassettes are needed. Also, to ensure complete processing into paraffin, tissues need to be placed in cassettes with enough room so that they do not touch each other. Large foci of erythrocytes (e.g., spleen in slide B1-1) have a few precipitates of acid hematin. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissueOrgans have no postfixation dehydration.

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

History of Communication

Date	To	Description
10/08/09 11:12 AM	Hewison, Tim - e-mail	bc report generated
10/13/09 12:46 PM	Microtek International In - e-mail	Case Invoiced

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

END OF REPORT

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Abbotsford BC V3G 2M3
Telephone : (604) 556-3003
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Toll-Free : 1-800-661-9903

Final Report AHC Case: 09-3892

Last Updated: 10/09/09 4:32 PM
Pathologist: Gary D. Marty
Received Date: 10/06/09
Collected Date: 10/01/09
Client Ref No: 7440

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

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

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

Case History

Submitted formalized tissues for histology and fresh tissues for PCR - IHN and VHS.

Atlantic salmon. Saltwater entry: 07 SO. Vaccinated. # in group - 2. Euthanized - no. Fish died Oct. 1/09.

Increased mortality on site. Suspected low dissolved oxygen event.

Final Diagnosis

1a. Heart: mural thrombosis, multifocal, mild (slides 4, 5)

1b. Heart: myocardial karyomegaly, multifocal, mild (slide 5)

2a. Gill (filament tips): cellular thrombi, multifocal, mild (slides 4, 5)

2b. Gill: lamellar hyperplasia and fusion, focal, mild (slide 5A)

3a. Liver: interstitial brown-yellow pigment, multifocal, mild (slides 4, 5)

3b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slides 4, 5)

4. Mesenteric adipose tissue: peritonitis, lymphohistiocytic, with fibrous fronds, multifocal, moderate (slides 4, 5)

5. Spleen: peritonitis, lymphohistiocytic, with fibrous fronds, regionally diffuse, mild (slide 5)

Final Comment: These fish have several lesions that might provide clues to their cause of death, but none are of sufficient severity to have caused mortality. Thrombi in the heart and gill are the most severe lesions in these fish. Thrombosis is a result of increased coagulability. This can result from endothelial damage related to toxin exposure, as well as inflammatory compounds released in viral, bacterial, or parasitic infections. The sections contain no obvious organisms. Comments on the other lesions follow:

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.4% of the 1609 Atlantic salmon hearts examined as part of the province's Fish Health Auditing and Surveillance Program from 2006 through 2008). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Gill lamellar hyperplasia with fusion may be a result of physical damage from exposure to an irritant (e.g., fine feed particles or toxins in the water, including ammonia); differentials include parasites or diatoms, but this lesion contains no organisms (they might have been lost during processing).

Yellow-brown interstitial pigment is a common finding in liver of fish that have been in the ocean more than a year. Pigment might be hemosiderin or lipofuscin. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants; it is more common in older fish. Hemosiderin accumulation in the liver might result from increased turnover of red blood cells.

Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). 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.

Peritonitis is consistent with a reaction to foreign material. Splenic peritonitis affected 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Histopathology

Formalin-fixed tissues from 2 fish were submitted in 4 cassettes for histopathology.

Slide 4 (7440-4) - brain, heart, spleen, liver, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue

Slide 5 (7440-5) - brain, heart, spleen, head kidney, trunk kidney (2 pieces), intestinal ceca

Slides 4A (7440-4A) and 5A (7440-5A) - gill

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: A Scouras Verified by: Dr. J. Robinson on 10/08/09 @ 10:25 AM

Specimen	ID	Test	Result
Tissue	org #3	PCR - IHNV	Negative
Tissue	org #4	PCR - IHNV	Negative

PCR - VHSV Resulted by: A Scouras Verified by: Dr. J. Robinson on 10/08/09 @ 10:25 AM

Specimen	ID	Test	Result
Tissue	org #3	PCR - VHSV	Negative
Tissue	org #4	PCR - VHSV	Negative

Staff Comments:

Fish Histopathology Fellow: Meritxell Diez Padrisa, DVM

History of Communication

Date	To	Description
10/09/09 4:34 PM	Morrison, Diane - fax	bc report generated
10/14/09 11:12 AM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-3893

Last Updated: 10/08/09 1:53 PM
Pathologist: Gary D. Marty
Received Date: 10/06/09
Collected Date:
Client Ref No: 7437

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

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

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

Case History

Submitted formalized Atlantic salmon tissues for histopathology.

Atlantic salmon. Freshwater. Saltwater entry: 09. Vaccinated. # in group - 5. Euthanized - yes.

Pre-smolt shipping check up.

Final Diagnosis

1a. Liver: hepatitis, granulomatous, multifocal, with multinucleate giant cells, mild (slide 3), and a few intralesional vacuoles, mild (slide 4)

1b. Liver: pericholangitis, lymphohistiocytic, multifocal, mild (slide 5)

2a. Spleen: splenitis, granulomatous, multifocal, with multinucleate giant cells and a few intralesional vacuoles, moderate (slide 2)

2b. Spleen: peritonitis, chronic, focal, with fibrocellular fronds, moderate (slides 1, 4)

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

4. Intestine, intestinal ceca, and mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild (slides 1, 5)

5. Gill: lamellar telangiectasis, multifocal, mild (slides 1A, 5A)

6. Brain: meningeal and neuropil hemorrhage, multifocal, mild (slide 5), moderate (slide 3)

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

Final Comment: These fish have several mild to moderate lesions. The inflammatory lesions are all consistent with vaccine reactions , and the hemorrhagic lesions are consistent with trauma (bonk on the head?) when the fish were sampled. Fish have no microscopic evidence of infectious organisms. Comments on specific lesions follow:

Foci of granulomatous inflammation in the liver and spleen are evidence of persistent immune stimulation . The most common cause in

cultured Atlantic salmon is a vaccine reaction. The primary differential is *Renibacterium salmoninarum* infection.

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.

Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe). Vacuoles are probably a result of vaccine material lost during tissue processing.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29% ; n = 469) and Pacific salmon (prevalence = 31% ; n = 118).

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

Telangiectasis in the gill (lamellar capillary aneurysms or ruptured lamellar capillaries) and hemorrhage in the brain most commonly results from trauma (e.g., handling, swimming into something in the environment, or euthanasia with a "bonk on the head").

Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 18% of the 467 Atlantic salmon hearts examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program.

Histopathology

Formalin-fixed tissues from 5 fish were submitted in 10 cassettes for histopathology. The gills (A cassettes) were immersed 2.5 h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

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

Organs included on most slides - gill, heart, liver, spleen, head kidney, trunk kidney, intestine/intestinal ceca, mesenteric adipose tissue, brain

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

Quality control: Liver autolysis: none (slides 1, 2, 3, 4, 5). Decalcification is 100% complete and differential staining is excellent. Organs have no postfixation dehydration and no acid hematin deposits.

Measures of physiologic condition:

Hepatocellular glycogen: none (slide 4), moderate amounts (slides 2, 3), abundant (slides 1, 5)

Mesenteric adipose tissue: abundant (all 5 fish)

These measures of physiologic condition are consistent with mostly healthy fish. Fish #4 might not have been eating normally in the 2 days before it was sampled.

History of Communication

Date	To	Description
10/08/09 1:53 PM	Morrison, Diane - fax	bc report generated
10/13/09 12:54 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Animal Health Centre

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Ministry of Agriculture and Lands
1767 Angus Campbell Road
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Toll-Free : 1-800-661-9903

Final Report AHC Case: 09-3990

Last Updated: 10/16/09 9:21 AM
Pathologist: Gary D. Marty
Received Date: 10/14/09
Collected Date:
Client Ref No: 7446

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

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

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

Case History

Submitted 9 fresh Atlantic fish tissues for PCR for IHN and VHS.

Saltwater entry 08 S0. Vaccinated - Yes. Insurance - No. # in group - 9. # dead - 9. Euthanized - No. Prior submission - No. # submitted dead - 9.

All fish had pet. hem in p.c and belly wall.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: A Scouras on 10/16/09 @ 9:21 AM

Specimen	ID	Test	Result
Tissue	ID#7446-2	PCR - IHNV	Negative
Tissue	ID#7446-4	PCR - IHNV	Negative
Tissue	ID#7446-7	PCR - IHNV	Negative
Tissue	ID#7446-8	PCR - IHNV	Negative
Tissue	ID#7446-10	PCR - IHNV	Negative
Tissue	ID#7446-17	PCR - IHNV	Negative
Tissue	ID#7446-18	PCR - IHNV	Negative
Tissue	ID#7446-19	PCR - IHNV	Negative
Tissue	ID#7446-20	PCR - IHNV	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: A Scouras on 10/16/09 @ 9:21 AM

Specimen	ID	Test	Result
Tissue	ID#7446-2	PCR - VHSV	Negative
Tissue	ID#7446-4	PCR - VHSV	Negative
Tissue	ID#7446-7	PCR - VHSV	Negative

Tissue	ID#7446-8	PCR - VHSV	Negative
Tissue	ID#7446-10	PCR - VHSV	Negative
Tissue	ID#7446-17	PCR - VHSV	Negative
Tissue	ID#7446-18	PCR - VHSV	Negative
Tissue	ID#7446-19	PCR - VHSV	Negative
Tissue	ID#7446-20	PCR - VHSV	Negative

History of Communication

Date	To	Description
10/16/09 2:01 PM	Morrison, Diane - fax	bc report generated
10/20/09 11:02 AM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-3991

Last Updated: 10/16/09 3:42 PM
Pathologist: Gary D. Marty
Received Date: 10/14/09
Collected Date:
Client Ref No: 7447

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

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

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

Case History

Submitted formalized Atlantic fish tissue for Histopathology.

Saltwater entry 08 S0. Vaccinated - Yes. # in group - 1. Euthanized - No. Prior submission - No. # submitted dead - 1.

Fish had swollen rounded liver and a mass in Atrium.

Final Diagnosis

1a. Heart, atrium: atrial dysplasia, with luminal and epicardial -vessel dilation, diffuse moderate (slide 2)
1b. Heart, atrium: endocarditis, diffuse, with endothelial cell hypertrophy, moderate numbers of eosinophilic granular cells, and multiple fibrinous, cellular, and organizing thrombi, diffuse, severe (slide 2)
1c. Heart, ventricle: endocarditis, diffuse, with endothelial cell hypertrophy, moderate (slide 2)
2a. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, moderate (slide 1)
2b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slide 1)
2c. Liver: hepatocellular cytoplasmic vacuoles, diffuse, moderate (slide 1)
3. Spleen and intestine: peritonitis, granulomatous, regionally diffuse, with intralesional vacuoles about 50 µm in diameter, moderate (slide 1)
4a. Trunk kidney: renal tubular casts of protein, yellow-brown pigment, and mineral, with tubular dilation, multifocal, mild (slide 1)
4b. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, moderate (slide 1)

Final Comment: This fish probably died of complications related to atrial dysplasia. Multiple thrombi in various stages of organization--up to 5 mm in diameter--provide evidence that this process has been ongoing for a long time (weeks to months?). This might be a congenital defect that the fish could accommodate when it was small, but it eventually grew too large for its dysfunctional heart. Sections contain no obvious organisms.

Endothelial cell hypertrophy in the heart is evidence of systemic disease. Differentials include a bacterial or viral infection (e.g., VHSV), or exposure to toxins (e.g., algal toxins); the cause is often not determined.

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

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

Hepatocellular 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.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting the spleens 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe). Moderate peritonitis is a bit unusual in a 2008 fish (i.e., it usually regresses in older fish). Vacuoles are probably a result of vaccine material lost during tissue processing.

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

Renal mineralization is common in cultured fish species, but it usually regresses soon after fish enter salt water (i.e., it is unusual in a 2008 fish). 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 Atlantic salmon (prevalence = 29%; n = 469) and Pacific salmon (prevalence = 31%; n = 118).

Histopathology

Formalin-fixed tissues from an Atlantic salmon were submitted in 2 cassettes and a bag (heart) for histopathology. The gill was decalcified in Protocol B (hydrochloric acid solution) for 2 hours before being rinsed in water and processed with other tissues into paraffin.
Slide 1 (7447) - brain, spleen, liver, head kidney, trunk kidney, skin/skeletal muscle, intestine, intestinal ceca, and mesenteric adipose tissue
Slides 2 and 3 - heart (3 pieces on each slide)
Slide G - gill
All organs were examined. Organs not listed elsewhere have no significant lesions.

Quality control: Liver autolysis: mild. Most other organs have a good preservation, but the intestine and gill have moderate autolysis. Organs have no postfixation dehydration and no acid hematin deposits.

History of Communication

Date	To	Description
10/16/09 3:43 PM	Morrison, Diane - fax	bc report generated
10/22/09 9:01 AM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-4114

Last Updated: 10/26/09 2:51 PM
Pathologist: Gary D. Marty
Received Date: 10/20/09
Collected Date: 10/01/09
Client Ref No: 7443

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

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

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

Case History

Submitted formalized Atlantic salmon tissues for Histopathology.

Saltwater entry: 07 S0. Vaccinated - Yes. # Submitted dead - 3.

Gills with mucous build up.

Final Diagnosis

1a. Gill, filaments: branchitis, granulomatous, with granulation tissue, multinucleated giant cells, and scattered hemorrhage and golden pigment, multifocal, severe (slide 2)

1b. Gill, filaments: branchitis, lymphohistiocytic, with granulation tissue, multinucleated giant cells, and hemorrhage, multifocal, moderate (slide 1)

2. Gill, lamellae: epithelial hyperplasia and hypertrophy, with fusion of adjacent lamellae, and mucous cell hyperplasia, multifocal, mild (slide 1); regionally diffuse, severe (slides 2, 3)

Final Comment: Severe gill lesions might have contributed to the death two of these fish. The multinucleated giant cells in the branchitis are evidence of persistent antigens; the reaction is similar to what occurs more commonly in viscera responding to vaccine material. However, gill is an unusual location for these morphologic changes. Exposure to algal toxins is unlikely due to the multifocal distribution of the lesions. There are no organisms in the lesions.

Gill lamellar hyperplasia with fusion may be a result of physical damage from exposure to an irritant (e.g., fine feed particles or toxins in the water, or mediators associated with inflammation in the filaments); differentials include parasites or diatoms, but this lesion contains no organisms. Hyperplasia of the mucous epithelium lining gill lamellae is a nonspecific response to irritation.

Histopathology

Formalin-fixed tissues were submitted in 3 cassettes for histopathology. All cassettes were immersed 2 h in Protocol B (hydrochloric acid

solution) for decalcification and then rinsed in water before being processed into paraffin blocks.

Slides 1 (7443-1), 2 (7443-2) and 3 (7443-3) - gill

A Twort's Gram stain was done for slide 2.

Quality control: Gill autolysis: mild (slides 1, 2, 3). Gills have no postfixation dehydration and no acid hematin deposits.

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

History of Communication

Date	To	Description
10/26/09 2:51 PM	Morrison, Diane - fax	bc report generated
10/27/09 4:18 PM	Marine Harvest Canada - e-mail	Case Invoiced

Gary D. Marty
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Final Report AHC Case: 09-4115

Last Updated: 10/26/09 1:54 PM
Pathologist: Gary D. Marty
Received Date: 10/20/09
Collected Date:
Client Ref No: 7409

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

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

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

Case History

Submitted 4 fresh and formalized Atlantic salmon tissues for Histopathology and PCR for IHN, VHS and ISA.(Insurance claim)

Saltwater entry: 08 S1. Vaccinated: Yes. Insurance - Yes. Euthanized - No. Prior submission - Yes case # 7399.

Fish experienced heterosigma bloom, with significant mortality. Four fresh morts sent in for sampling. This will be an insurance claim.

Final Diagnosis

1. Multiple organs (spleen, kidney, intestine, mesenteric adipose tissue): peritonitis, fibrocellular, diffuse, moderate (slides 1, 2, 3, 4)

2a. Liver: interstitial brown-yellow pigment (lipofuscin or hemosiderin?), diffuse, small amounts (slides 1, 2, 3, 4)

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

2c. Liver: basophilic hepatocellular cytoplasm, diffuse, mild (slide 3)

2d. Liver: sinusoidal congestion, diffuse, mild (slide 1)

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

3. Spleen: parenchymal golden pigment (lipofuscin?), scattered, mild (slides 2, 3, 4)

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

5. Intestine, lamina propria: vascular congestion, diffuse, moderate (slide 1)

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

7a. Brain: encephalitis, leukocytic, multifocal, mild (slide 4)

7b. Brain: capillary congestion, diffuse, mild (slide 3)

Final Comment: These fish have several lesions, but none of sufficient severity to have caused mortality. The lack of significant lesions in the submitted organs is consistent with mortality due to *Heterosigma sp.* (i.e., *Heterosigma sp.* usually causes mortality without morphologic lesions in internal organs, Kent & Poppe, 1998). Comments on specific lesions follow:

All fish have peritonitis of multiple organs, which is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Peritonitis of intestine and adjacent mesenteries due to foreign material affected 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).

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

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins or inflammatory mediators) or come from outside the fish (e.g., from the water or the feed). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 14% of the 468 Atlantic salmon livers examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2008 was sufficient to identify a trend towards greater prevalence in the winter and spring (21-30%) than in the summer and fall (1.9-4.4%).

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.

Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion; diffuse congestion sometimes occurs as a postmortem artifact. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined.

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.

Renal congestion is commonly associated with circulating toxins (e.g., from inflammatory cells or bacterial or viral infections). Renal congestion and hemorrhage in BC has been associated with VHSV and bacteria; in euthanized fish it can be a sampling artifact. Renal congestion is one of the classic signs of infectious salmon anemia (ISA), but PCR results rule out ISAV in this case (also, ISAV has never been identified in fish in BC).

Distension of capillaries in the intestinal lamina propria is a distinctive change (called "red gut") that is sometimes attributed to a nutritional (or rather an anti- nutritional) colitis exacerbated by opportunistic colonizing bacteria: mostly vibrios of various types. This type of reaction is recognized in salmon, yellowtail, and *Seriola* (source: Dr. Mark Sheppard, BC-MAL, personal communication).

Mild myocardial karyomegaly is somewhat common in cultured salmonids (e.g., 8.4% of the 1609 Atlantic salmon hearts examined as part of the province's Fish Health Auditing and Surveillance Program from 2006 through 2008). The cause and significance is unknown, but there might be a genetic predisposition to developing the lesion. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).

Encephalitis is evidence of immune stimulation. Leucocytic inflammation is most consistent with a virus, but consider bacteria as a possible differential. Encephalitis of unknown cause occurs occasionally in fish sampled as part of the BC Fish Health Auditing and Surveillance Program, affecting 7% of the 644 Atlantic salmon examined in 2007 (5.9% were mild, and 1.1% were moderate or severe).

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 often occurs as part of postmortem change. 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.

Histopathology

Formalin-fixed tissues from 4 fish were submitted in 4 cassettes for histopathology.

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

Organs included on most slides - heart, liver, spleen, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue, brain.

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

Quality control: Liver autolysis: mild (slides 1, 2), severe (slides 3, 4). Intestinal autolysis: severe (slides 1, 2, 3, 4). Erythrocytes in large vessels in the spleen (slides 1, 2, 3) and liver (slide 3) have abundant acid hematin deposits. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Sean Byrne on 10/22/09 @ 9:31 AM

Specimen	ID	Test	Result
Tissue	7409-1	PCR - IHNV	Negative
Tissue	7409-2	PCR - IHNV	Negative
Tissue	7409-3	PCR - IHNV	Negative
Tissue	7409-4	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Sean Byrne on 10/22/09 @ 9:31 AM

Specimen	ID	Test	Result
Tissue	7409-1	PCR - ISA	Negative
Tissue	7409-2	PCR - ISA	Negative
Tissue	7409-3	PCR - ISA	Negative
Tissue	7409-4	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Sean Byrne on 10/22/09 @ 9:32 AM

Specimen	ID	Test	Result
Tissue	7409-1	PCR - VHSV	Negative
Tissue	7409-2	PCR - VHSV	Negative
Tissue	7409-3	PCR - VHSV	Negative
Tissue	7409-4	PCR - VHSV	Negative

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

History of Communication

Date	To	Description
10/26/09 1:55 PM	Morrison, Diane - fax	bc report generated
10/27/09 4:18 PM	Marine Harvest Canada - 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: 09-4116

Last Updated: 10/27/09 8:50 AM
Pathologist: Gary D. Marty
Received Date: 10/20/09
Collected Date: 10/19/09
Client Ref No: PO #7408

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

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

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

Case History

Submitted fresh and formalized Atlantic salmon tissues for histopathology and PCR for IHN, VHS and ISA.

Fish experienced heterosigma bloom with high mortality. Four fresh morts sent in for sampling. This will be an insurance claim.

Atlantic salmon. Saltwater entry - 08 S1. Vaccinated: yes. Euthanized: no. Prior submission: yes, 7397.

Final Diagnosis

1. Brain: meningoencephalitis, granulomatous, with intralesional bacteria (*Renibacterium salmoninarum* ?), multifocal, severe (slide 3)

2a. Heart: myocarditis, lymphohistiocytic, multifocal, severe (slide 4)

2b. Heart: epicarditis, lymphohistiocytic, mild (slides 1, 2), moderate (slide 4)

2c. Heart: myocarditis, histiocytic, focal, mild (slide 3)

3a. Liver: hepatitis, granulomatous, multifocal (probable *Renibacterium salmoninarum*), severe (slide 3)

3b. Liver: sinusoidal congestion, multifocal, mild (slide 2, 4), moderate (slide 1)

3c. Liver: interstitial yellow-brown pigment (hemosiderin or lipofuscin?), scattered, small amounts (slides 1, 2, 3, 4)

3d. Liver: hepatocellular vacuoles, diffuse, mild (slides 3, 4)

3e. Liver: intravascular cellular thrombus, focal, mild (slide 4)

4a. Trunk kidney: nephritis, granulomatous, multifocal (probable *Renibacterium salmoninarum*), severe (slide 3)

4b. Trunk kidney: interstitial congestion, diffuse, mild (slide 4), moderate (slide 1)

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

5a. Intestine and adjacent mesenteries: peritonitis, fibrocellular, diffuse, mild (slide 4), moderate (slide 3)

5b. Intestine, lamina propria: vascular congestion, multifocal, mild (slides 1, 2), moderate (slide 3)

6. Mesenteric adipose tissue: steatitis, lymphohistiocytic, multifocal, mild (slide 3)

7. Spleen: parenchymal golden pigment (lipofuscin?), scattered, mild (slide 1)

Final Comment: Granulomatous inflammation in the brain, liver and kidney of fish #3 is evidence that this fish died from disseminated infection with *Renibacterium salmoninarum* , the cause of bacterial kidney disease. Lymphohistiocytic inflammation in the heart of fish #4 was probably not of sufficient severity to have killed the fish, but it would have increased its susceptibility to death from exposure to *Heterosigma* . . Lesions in fish #1 and #2 are not of sufficient severity to have caused mortality, and based on the clinical history their death might have been mostly a result of *Heterosigma* exposure. *Heterosigma sp.* usually causes mortality without morphologic lesions in internal organs [Reference: Kent, M.L., and T.T. Poppe. 1998. Diseases of seawater netpen-reared salmonid fishes. Quadra Printers, Ltd. Nanaimo, B.C., Canada.] Comments on specific lesions follow:

Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 18% of the 467 Atlantic salmon hearts examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program.

Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 28% of the 467 Atlantic salmon hearts examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program.

Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion; diffuse congestion sometimes occurs as a postmortem artifact. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infection, but PCR results rule out ISAV in this case; also, ISAV has never been identified in British Columbia.

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

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.

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

Renal congestion in autolyzed fish is probably a result of postmortem passive congestion, but it might be a result of circulating vasodilators. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; PCR results in this case rule out VHSV, IHNV, and ISAV.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29% ; n = 469) and Pacific salmon (prevalence = 31% ; n = 118).

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

Distension of capillaries in the intestinal lamina propria is a distinctive change (called "red gut") that is sometimes attributed to a nutritional (or rather an anti- nutritional) colitis exacerbated by opportunistic colonizing bacteria: mostly vibrios of various types. This type of reaction is recognized in salmon, yellowtail, and *Seriola* (source: Dr. Mark Sheppard, BC-MAL, personal communication).

Steatitis is an uncommon lesion in salmonids. In this case (fish #3) it is probably a result of *Renibacterium salmoninarum* 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. 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%). Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

Histopathology

Formalin-fixed tissues from 4 fish were submitted in 4 cassettes for histopathology.

Slide #s 1-4 are labeled in the same order as client #s 1 - 4. Organs included on most slides - heart, liver, spleen, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue, brain.

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

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

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Sean Byrne on 10/22/09 @ 9:33 AM

Specimen	ID	Test	Result
Tissue	7408-1	PCR - IHNV	Negative
Tissue	7408-2	PCR - IHNV	Negative
Tissue	7408-3	PCR - IHNV	Negative
Tissue	7408-4	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Sean Byrne on 10/22/09 @ 9:33 AM

Specimen	ID	Test	Result
Tissue	7408-1	PCR - ISA	Negative
Tissue	7408-2	PCR - ISA	Negative
Tissue	7408-3	PCR - ISA	Negative
Tissue	7408-4	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Sean Byrne on 10/22/09 @ 9:34 AM

Specimen	ID	Test	Result
Tissue	7408-1	PCR - VHSV	Negative
Tissue	7408-2	PCR - VHSV	Negative
Tissue	7408-3	PCR - VHSV	Negative
Tissue	7408-4	PCR - VHSV	Negative

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

History of Communication

Date	To	Description

10/27/09 8:51 AM	Morrison, Diane - fax	bc report generated
10/27/09 4:19 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Animal Health Centre

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Final Report AHC Case: 09-4245

Last Updated: 11/20/09 10:24 AM
Pathologist: Gary D. Marty
Received Date: 10/28/09
Collected Date: 10/24/09
Client Ref No: 7474

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

Submitter: **Tiffany MacWilliam - Marine Harvest Can.**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

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

Case History

Submitted from 5 Atlantic salmon: formalized tissues for histopathology, and fresh tissues for tissue culture and PCR for IHNV, VHSV and ISAV.

Saltwater entry: 07 S0. Vaccinated - Yes. Insurance: Yes. # Submitted Dead - 5. DOD: Oct 24/09.

Do event. 5 fish submitted dead on ice (to Marine Harvest fish health staff; fish were not fresh dead). Some colour left in gill, firm internal organs. 5 histo samples taken - gills submitted separately. 5 tissue samples taken for tissue culture and PCR. No visible lesions.

Final Diagnosis

1. Heart: epicarditis, leukocytic, diffuse, severe (slide 1B)

Final Comment: Submitted organs in these fish have no lesions that could have caused mortality. These findings are consistent with an acute process that caused mortality without inducing morphologic changes in the submitted organs; differentials include low levels of dissolved oxygen, toxic algal blooms, or brain lesions (e.g., hemorrhage or inflammation).

Epicarditis in fish #1 is evidence of chronic immune stimulation ; differentials include a low grade bacterial infection and reaction to a vaccine. Severe epicarditis is rare in Atlantic salmon "fresh silvers" that die in marine net pens, affecting none of the 467 Atlantic salmon hearts examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program; by comparison, 28% of these fish had mild or moderate epicarditis.

Histopathology

Formalin -fixed tissues from 5 fish were submitted in 10 cassettes for histopathology.

Slide #s 1A - 5A are labeled in the same order as client #s 1A - 5A and slide #s 1B - 5B are labeled in the same order as client #s 1-5.

Organs included on most slides - gill, heart (except in slides 4B and 5B), liver, spleen, head kidney, trunk kidney, intestinal ceca, mesenteric adipose tissue.

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

Quality control: Liver autolysis: severe (slides 1B, 2B, 3B, 4B, 5B). Gill autolysis: severe (slides 1A, 2A, 3A, 4A, 5A). The level of autolysis in these fish is greater than what would be considered acceptable for the provincial government's Fish Health Auditing and Surveillance Program. Large foci of perivascular collagen in the kidney (slide 4B) have acid hematin deposits. Acid hematin accumulates as brown birefringent deposits when tissues are not fixed in neutral buffered formalin and when tissues become acidic before or during fixation (as can happen with thick bloody pieces of tissue or with acid decalcification). Organs have no postfixation dehydration.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/30/09 @ 11:46 AM

Specimen	ID	Test	Result
Tissue	7474-1	PCR - IHNV	Negative
Tissue	-2	PCR - IHNV	Negative
Tissue	-3	PCR - IHNV	Negative
Tissue	-4	PCR - IHNV	Negative
Tissue	-5	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/30/09 @ 11:46 AM

Specimen	ID	Test	Result
Tissue	7474-1	PCR - ISA	Negative
Tissue	-2	PCR - ISA	Negative
Tissue	-3	PCR - ISA	Negative
Tissue	-4	PCR - ISA	Negative
Tissue	-5	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 10/30/09 @ 11:46 AM

Specimen	ID	Test	Result
Tissue	7474-1	PCR - VHSV	Negative
Tissue	-2	PCR - VHSV	Negative
Tissue	-3	PCR - VHSV	Negative
Tissue	-4	PCR - VHSV	Negative
Tissue	-5	PCR - VHSV	Negative

Virology

Tissue Culture Resulted by: Melissa Trapp Verified by: Dr. J. Robinson on 11/20/09 @ 10:24 AM

Specimen	ID	Isolate	Result	Level
Tissue	7474-1		No viruses isolated	
Tissue	-2		No viruses isolated	
Tissue	-3		No viruses isolated	
Tissue	-4		No viruses isolated	
Tissue	-5		No viruses isolated	

Staff Comments:
Fish Histopathology Fellow: Meritxell Diez Padrisa, DVM Histopathology and PCR results sent to Marine Harvest fish health staff via Outlook on Monday 2009-11-02 2:05 PM.

History of Communication

Date	To	Description
11/20/09 11:45 AM	Morrison, Diane - fax	bc report generated
11/25/09 8:44 AM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-4601

Last Updated: 11/26/09 12:43 PM
Pathologist: Gary D. Marty
Received Date: 11/23/09
Collected Date: 11/18/09
Client Ref No: CB181109

Veterinarian: **Dr. Peter McKenzie**
Clinic:
Phone:
Fax:

Submitter: **Nathan Cassan - Mainstream**
Phone:
Fax:
Owner: **Mainstream Canada**
Phone:
Fax:(250) 286-0042

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

Case History

Submitted 4 formalized Atlantic salmon tissues for Histopathology and 2 plates for Bacteriology.

Saltwater. Vaccinated - Yes. Insurance - No. Euthanized - Yes, TMS. Prior submission: No. # submitted dead - 4. DOD: Nov. 18/09.

Slow swimmers observed in corners displaying exothalmia. 4 pcs from varying pens dipped, euthanized and examined. Plated kidney and brain from each for Bacteriology over 2 plates. 4 cassettes submitted formalized with above noted tissues labelled per fish for Histopathology.

Final Diagnosis

1a. Brain: meningoencephalitis (probably *Yersinia ruckeri*), granulomatous, diffuse, mild (slide 1A), severe (slides 2A, 4A)

1b. Brain: encephalitis, focal, mild (slide 1A)

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

2b. Heart: epicarditis, lymphohistiocytic, regionally diffuse, mild (slides 1A, 3A, 4A)

3a. Trunk kidney: renal tubular intraluminal necrotic cells, with luminal dilation, multifocal, mild (slides 1A, 3A)

3b. Trunk kidney: renal tubular casts of yellow-brown pigment (lipofuscin?), focal, mild (slide 2A)

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

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

4b. Liver: biliary preductular cells hyperplasia, diffuse, mild (slide 1A)

4c. Liver: hepatocellular cytoplasmic lipofuscin, multifocal, mild (slide 4A)

5. Intestinal ceca: peritonitis, granulomatous, multifocal, mild (slide 4A), moderate (slides 1A, 2A)

6. Spleen: peritonitis, granulomatous, diffuse, moderate (slides 1A, 4A)

7. Gill: lamellar epithelial cell hypertrophy and hyperplasia, multifocal, mild (slide 2G)

Final Comment: Two of these fish were moribund as a result of a severe meningoencephalitis. Granulomatous inflammation in the brain is consistent with a bacterial septicemia. A chronic infection by *Yersinia ruckeri* is the most likely differential in this case. Two out of four brain swabs and two out of four kidney swabs cultured are positive for *Yersinia ruckeri*. Although the brain lesions have no obvious organisms on the standard H&E stain, this finding is not unusual for *Yersinia ruckeri*. These fish have other lesions consistent with a bacterial septicemia: meningoencephalitis, cardiac endothelial cell hyperplasia and hypertrophy, and renal tubular necrotic epithelial cells.

Other lesions are not significant enough to have caused morbidity, but might provide clues to the cause of disease. Comments on each of them follow:

Pigment in the renal tubules 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.

Renal tubular epithelial protein droplets might be an indication of stress (e.g., recent vaccination or handling) or glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Renal tubular intracytoplasmic protein droplets were common among fish sampled in 2008 as part of the Ministry's Fish Health Auditing and Surveillance Program in Atlantic salmon (prevalence = 29% ; n = 469) and Pacific salmon (prevalence = 31% ; n = 118).

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

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins or inflammatory mediators) or come from outside the fish (e.g., from the water or the feed). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 14% of the 468 Atlantic salmon livers examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2008 was sufficient to identify a trend towards greater prevalence in the winter and spring (21-30%) than in the summer and fall (1.9-4.4%).

A few hepatocytes throughout the liver contain cytoplasmic red-orange globular inclusions (on H&E) that are consistent with lipofuscin. Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. Hepatic lipofuscin is uncommon in young fish.

Peritonitis in the spleen and intestinal ceca is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Splenic peritonitis affected 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Hypertrophy and hyperplasia of the epithelium lining gill lamellae is a nonspecific response to irritation. Inciting causes include parasites, bacteria, viruses, and toxins. Chronic cases may have fusion of adjacent lamellae.

Histopathology

Formalin-fixed tissues from 4 fish were submitted in 4 cassettes for histopathology. Gills were removed from the original (A) cassettes and placed in separate (G) cassettes. The gills were then immersed 2h in Protocol B (hydrochloric acid solution) for decalcification and then rinsed in water before being processed with other cassettes into paraffin.

Slide #s 1A-4A and 1G-4G are labeled in the same order as client #s Fish #1 - Fish #4.

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

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

Quality control: Liver autolysis: none (slides 1A, 2A, 3A, 4A). 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 of the gills is 100% complete and differential staining is good. Organs have no postfixation dehydration and no acid hematin deposits.

Bacteriology

Aerobic Culture - Prod Resulted by: Erin Zabek Verified by: Sean Byrne on 11/26/09 @ 12:08 PM

Specimen	ID	Isolate	Result	Level
Kidney	1	Yersinia ruckeri	Positive	
Brain	1		No Bacteria Isolated	
Kidney	2	Yersinia ruckeri	Positive	
Brain	2	Yersinia ruckeri	Positive	
Kidney	3		No Bacteria Isolated	
Brain	3		No Bacteria Isolated	
Kidney	4		No Bacteria Isolated	
Brain	4	Yersinia ruckeri	Positive	

Fish Resulted by: Erin Zabek Verified by: Sean Byrne on 11/26/09 @ 12:09 PM

Organism	ID	e	ffc	s3	sxt	ot
Yersinia ruckeri	1	r	s	s	s	s

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

Staff Comments:
Please Note: Plates were contaminated in a splash pattern; therefore only isolates growing on a distinct streak line were worked up. ---- - ----
Fish Histopathology Fellow: Meritxell Diez Padrisa, M.Sc., DVM

History of Communication

Date	To	Description
11/26/09 12:52 PM	Mainstream Canada - e-mail	bc report generated
11/26/09 12:52 PM	Dr. Peter McKenzie - e-mail	bc report generated
11/30/09 3:28 PM	Mainstream Canada - 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: 09-4748

Last Updated: 12/07/09 12:27 PM
Pathologist: Gary D. Marty
Received Date: 12/02/09
Collected Date: 11/17/09
Client Ref No: 7507/7499

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

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

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

Case History

Ref. # 7507 - Submitted formalized Atlantic salmon tissues for Histopathology.

Saltwater entry: 08 S0. Vaccinated - Yes. Insurance legal: No. Euthanized - Yes - TMS. DOD: Nov. 17/09.

Gill with mucous build up and visible damage.

Dec. 7/09 - Additional history (Ref. #7499) - Saltwater entry 08 SO. Vaccinations: Yes. Insurance/Legal: No. Euthanized: Yes, TMS. Date fish died: Nov. 17/09. Gill with mucous build-up and visible damage.

Final Diagnosis

1a. Liver: hepatitis, granulomatous, multifocal, with intralesional necrosis and small numbers of Gram - positive rods (*Renibacterium salmoninarum*), severe (slide 1A)

1b. Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, focal, mild (slide 1A)

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

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

1e. Liver: hepatocellular single cell necrosis (apoptosis?), disseminated, mild (slide 1A)

2a. Gill: branchitis, granulomatous, with intralesional multinucleated giant cells, multifocal, mild (slide 2), severe (slide 3)

2b. Gill: lamellar epithelial cell hypertrophy and hyperplasia, with lamellar fusion and mucous cell hyperplasia, multifocal, moderate (slide 2), diffuse, severe (slide 3)

2c. Gill: lamellar capillary thrombosis, focal, mild (slides 2, 3)

3a. Heart: myocarditis, granulomatous, bifocal, moderate (slide 1A)

3b. Heart; endothelial cell hypertrophy, diffuse, moderate (slide 1A)

3c. Heart: epicarditis, lymphohistiocytic, diffuse, mild (slide 1A)

4a. Head kidney: nephritis, granulomatous, multifocal, mild (slide 1A)

4b. Head kidney: peritonitis, fibrinous, granulomatous, focal, moderate (slide 1A)

5. Spleen: peritonitis, granulomatous, diffuse, moderate (slide 1A)

Final Comment: These fish have several lesions that might have contributed to morbidity. Granulomatous inflammation in multiple organs is consistent with a chronic bacterial infection, and *Renibacterium salmoninarum* is the most common organism associated with these lesions; the liver is the only organ with obvious bacteria on the Gram stain. By comparison, granulomatous inflammation on the spleen (peritonitis) is more consistent with a reaction to foreign material: common in fish that have been vaccinated.

Comments on other lesion follow:

Sinusoidal congestion in the liver is a nonspecific vascular lesion; diffuse congestion sometimes occurs as a postmortem artifact. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

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.

Biliary preductular cell hyperplasia is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins or inflammatory mediators) or come from outside the fish (e.g., from the water or the feed). Biliary preductular cell hyperplasia is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 14% of the 468 Atlantic salmon livers examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program; the sample size of Atlantic salmon in 2008 was sufficient to identify a trend towards greater prevalence in the winter and spring (21-30%) than in the summer and fall (1.9-4.4%).

Causes of hepatocellular single cell necrosis have not been well defined in fish. Possible differentials include exposure to toxins (endogenous or exogenous), or a viral infection (VHSV).

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

Thrombosis in the gill is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection, or exposure to toxins from harmful algal blooms.

Hypertrophy of endothelial cells in the heart is consistent with accumulation of foreign material. It is common in females of some fish species, related to follicular degeneration or resorption, but it can occur in either gender with bacterial infections and parasites.

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

Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology. Gills were decalcified in protocol B (hydrochloric acid) for 2.5 hours before being processed into paraffin blocks.

Slide 1A (7499-1) - heart, liver, spleen, head kidney, intestinal ceca, mesenteric adipose tissue. Gram -Twort's stain done.

Slides 1B (7507-1), 2 (7507-2), 3 (7507-3) - gill

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

Quality control: Liver autolysis: mild (slide 1A). Gill autolysis: none (slide 2), mild (slide 3), moderate (slide 1B) Decalcification of the gills is 100% complete and differential staining is good. Large foci of erythrocytes (e.g., gill in slide 3) 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.

Staff Comments:
Fish Histopathology Fellow: Meritxell Diez Padrisa, M.Sc., DVM

History of Communication

Date	To	Description
12/07/09 12:27 PM	Morrison, Diane - fax	bc report generated
12/10/09 9:16 AM	Marine Harvest Canada - 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: 09-4749

Last Updated: 12/08/09 2:54 PM
Pathologist: Gary D. Marty
Received Date: 12/02/09
Collected Date: 11/25/09
Client Ref No: 7518

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

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

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

Case History

Submitted fresh and formalized Atlantic salmon tissues for Histopathology and PCR for IHN, ISA and VHS.

Saltwater entry: 09 S1. Insurance legal: No. Euthanized - Yes. DOD: Nov. 25/09.

6 samples labelled: 3-1, 4-1, 5-1, 5-2, 10-1 and 12-1 for PCR for IHN, ISA and VHS. 4 cassettes for histology taken from 2 fish labelled 4-1 and 12-1. Fish #4-1 with hem of liver. Fish # 12-1 with hem of intestine. Bacteriology resulted in no significant growth.

Final Diagnosis

1a. Trunk kidney: glomerular capillary congestion, multifocal, moderate (slide 4A)

1b. Trunk kidney: distended urinary space, multifocal, mild (slide 4A)

1c. Trunk kidney: tubular epithelial cell hyperplasia, multifocal, mild (slide 4A)

2a. Liver: sinusoidal congestion, with acid hematin granules and intracytoplasmic spherical golden to amphophilic inclusions, acute, multifocal, moderate (slide 4A)

2b. Liver: basophilic cytoplasm, diffuse, moderate (slide 4A)

2c. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slide 12A)

2d. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slide 12A)

3. Mesenteric adipose tissue: vascular congestion, multifocal, mild (slide 4A)

4. Spleen: peritonitis, granulomatous, focal, mild (slide 4A)

5. Intestinal ceca: peritonitis, granulomatous, multifocal, mild (slide 4A)

Final Comment: Several lesions in fish #4 provide evidence of systemic vascular damage: glomerular capillary congestion, distended urinary spaces, renal tubular epithelial cell hyperplasia, sinusoidal congestion in the liver (consistent with gross findings), and congestion in the mesenteric adipose tissue. Vasoactive substances might be from exposure to toxins in the water or inside the fish (e.g. bacterial toxins, or substances released from inflammatory cells in a bacterial or viral infection). Lesions in fish 12# are not severe enough to impair this fish health.

Comment on less significant lesions in these fish follow:

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

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.

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

Splenic and intestinal peritonitis are consistent with a reaction to foreign material, and are common in fish that have been vaccinated. Splenic peritonitis affects 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Histopathology

Formalin-fixed tissues were submitted in 4 cassettes for histopathology.

Slide 4A (4-1 7518) - heart, liver, spleen, head kidney, trunk kidney, skeletal muscle, intestinal ceca, mesenteric adipose tissue

Slides 4B (4-1 7518) and 12B (12-1 7518) - gill

Slide 12A (12-1 7518) - skin, heart, liver, head kidney, trunk kidney, skeletal muscle, intestinal ceca, mesenteric adipose tissue

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

Quality control: Liver autolysis: none (slide 4A), moderate (slide 12A). Organs have no postfixation dehydration and no acid hematin deposits.

Molecular Diagnostics

PCR - IHNV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 12/08/09 @ 2:54 PM

Specimen	ID	Test	Result
Tissue	7518 3-1	PCR - IHNV	Negative
Tissue	7518 4-1	PCR - IHNV	Negative
Tissue	7518 5-1	PCR - IHNV	Negative
Tissue	7518 5-2	PCR - IHNV	Negative
Tissue	7518 10-1	PCR - IHNV	Negative
Tissue	7518 12-1	PCR - IHNV	Negative

PCR - ISA Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 12/08/09 @ 2:54 PM

Specimen	ID	Test	Result
Tissue	7518 3-1	PCR - ISA	Negative
Tissue	7518 4-1	PCR - ISA	Negative
Tissue	7518 5-1	PCR - ISA	Negative
Tissue	7518 5-2	PCR - ISA	Negative
Tissue	7518 10-1	PCR - ISA	Negative
Tissue	7518 12-1	PCR - ISA	Negative

PCR - VHSV Resulted by: Ken Sojonky Verified by: Dr. J. Robinson on 12/08/09 @ 2:54 PM

Specimen	ID	Test	Result
Tissue	7518 3-1	PCR - VHSV	Negative
Tissue	7518 4-1	PCR - VHSV	Negative
Tissue	7518 5-1	PCR - VHSV	Negative
Tissue	7518 5-2	PCR - VHSV	Negative
Tissue	7518 10-1	PCR - VHSV	Negative
Tissue	7518 12-1	PCR - VHSV	Negative

Staff Comments:
Fish Histopathology Fellow: Meritxell Diez Padrisa, M.Sc., DVM

History of Communication

Date	To	Description
12/07/09 12:20 PM	Morrison, Diane - fax	bc report generated
12/10/09 9:17 AM	Marine Harvest Canada - e-mail	Case Invoiced
01/18/10 3:07 PM	Morrison, Diane - fax	bc report generated

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

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Final Report AHC Case: 09-4964

Last Updated: 12/21/09 2:49 PM
Pathologist: Gary D. Marty
Received Date: 12/17/09
Collected Date: 12/15/09
Client Ref No: #7549 - PCR

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

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

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

Case History

Submitted fresh Atlantic salmon tissues for PCR - IHN, VHS, ISA.

Atlantic salmon. Saltwater entry: 08 SO. Vaccinations: Yes. # in group: 2. Euthanized: No.

Increased mortality in pen. NVL. Only severe hem. in lower intestine. Fish had food in intestine.

Histopathology from MH case 7549 is on AHC case 2009-4965.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/21/09 @ 2:49 PM

Specimen	ID	Test	Result
Tissue	7549-1	PCR - IHNV	Negative
Tissue	7549-2	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/21/09 @ 2:49 PM

Specimen	ID	Test	Result
Tissue	7549-1	PCR - ISA	Negative
Tissue	7549-2	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/21/09 @ 2:49 PM

Specimen	ID	Test	Result
Tissue	7549-1	PCR - VHSV	Negative
Tissue	7549-2	PCR - VHSV	Negative

History of Communication

Date	To	Description
12/21/09 4:14 PM	Morrison, Diane - fax	bc report generated
12/24/09 1:38 PM	Marine Harvest Canada - e-mail	Case Invoiced

Gary D. Marty
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Final Report AHC Case: 09-4965

Last Updated: 12/21/09 11:54 AM
Pathologist: Gary D. Marty
Received Date: 12/17/09
Collected Date: 12/15/09
Client Ref No: #7549 - Histo

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

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

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

Case History

Submitted formalized Atlantic salmon tissues for histopathology.

Atlantic salmon. Saltwater entry: 08 SO. Vaccinations: Yes. # in group: 2. Euthanized: No.

Increased mortality in pen. NVL. Only severe hem. in lower intestine. Fish had food in intestine.

PCR from MH case 7549 is on AHC case 2009-4964.

Final Diagnosis

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

2. Intestine: peritonitis, chronic, diffuse, with abundant congested fibrocellular fronds, severe (slide 3)

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

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

Final Comment: Distension of capillaries in the intestinal lamina propria is a distinctive change (called "red gut") that is sometimes attributed to a nutritional (or rather an anti-nutritional) colitis exacerbated by opportunistic colonizing bacteria: mostly vibrios of various types. This type of reaction is recognized in salmon, yellowtail, and *Seriola* (source: Dr. Mark Sheppard, BC-MAL, personal communication). Among affected fish in your experience, do live- sampled or moribund fish have the lesion? [Some of the congestion might be a result of postmortem change.] If the lesion seems to be associated with an infectious disease, it might be the result of a virus that cannot be readily cultured. Consider contacting Kyle Garver at the Nanaimo DFO lab; he has been working with BC-CDC using a microarray that has the ability to amplify sequences from all known pathogenic viruses (at least those described from vertebrates).

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. In this case, it might be related to the proprial-submucosal congestion.

Lymphohistiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 18% of the 467 Atlantic salmon hearts examined in 2008 as part of the Province's Fish Health Auditing and Surveillance Program.

Histopathology

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

Slides 1 (7549-1), 2 (7549-2), and 3 (7549-3) - heart, liver, spleen, trunk kidney (3 pieces), intestine, intestinal ceca, and mesenteric adipose tissue. All organs were examined. Organs not listed elsewhere have no significant lesions.

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

History of Communication

Date	To	Description
12/21/09 11:55 AM	Morrison, Diane - fax	bc report generated
12/24/09 1:39 PM	Marine Harvest Canada - e-mail	Case Invoiced

Gary D. Marty
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Final Report AHC Case: 09-4966

Last Updated: 12/21/09 2:49 PM
Pathologist: Gary D. Marty
Received Date: 12/17/09
Collected Date: 12/15/09
Client Ref No: #7548 - PCR

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

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

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

Case History

Submitted fresh Atlantic salmon tissues for PCR - IHN, VHS, ISA.

Atlantic salmon. Saltwater entry: 08 SO. Vaccinations: Yes. Insurance/Legal: No. # in group: 3. Prior submission: No. Dead - 3.

Petechial hem. on pyloric ceca, hem. on liver and along belly wall. Histopathology from MH case 7548 is on AHC case 2009-4967.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/21/09 @ 2:49 PM

Specimen	ID	Test	Result
Tissue	7548-1	PCR - IHNV	Negative
Tissue	7548-2	PCR - IHNV	Negative
Tissue	7548-3	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/21/09 @ 2:49 PM

Specimen	ID	Test	Result
Tissue	7548-1	PCR - ISA	Negative
Tissue	7548-2	PCR - ISA	Negative
Tissue	7548-3	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/21/09 @ 2:49 PM

Specimen	ID	Test	Result
Tissue	7548-1	PCR - VHSV	Negative
Tissue	7548-2	PCR - VHSV	Negative
Tissue	7548-3	PCR - VHSV	Negative

History of Communication

Date	To	Description
12/21/09 4:22 PM	Morrison, Diane - fax	bc report generated
12/24/09 1:39 PM	Marine Harvest Canada - e-mail	Case Invoiced

Gary D. Marty
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Final Report AHC Case: 09-4967

Last Updated: 12/21/09 4:53 PM
Pathologist: Gary D. Marty
Received Date: 12/17/09
Collected Date: 12/14/09
Client Ref No: #7548 - Histo.

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

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

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

Case History

Submitted formalized Atlantic salmon tissues for histopathology.

Atlantic salmon. Saltwater entry: 08 SO. Vaccinations: Yes. Insurance/Legal: No. # in group: 1. Prior submission: No. Dead - 1.

Petechial hem. on pyloric ceca, hem. on liver and along belly wall. PCR from MH case 7548 is on AHC case 2009-4966.

Final Diagnosis

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

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

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

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

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

2a. Kidney: interstitial congestion/hemorrhage , diffuse, with hematopoietic cell depletion, moderate (slide 2)

2b. Kidney: glomerular capillary congestion, generalized, diffuse, moderate (slide 1)

4. Heart: endocarditis, diffuse, with endothelial cell hypertrophy, moderate (slide 2)

3a. Spleen and intestine: peritonitis, chronic, multifocal, with fibrocellular fronds, mild (slides 1, 2)

3b. Spleen: parenchymal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin?), disseminated, moderate (slide 1)

Final Comment: Both fish have lesions consistent with death due to systemic disease, but PCR results rule out a viral cause. Bacteria are a possible differential. Comments on specific lesions follow:

Multifocal sinusoidal congestion in the liver is a nonspecific vascular lesion; diffuse congestion sometimes it occurs as a postmortem artifact. Differentials include algal toxins, substances released from inflammatory cells or bacteria, and infection with VHSV; the cause is usually not determined. Sinusoidal congestion is one of the classic lesions associated with ISAV infections, but ISAV has never been identified in British Columbia. Consider bacteriology (if not already done). I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). The golden to amphophilic cytoplasmic inclusions in hepatocytes are large, up twice the size of hepatocyte nuclei. The inclusions are probably remnants of ingested erythrocytes (this type of inclusion has not been described with any salmon virus). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the liver, are evidence that the congested focus was acidic. This could have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.

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

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

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

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

Renal interstitial congestion/hemorrhage with depletion of interstitial hematopoietic cells is an uncommon manifestation of infection with viral hemorrhagic septicemia virus (VHSV), but if the PCR tests were done on tissues from this fish, then the negative results render a viral cause less likely in this case. Many of the remaining hematopoietic cells have prominent nucleoli: evidence of active regeneration.

Glomerular capillary congestion is a distinctive lesion that occurs occasionally in Atlantic salmon. The change is not associated with hemorrhage or mesangial changes, but it might be a result of altered glomerular vascular function. It is not described in major fish pathology textbooks (i.e., significance unknown).

Endothelial cell hypertrophy in the heart is evidence of systemic disease. Differentials include a bacterial or viral infection (e.g., VHSV), or exposure to toxins (e.g., algal toxins); the cause is often not determined. Hypertrophic endothelial cells are basophilic and up to 10 µm thick.

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting the spleen of 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% were severe).

Histopathology

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

Slide 1 (7548-1) - heart, liver, spleen, head kidney, trunk kidney (2 pieces), intestine, intestinal ceca, and mesenteric adipose tissue.

Slide 2 (7548-2) - heart, liver, spleen, trunk kidney (3 pieces), intestine (2 pieces), intestinal ceca, and mesenteric adipose tissue.

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

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

History of Communication

Date	To	Description
12/21/09 4:54 PM	Morrison, Diane - fax	bc report generated
12/24/09 1:40 PM	Marine Harvest Canada - e-mail	Case Invoiced

Gary D. Marty
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Final Report AHC Case: 09-4969

Last Updated: 12/21/09 2:50 PM
Pathologist: Gary D. Marty
Received Date: 12/17/09
Collected Date: 12/15/09
Client Ref No: #7550

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

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

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

Case History

Submitted fresh Atlantic salmon tissues for PCR - IHN, VHS, ISA.

Atlantic salmon. Saltwater entry 07 SO. Vaccinations: Yes. Insurance/Legal: No. # in group: 2. Prior submission: No. Dead - 2.

Petechial hem. on liver, p.c and belly wall.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/21/09 @ 2:50 PM

Specimen	ID	Test	Result
Tissue	7550-1	PCR - IHNV	Negative
Tissue	7550-2	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/21/09 @ 2:50 PM

Specimen	ID	Test	Result
Tissue	7550-1	PCR - ISA	Negative
Tissue	7550-2	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/21/09 @ 2:50 PM

Specimen	ID	Test	Result
Tissue	7550-1	PCR - VHSV	Negative
Tissue	7550-2	PCR - VHSV	Negative

History of Communication

Date	To	Description
12/21/09 4:25 PM	Morrison, Diane - fax	bc report generated
12/24/09 1:41 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-4970

Last Updated: 12/21/09 2:50 PM
Pathologist: Gary D. Marty
Received Date: 12/17/09
Collected Date: 12/15/09
Client Ref No: #7553

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

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

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

Case History

Submitted fresh Atlantic salmon tissues for PCR - IHN, VHS, ISA.

Atlantic salmon. Saltwater entry 09 S1. Vaccinations: Yes. Insurance/Legal: No. Prior submission: No.

Hem. in liver and blood on swim bladder.

Molecular Diagnostics

PCR - IHNV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/21/09 @ 2:50 PM

Specimen	ID	Test	Result
Tissue	7553	PCR - IHNV	Negative

PCR - ISA Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/21/09 @ 2:50 PM

Specimen	ID	Test	Result
Tissue	7553	PCR - ISA	Negative

PCR - VHSV Resulted by: Julie Bidulka Verified by: Dr. J. Robinson on 12/21/09 @ 2:50 PM

Specimen	ID	Test	Result
Tissue	7553	PCR - VHSV	Negative

History of Communication

Date	To	Description
12/21/09 4:27 PM	Morrison, Diane - fax	bc report generated
12/24/09 1:41 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

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Final Report AHC Case: 09-5072

Last Updated: 12/24/09 5:36 PM
Pathologist: Gary D. Marty
Received Date: 12/23/09
Collected Date:
Client Ref No: 7559

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

Submitter: **Gerry Burry - Marine Harvest Can.**
Phone:
Fax:
Owner: **Marine Harvest Canada**
Phone:
Fax:(250) 850-3275

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

Case History

Submitted formalized Atlantic salmon tissues for Histopathology.

Saltwater entry: 08S0. Vaccinated: Yes. Insurance legal: No.Euthanized: Yes, Percussion. Prior submission: No. # dead - 3.

Moribund fish collected with large lesions externally. Increase in mortality in pens. No visible lesion internally.

Final Diagnosis

1. Skin (?, epidermis not included): dermatitis, ulcerative, diffuse, with superficial bacterial rods (*Vibrio sp*.?), severe

2a. Liver: hepatocellular hydropic degeneration, disseminated, acute, mild (slide 5)

2b. Liver: basophilic hepatocellular cytoplasm, diffuse, moderate (slides 1, 3, 5)

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

2d. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slide 3)

3. Spleen: parenchymal golden pigment (lipofuscin?), scattered, intracellular, mild (slides 1, 3, 5)

4. Spleen and intestinal mesenteries: peritonitis, chronic, regionally diffuse, with fibrocellular fronds, mild (slides 3, 5)

5. Trunk kidney: interstitial cell hyperplasia, diffuse, mild (slide 3)

Final Comment: The ulcer in fish #1 is consistent with the clinical history of external lesions. Bacterial rods commonly invade ulcers, but they are not necessarily the cause of the ulcer. Ulcers are often associated with some type of underlying stress (e.g., crowding, suboptimal water quality, other infection like VHSV). Consider bacteriology and virology, if not already done. Other lesions provide clues to morbidity, but alone are not as significant:

Hydropic degeneration among small numbers of hepatocytes (slide 5) 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. 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.]

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

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

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

Peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated, affecting spleens of 60% of the 460 Atlantic salmon fresh mortalities ("fresh silvers") examined in 2008 as part of the British Columbia Fish Health Auditing and Surveillance Program (42% were mild, 16% were moderate, and 2.6% 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).

Histopathology

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

Slide 1 (7559-1) - heart, liver, spleen, trunk kidney (2 pieces, one of which is transition with head kidney), intestine, intestinal ceca, mesenteric adipose tissue, skin/skeletal muscle, brain

Slides 3 (7559-3) and 5 (7559-5 - heart, liver, spleen, trunk kidney, head kidney, intestine, intestinal ceca, mesenteric adipose tissue, brain

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

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

History of Communication

Date	To	Description
12/24/09 5:36 PM	Morrison, Diane - fax	bc report generated
01/06/10 3:26 PM	Marine Harvest Canada - e-mail	Case Invoiced

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

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