HISTOPATHOLOGY REPORT
Case #: 2010-4517
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Histopathology: Formalin-fixed tissues from 6 fish were submitted in 12 cassettes for histopathology. The gills (B and G) cassettes were immersed 2.25 h in Protocol B (hydrochloric acid solution) for decalcification and rinsed in water before being processed with other cassettes into paraffin.
Slide #s - 1A/1B (Fish 1 Oct 27/10), 2A/2B (Fish 2 Oct 27/10), 102/102G (Sept 20/10 102), 103/103G (Sept 20/10 Pen 103), 106/106G (Sept 20/10 106), 109/109G (Sept 20/10 109)
Organs included on most 'A' 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 2A, 103), moderate (slides 1A, 102), severe (slides 106, 109). Large foci of erythrocytes (e.g., liver in slide 103) 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.

Diagnoses:
1a. Brain: meningitis, lymphohistiocytic, focal, with intracellular bacteria characteristic of Piscirickettsia salmonis, moderate (slide 1A)
1b. Brain: meningoencephalitis, multifocal, coalescing, with necrosis and foci of intralesional bacteria consistent with Renibacterium salmoninarum, severe (slide 2A)
1c. Brain: meningoencephalitis, perivascular, with hemorrhage and rare foci of intralesional bacteria consistent with Renibacterium salmoninarum, moderate (slide 103)
2a. Liver: hepatic necrosis, acute, multifocal, moderate (slides 2A, 109)
2b. Liver: hepatocellular cytoplasmic vacuoles, diffuse, mild (slide 2A), moderate (slide 1A)
2c.Liver: granulomas, multifocal (up to 600 µm in diameter), with intralesional multinucleate giant cells, focal, moderate (slide 10B)
2d. Liver: sinusoidal macrophages with cytoplasmic yellow-brown to yellow-green pigment (lipofuscin and hemosiderin?), disseminated, mild (slide 103)
3a. Heart: endocarditis, diffuse, with endocardial cell hypertrophy, mild (slide 2A)
3b. Heart: endocarditis, multifocal, lymphohistiocytic, mild (slide 1A)
4a. Head kidney: abundant interstitial intracytoplasmic eosinophilic granules, diffuse (slide 2A)
4b. Head kidney: nephritis, interstitial, granulomatous, bifocal (60 - 100 µm in diameter), with focal intralesional radiating globular pale eosinophilic material (vaccine origin?), focal, mild (slide 1A)
5. Trunk kidney: tubular epithelial intracytoplasmic protein droplets, multifocal, mild (slides 2A, 103)

6. Spleen: peritonitis, granulomatous, lymphocytic, regionally diffuse, with occasional fine fibrocellular fronds, mild (slide 103), moderate (slide 2A)
7. Intestinal ceca and mesenteries: peritonitis, chronic, focal, with fibrocellular fronds, mild (slide 106)

Final Comment: These fish died from a range of lesions including hepatic necrosis and brain infections with Piscirickettsia salmonis or Renibacterium salmoninarum. The low prevalence of P. salmonis in these samples is consistent with the clinical history of an occasional dead fish with suspicious lesions. Comments on specific lesions follow:

Fish 1 has brain lesions characteristic of Piscirickettsia salmonis infection, but other organs have no lesions specific for this disease. The brain form of Piscirickettsia salmonis occurs occasionally in farmed Atlantic salmon in BC, and when it does, other organs are routinely negative by PCR for Piscirickettsia salmonis; therefore, the negative PCR result from non-brain tissues is consistent with the brain form of Piscirickettsia salmonis (i.e., it is very specific to the brain).

Fish #2 (and possibly, fish 103) died of complication related to a fulminating infection of the brain with Renibacterium salmoninarum, the cause of bacterial kidney disease. Among 2311 Atlantic salmon examined as part of the BC Fish Health Auditing and Surveillance Program (from 2007 through the 3rd quarter of 2010), 8.0% had Renibacterium salmoninarum infection; half of these cases involved the brain and a quarter of these cases involved only the brain. The negative PCR results rule out the presence of Renibacterium salmoninarum in the tested tissues (which did not include brain), but these PCR results do not provide any information about the brains with R. salmoninarum.

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. Lesions in slide 2A might be related to encephalitis, and lesions in slide 109 might have been the primary cause of death. Lack of proliferative lesions in the biliary system is evidence against a chronic toxic cause for the hepatic necrosis. Hepatic necrosis is somewhat common in salmon that die in marine net pens, in 2009 affecting 12% of the 514 Atlantic salmon examined as part of the Province's Fish Health Auditing and Surveillance Program.

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

Granulomas in the liver (slide 103) and head kidney (slide 1A) are evidence of persistent

antigen. Vaccine material is the most common cause in farmed Atlantic salmon in BC.

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.

Endocardial 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 but in this case (slide 2A) it might be the infection with R. salmoninarum. Hypertrophic endocardial cells are basophilic and up to 7 µm thick. The enlarged cells might be endothelial cells or inflammatory cells.

Lymphohistiocytic inflammation in the heart (endocarditis) is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. It is fairly common in Atlantic salmon "fresh silvers" that die in marine net pens, affecting 17% of the 512 Atlantic salmon hearts examined in 2009 as part of the Province's Fish Health Auditing and Surveillance Program.

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 also previously seen them with severe cerebral Renibacterium salmoninarum (i.e., a similar pattern to slide 2a).

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

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

End of Report