3.2.4 Salmonid Ceratomyxosis

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A. Name of Disease and Etiological Agent

Salmonid ceratomyxosis is an intestinal infection caused by the myxozoan *Ceratomyxa shasta*.

B. Known Geographic Range and Host Species of the Disease

1. Geographical Range

The parasite has a complex life cycle and is established only in the Pacific Northwest of the United States, British Columbia, Canada and Alaska. In the United States Pacific Northwest: Lake Washington; Chehalis River; Columbia River basin including Cowlitz, Lewis (east fork), and Washougal rivers; LaCamas Lake; Snake River from its confluence with the Columbia River to approximately 440 miles upstream; Deschutes River basin including Crooked and Metolius rivers and Davis, Odell, Crescent, Wickiup and Suttle lakes; Willamette River from the mouth to 100 miles upstream and including the Clackamas River: Nehalem, Siletz, Rogue, and Klamath rivers; Klamath Lake; Sacramento River basin including the Mokelumne, Feather, Butte and Pit River systems. In B. C. Canada: Fraser River. In Alaska: Tanana and Naknek River systems on the Alaskan Peninsula, Russell Creek (Cold Bay) in the Aleutian chain, the Togiak and Wood Rivers in Bristol Bay, and Lower Talarik Creek (Lake Iliamna). Additional isolations have been made in Alaska; however, it is unclear if these represent an established parasite presence.

Anadromous salmon may come in contact with *C. shasta* during migration and infected juvenile and adult fish have been reported in freshwater and marine environments outside of the parasite's established range.

2. Host Species

Natural infections of *C. shasta* are known to occur in the following native salmonid species: rainbow/steelhead trout *Oncorhynchus mykiss*, cutthroat trout *O. clarkii,* pink salmon *O. gorbuscha,* chum salmon *O. keta,* coho salmon *O. kisutch*, sockeye salmon *O. nerka,* Chinook salmon *O. tshawytscha* and Dolly Varden *Salvelinus malma*. Infections have also been reported from non-native Atlantic salmon *Salmo salar,* brown trout *S. trutta* and brook trout *Salvelinus fontinalis.*

Strains of salmonids within the same species may show different susceptibilities to *C. shasta* (Zinn *et al*. 1977; Buchanan *et al*. 1983; Ching and Munday 1984a-c; reviewed in Bartholomew.

1998). Salmonids that originate from enzootic waters are relatively resistant to infection and disease.

C. Epizootiology

Ceratomyxosis causes losses in wild and domestic trout and salmon of all ages and sizes and has been reported as a contributor to prespawning mortality among infected adult fish. *Ceratomyxa shasta* has been considered as a single species based on similarities in the site of infection in fish, disease manifestations and myxospore morphology. However, recent studies document the presence of multiple parasite strains with different host specificities (Atkinson and Bartholomew 2010 a,b).

Bartholomew et al. (1997) demonstrated that completion of the parasite life cycle requires development of actinosporean stages in the freshwater polychaete worm, *Manayunkia speciosa* (Figure 1). Natural transmission occurs when the waterborne actinospore attaches to the fish gill and penetrates the gill epithelium (Bjork and Bartholomew 2010). It reaches the intestine and other organs via the circulatory system. Distribution of the polychaete is likely the principal factor that has defined the geographic distribution of the parasite.

Mortality generally occurs when water temperatures exceed 10°C; however, fish can become subclinically infected at temperatures as low as 4°C. Infections with *C. shasta* are prevented at salinities greater than 15 ppt; however, if fish are infected when they enter salt water the disease may still progress.

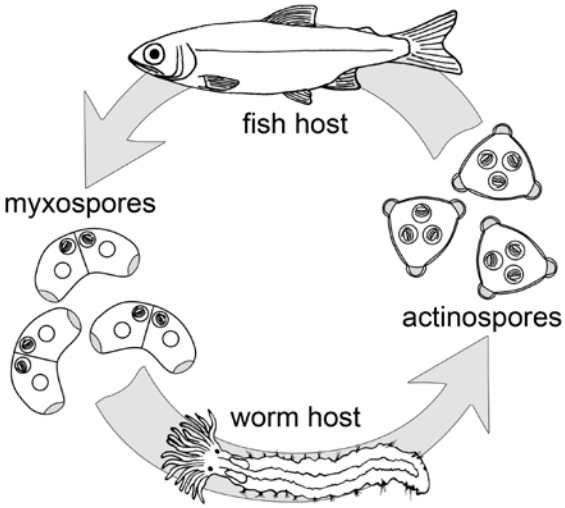

Figure 1. Life cycle of *Ceratomyxa shasta* showing salmonid and polychaete host with alternating myxospore and actinospore stages. Diagram: Stephen Atkinson, Oregon State University.

D. Disease Signs

Clinical signs of ceratomyxosis vary with fish species and fish age. In most cases, at least some of the following will be seen: anorexia, lethargy, marked darkening (especially in rainbow trout/steelhead), distended abdomen, exopthalmia, a swollen and hemorrhagic vent, and emaciation (Figure 2). In juvenile salmonids, the digestive tract may be grossly swollen, necrotic, and hemorrhagic with mucoid contents and ascites may accumulate in the body cavity. The intestine and pyloric caeca may be lined with caseous material.

Parasite development in other organs, such as the kidney, liver and pyloric caeca, occurs after initial proliferation in the intestine. Pathological changes in these tissues include kidney lesions (fluid filled blebs/pustules to firm creamy white nodules), and hemorrhaging and (or) necrosis of liver, gall bladder, spleen, gonads, kidney, heart, gills, skeletal musculature and the eye.

Figure 2. Clinical ceratomyxosis: Clockwise from top: juvenile rainbow trout with distended abdomen and swollen and hemorrhaged vent; pyloric caeca; intestine; liver from a heavily infected adult salmon. Photos: Craig Banner, Oregon Dept. of Fish and Wildlife.

In adult salmonids the walls of the intestine and pyloric caeca may be thickened and hemorrhagic. Nodular lesions may develop in the intestinal wall perforating the intestine in Chinook salmon. Gross lesions (which may abscess) can occur in liver, kidney, spleen, or musculature (Figure 2). Abscesses of the body musculature are particularly common in coho salmon.

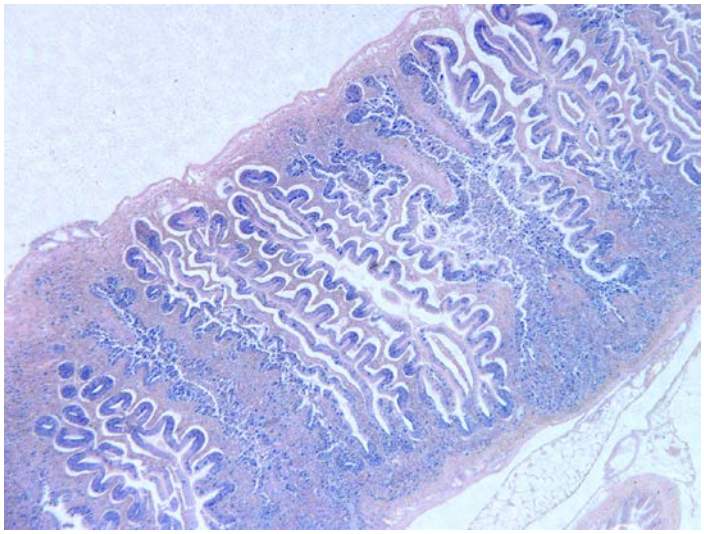

Figure 3. Histological cross-section of intestine showing inflammation, erosion of villi and proliferation of parasite stages. Giemsa-stained. Photo: Jerri Bartholomew.

Development of *C. shasta* infections in the posterior intestine typically triggers acute inflammation involving polymorphonuclear leukocytes (PMNs), fibroblasts, and macrophages. The epithelial lining necrotizes, fragments, and ultimately sloughs, and is replaced by fibrous connective tissue containing

host cells and trophozoites (Figure 3). The intestinal lumen may contain epithelial cells, epithelial cell fragments, PMNs, fibroblasts, trophozoites, pansporoblasts, and spores in later stages (Bjork and Bartholomew 2010).

Pathological changes are less pronounced in the pyloric caeca. Trophozoites are often abundant between epithelial cells and in the muscularis externa. There may be separation of muscle layers due to the large number of trophozoites, but muscle necrosis is normally not severe.

Infection in a host resistant to damage from the disease is not as well characterized but includes granulomas surrounding degenerative parasite stages, replicating parasite stages in the lumen of the intestinal tissues or the absence of any parasite stages (Bartholomew *et al*. 1989c; Ibarra *et al*. 1992, 1994; Bjork and Bartholomew 2010).

E. Disease Diagnostic Procedures

1. Presumptive Diagnosis

Wet mounts can be prepared from the wall of the posterior intestine or from ascites if present. Material obtained via intestinal lavage is acceptable (Coley et al. 1983). Lesions present in any tissue should also be examined. Wet mounts can be scanned in a systematic manner under phase contrast or brightfield microscopy at 250× to 400× magnification. Presumptive diagnosis is based on identification of multicellular presporogonic stages (trophozoites) in salmonids showing signs of ceratomyxosis (Figure 4). An alternative to wet mounts are tissue imprints or histological sections of intestinal or other grossly infected tissues. These may be stained with either Giemsa or hematoxylin and eosin.

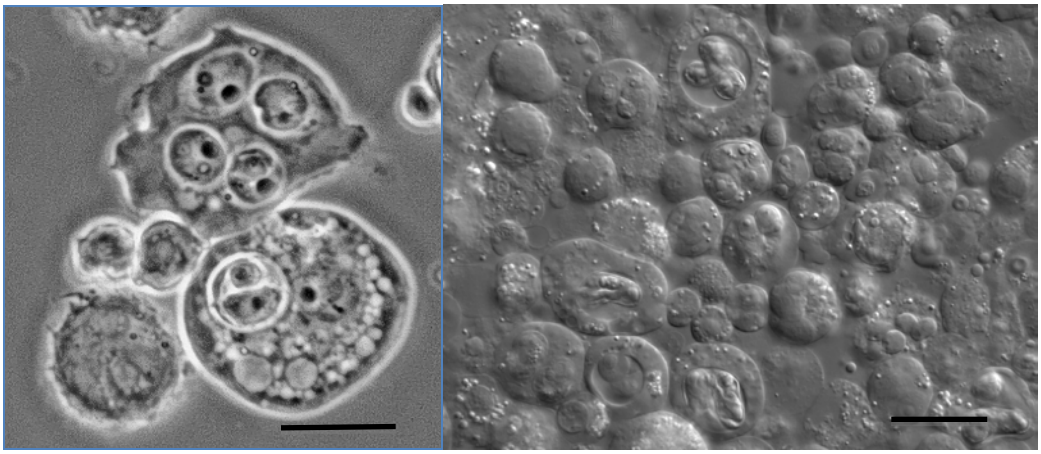

Figure 4. Trophozoites: Left to right: in wet mount (phase contrast); in imprint (Nomarski). Scale bars = 10 µm. Photos: Stephen Atkinson, OSU, and Jerri Bartholomew.

Histological sections of intestine or other grossly infected tissues may be stained with either Giemsa or hematoxylin and eosin. In Giemsa-stained sections, multicellular trophozoites stain light blue with the nuclei containing a dark-staining karyosome surrounded by a clear halo (Figure 5).

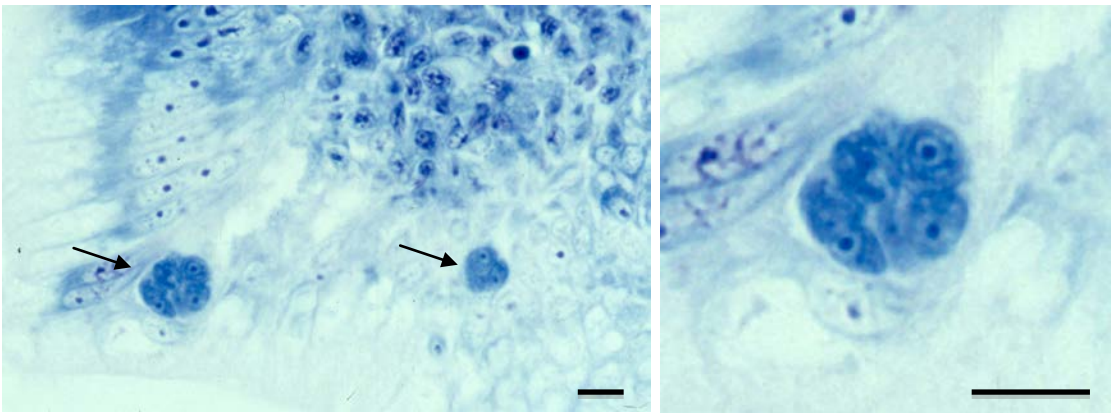

Figure 5. Giemsa stained histological section of intestine from an infected rainbow trout, arrows indicate presporogonic stages. Scale bars = 10 µm. Photos: Jerri Bartholomew.

2. Confirmatory Diagnosis

Confirmatory diagnosis of ceratomyxosis can be based on the presence of the characteristic kidney bean-shaped mature spores of *C. shasta* in wet mounts or histological sections. Spores observed in wet mounts are about 14 to 17 µm long by 6 to 8 µm wide at the suture line (Figure 6). Spores are most likely found in the posterior intestine but are often found in other tissues as well, particularly the kidney, liver, gall bladder, and pyloric caeca.

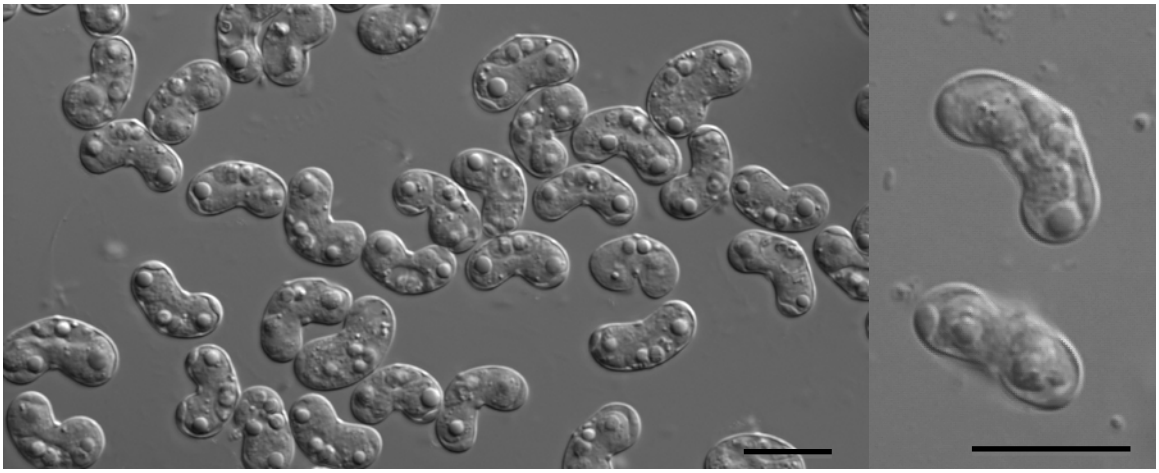

Figure 6. Mature *Ceratomyxa shasta* myxospores from fish (Nomarski). Scale bars = 10 µm. Photos: Stephen Atkinson, OSU.

Serological identification of *C. shasta* can be accomplished using monoclonal antibodies (Figure 7) (Bartholomew et al. 1989b), however, parasite-specific antibodies are not widely available and molecular techniques have become standard.

Molecular diagnosis of *C. shasta* can be accomplished using parasite-specific primers in a polymerase chain reaction assay [PCR; Palenzuela et al. 1999; Palenzuela and Bartholomew 2002; Section 1, 3.2.4.1 Appendix 1]. A modification of the PCR assay has been made to allow for non-lethal sampling of fish (Fox et al. 2000) and *in situ* hybridization techniques provide an alternative to monoclonal antibodies for examining the pathogenesis of the infection histologically (Figure 7) (Palenzuela and Bartholomew 2002).

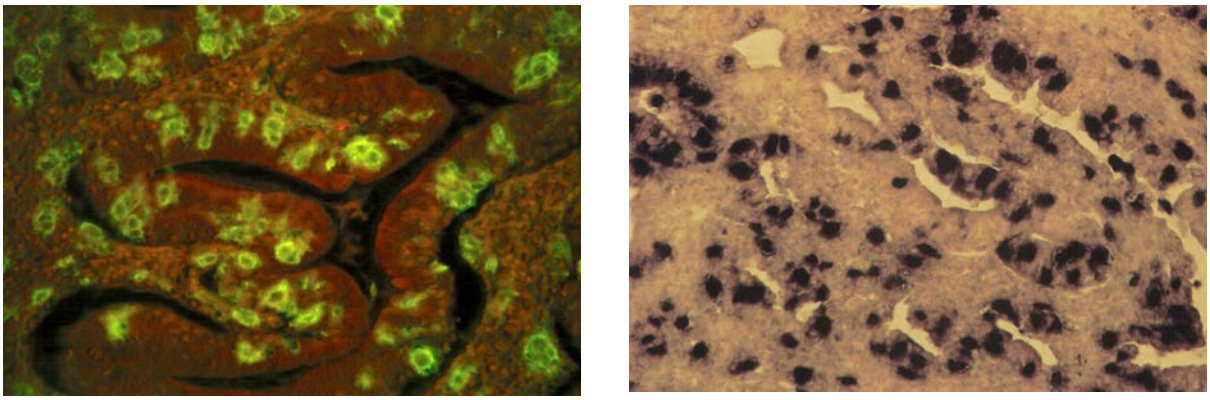

Figure 7. Trophozoite stages in histological sections of intestinal tissue labeled using a fluorescein-conjugated monoclonal antibody specific for *Ceratomyxa shasta* (left) and an enzyme labeled DNA probe (right). Photos: Jerri Bartholomew.

F. Procedures for Detecting Subclinical Infections

Spore formation usually does not occur until late in the infection; therefore, diagnosis of ceratomyxosis in early or subclinical infections should rely on serological or molecular detection of the parasite.

G. Procedures for Determining Prior Exposure to the Etiological Agent

No procedures have been reported.

H. Procedures for Transportation and Storage of Samples to Ensure Maximum Viability and Survival of the Etiological Agent

Although spores can be detected in frozen samples, trophozoites are fragile and easily destroyed by freezing or heat. Therefore, samples for visual examination should consist of living, moribund, or dead fish (or tissues) held at low temperatures or on ice but not frozen. Samples may also be processed routinely for histology. Samples for molecular analysis should be frozen or stored in 100% ethanol.

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