3.2.17 Salmonid Intranuclear Microsporidosis

R.P. Hedrick[1], M.K. Purcell[2], T. Kurobe[1]

[1] Department of Medicine and Epidemiology
School of Veterinary Medicine
University of California, Davis
Davis, CA 95616
530/752-3411
rphedrick@ucdavis.edu, tkurobe@ucdavis.edu

[2]Western Fisheries Research Center
USGS
6505 NE 65th St
Seattle, WA 98115
206/526-6282 x 252
mpurcell@usgs.gov

A. Etiological Agent

Nucleospora salmonis is an intra-nuclear microsporidian parasite in the family *Enterocytozoonidae* (Docker et al. 1997). Prespore stages of the parasite were first observed among adult and then juvenile Chinook salmon (*Oncorhynchus tshawytscha*) by Elston et al. (1987) and Morrison et al. (1990), respectively in Washington, U.S.A. The microsporidian nature of the parasite was subsequently confirmed by the observation of spores in lymphoblasts of juvenile Chinook salmon from California (Hedrick et al. 1991). The principal target cell for *N. salmonis* are hematopoietic cells which, upon infection, undergo proliferative changes leading to a leukemia-like condition with an accompanying anemia (Wongtavatchai et al. 1995).

B. Known Geographic Range and Host Species of the Disease

1. Geographic Range

The parasite has a wide geographic range with reports of infections among salmonid fishes in North and South America and Europe. In N. America, the parasite has been reported from California, Oregon, Washington, Idaho, Colorado, Montana, Missouri, Nebraska in the

U.S.A. and British Columbia, Nova Scotia, New Brunswick and Newfoundland in Canada (Gresoviac et al. 2000).

2. Host Species

Natural infections have been observed among a broad range of salmonids, including steelhead and rainbow trout (*Oncorhynchus mykiss*), golden trout (*O. aquabonita*), Coho salmon (*O. kisutch*), Atlantic salmon (*Salmo salar*), lake trout (*Salvelinus namaycush*), and brook trout (*S. fontinalis*) (MacConnell et al. 1991,Grésoviac et al. 2000, El Alaoui et al. 2006). Infections with *N. salmonis* related organisms have been reported from non-salmonid species such as Atlantic halibut (*Hippoglossus hippoglossus*) (Norway) and lumpfish (*Cyclopterus lumpus*) (Nova Scotia) (Mullins et al. 1994; Nilsen et al. 1995). A *Nucleospora*-like parasite has also been observed in X-cell pseudotumors of English sole (*Parophrys vetulus*) from Washington state (Khattra et al. 2000). An intranuclear microsporidian *Microsporidia rhabdophilia* infecting rodlet cells was described from several salmonid species in California by Modin (1981). Although this parasite has affinities to *N. salmonis,* it was not associated with a clinical syndrome and the lack of more recent occurrences has prevented comparisons to *N. salmonis.* A second species assigned to the genus *Nucleospora,* based upon morphological properties, *N. secunda,* has been identified as an intranuclear parasite of enterocytes of the aquarium fish, *Nothobranius rubripinnis,* originating from Tanzania (Lom and Dyková 2002). Most recently, *Paranucleospora theridion*, a parasite related but distinct from *N. salmonis*, has been identified as infecting both Atlantic salmon and their copepod parasite *Lepeophtheirus salmonis* in Norway (Nylund et al. 2010).

C. Epizootiology

The life cycle of *N. salmonis* is not completely known, but likely involves transmission routes and persistence in salmonid populations by horizontal, vertical or both types of parasite transmission. Horizontal transmission of *N. salmonis* has been demonstrated experimentally following the feeding of infected tissues to Chinook salmon (Baxa-Antonio et al. 1992). Intraperitoneal injections of whole cell preparations from the kidney and spleen of infected fish into juvenile Chinook salmon is also a reliable means of maintaining the parasite in the laboratory (Hedrick et al. 1990, 1991). Infections among spawning adult Chinook salmon and cutthroat trout (*O. clarkii*) and the appearance of *N. salmonis* infections in their progeny provides suggestive evidence for vertical transmission. Recent laboratory studies support vertical transmission of the parasite in cutthroat trout populations despite the application of egg disinfection procedures. The spores formed in infected salmonids are the suspected stages that are directly infectious for other salmonids. During active infections in juvenile salmonids, autoinfection with spores may occur, but proliferation of merogonic (prespore) stages and their dispersion with daughter cells of dividing lymphoblasts is an additional means by which the parasite may multiply in the salmonid host. Presently, there is no evidence for the requirement of intermediate or alternate hosts in the life cycle of *N. salmonis*. Plasmocytoid leukemia (PL), a disease detected among net pen reared Chinook salmon in British Columbia Canada, has many features in common with *N. salmonis* infected salmonids (Kent et al. 1990). Evidence to date

suggests that the proliferative changes associated with PL are due to a putative retrovirus (Eaton et al. 1992) even though concurrent infections with *N. salmonis* may occur. Conversely, experimental studies demonstrate that 0.45 and 0.2 μm filtration effectively eliminates transmission of *N. salmonis* infectious stages in experimental studies with juvenile Chinook salmon (unpublished data).

D. Disease Signs

1. Behavioral Changes Associated with the Disease

The main clinical presentation associated with *N. salmonis* infection is anemia with affected fish demonstrating lethargy and often secondary bacterial or parasitic infections. Infected fish may continue to feed and increased mortality following feeding or handling was reported among infected juvenile Chinook salmon in California (Hedrick et al. 1990).

2. External Gross Signs

There are no specific external signs of infection. Pale gills indicating anemia and occasional exophthalmos may accompany acute infections. Chronic and subclinical infections in the absence of external signs may be the most common means by which *N. salmonis* persists in salmonid populations.

3. Internal Gross Signs

The major internal changes observed during acute *N. salmonis* infections are moderate enlargement of the kidney and spleen and often swelling of the posterior intestine (Figure 1). The swelling of the kidney and spleen is a common clinical sign seen in other diseases including proliferative kidney disease (PKD) and bacterial coldwater disease. The kidney seldom enlarges sufficiently during *N. salmonis* infections to provide the corrugated appearance of the capsule characteristic during advanced episodes of PKD.

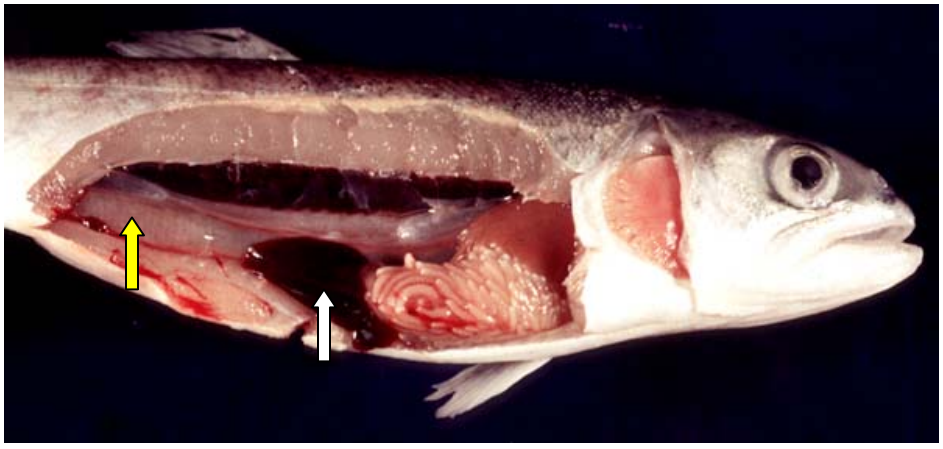

Figure 1. A juvenile Chinook salmon with *N. salmonis* infection. The pale gills indicating anemia, enlarged spleen (white arrow) and slight swelling of the kidney and posterior intestine (yellow arrow) are internal signs that may be observed in acute infections.

4. Histopathological Changes Associated with the Disease

Microscopic pathological features of *N. salmonis* infections are tied to the rapid proliferation and then dispersion of infected hematopoietic cells (e.g. lymphoblasts). Infected cells can be seen in the blood, as well as other tissues where they may collect around major vessels or sinuses (Figure 2). Perivascular accumulations of *N. salmonis* infected cells may be observed in the major veins of the liver, heart, body musculature, choroid gland of the eye and the lamina propria of the small and large intestine (Hedrick et al. 1991). Aggregates of infected cells may also be seen in the dermis and epidermis, as well as associated with the meninges, myelencephalon and metencephalon. In one case involving hatchery rainbow trout, *N. salmonis*-infected cells were detected in cranial nervous tissues of fish showing an abnormal spiral swimming behavior.

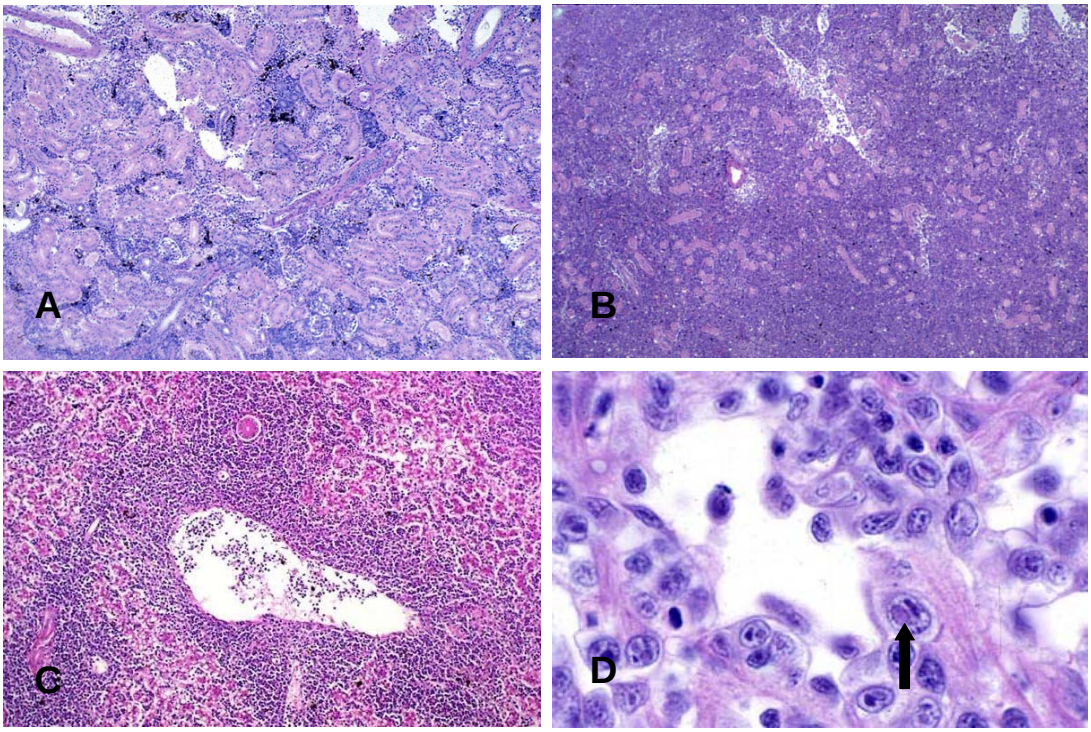

Figure 2. Microscopic pathological changes in Chinook salmon with acute *N. salmonis* infections. (A) normal kidney showing moderate amounts of interstitial hematopoietic cells, (B) kidney with *N. salmonis* infection with greatly increased hematopoietic cells between nephrons, (C) perivascular accumulations of hematopoietic cells in the liver and (D) around a sinus in the heart. Arrow indicates a merogonic stage of the parasite present in the nucleus of the infected host cell. All stains are hematoxylin and eosin.

E. Disease Diagnostic Procedures

1. Presumptive Diagnosis

Observation of external and internal signs in affected fish and presence of merogonic or sporogonic parasite stages in the nucleus of hematopoietic cells in Giemsa-stained blood films or kidney/spleen imprints or hematoxylin and eosin (H&E) stained tissue sections provides a presumptive diagnosis of *N. salmonis* infection (Figure 3). Gram-staining may also be used with fresh tissue smears/imprints. If tissues have previously been frozen, other stains, such as Calcofluor, may be more effective in revealing parasite stages (Guzmán et al. 2001). The Warthin-Starry stain has been shown to improve parasite detection as compared to H&E staining of tissue sections from *N. salmonis*-infected fish (Kent et al. 1995).

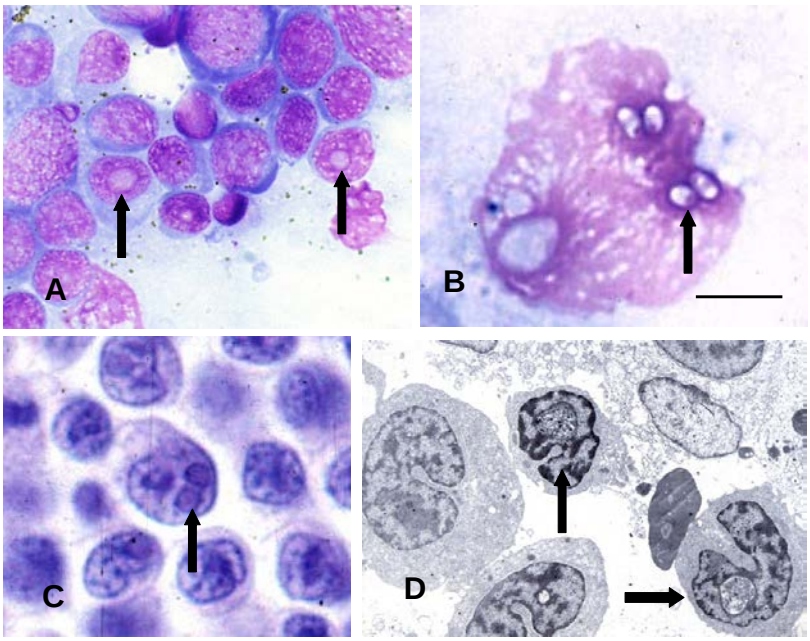

Figure 3. Detection of *N. salmonis*. Kidney imprints stained with Giemsa showing (A) 4 – 7 µm circular and opaque merogonic stages (arrows) in the host cell nucleus, (B) a group of 4 ovoid spores approximately 1 x 2 µm in size (arrow) with bar = 5 µm, (C) two meronts as present in the nucleus of an interstitial hematopoietic cell in the kidney of an H&E stained tissue section and (D) an electron micrograph with two leukocytes (arrows) with developing intranuclear stages (prespore) of *N. salmonis.*

2. Confirmatory Diagnosis

DNA detection procedures, including in situ hybridization, several described PCR assays and LAMP (loop-mediated isothermal amplification) methods, may be used to confirm diagnoses of *N. salmonis* infection. Tissue sections from fish with suspected infections can used for in situ hybridization with labeled probes designed to bind to unique regions of the ribosomal gene of *N.*

salmonis (Grésoviac et al. 2007). However, PCR is more commonly used for confirmation of *N. salmonis* infection (see below). T. Kurobe's recent modification of the PCR assay, originally described by Barlough et al. (1995) (see Appendix 3.18A1), has undergone the initial stages of the test validation process (Georgiadis et al. 1998) and is the most broadly applied of the described procedures. Most recently Sakai et al. (2009) has developed a LAMP assay, and Foltz et al. (2009) and Badil et al. 2011 have described a quantitative PCR assay for detecting DNA from *N. salmonis*. The presence of characteristic intranuclear spores of *N. salmonis* in leukocytes, as detected by electron microscopy, is an additional method of confirmation of parasite infection (Chilmonczyk et al. 1991).

F. Procedures for Detecting Subclinical Infections

Since subclinical infections or mild infections with *N. salmonis* are difficult to detect by light microscopic methods, PCR or LAMP are the preferred approaches to screening of healthy fish populations. Single round, nested and quantitative PCR tests have been described using ribosomal genes as the target sequences (Barlough et al. 1995, Docker et al. 1997, Brown and Kent 2002, Foltz et al. 2009, Badil et al. 2011). The nested PCR protocol originally developed by Barlough et al. (1995) and then as modified by T. Kurobe is described in Appendix 3.18A1 and the quantitative PCR protocol of Badil et al. 2011 is described in Appendix 3.18A2. Permission was obtained from the senior authors of the publications to include these protocols.

G. Procedures for Determining Prior Exposure to 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

Fresh or formalin fixed tissues from fish showing clinical signs such as pale gills and/or moribund fish can be submitted for routine histology processing. Kidney tissue imprints can be heat-fixed or air-dried and fixed in acetone or methanol for 5 min. Samples for molecular analysis should be frozen at -20 or -80^{o}C or preserved in 95% ethanol.

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