

2.2.2 Erythrocytic Inclusion Body Syndrome

J. R. Winton and M. K. Purcell

Western Fisheries Research Center
U.S. Geological Survey
6505 NE 65th Street
Seattle, WA 98115
206-526-6282, Ext. 6587 (JW) or 2052 (MP)
jwinton@usgs.gov; mpurcell@usgs.gov

A. Name of Disease and Etiological Agent

Erythrocytic inclusion body syndrome (EIBS) is an infectious disease of salmonid fish that is caused by a yet uncharacterized virus. Additional host and environmental factors may contribute to EIBS severity and the syndrome is often associated with secondary infections.

B. Known Geographical Range and Host Species

1. Geographical Range

The virus associated with erythrocytic inclusion body syndrome has been reported to occur in wild or captive-reared salmonids from the west coast of North America, Norway, Ireland, Scotland, and Japan (Foott et al. 1992; Leek 1987; Lunder et al. 1990; Okamoto et al. 1992a; Rodger 2007; Rodger and Richards 1998; Rodger et al. 1991; Takahashi et al. 1992a).

2. Host Species

Natural infections with the EIBS virus have been reported in Coho Salmon *Oncorhynchus kisutch*, Chinook Salmon *O. tshawytscha*, Rainbow Trout or steelhead *O. mykiss*, and Atlantic Salmon *Salmo salar* (Leek, 1987; Michak et al. 1992; Rodger et al. 1991; Takahashi et al. 1992a). Experimentally, Cutthroat Trout *O. clarkii*, Chum Salmon *O. keta*, and Masu Salmon *O. masou* have been shown to be susceptible by intraperitoneal injection (Okamoto et al. 1992b; Piacentini et al. 1989).

C. Epizootiology

The hallmarks of EIBS include a moderate to severe anemia, the presence of cytoplasmic inclusions in erythrocytes and frequent co-infections with other pathogens such as *Flavobacterium psychrophilum*, *Renibacterium salmoninarum* and various fungi (Piacentini et al. 1989). Outbreaks of EIBS are especially problematic during the rearing of juvenile and smolt stages of Pacific salmon in freshwater hatcheries of western North America (Piacentini et al. 1989) and in smolt or larger stages of coho and Atlantic Salmon reared in freshwater and marine net pens in Asia and Europe (Lunder et al. 1990; Takahashi et al. 1992a). The disease is more common during periods of cool water in the fall, winter, and spring, possibly because of a reduced host immune response. At lower temperatures, epizootics can

2.2.2 Erythrocytic Inclusion Body Syndrome - 2

have long durations (up to 5 months) because the progress of infection is slowed as shown by Piacentini et al. (1989). Fish recovering from infections are more resistant to re-infection, presumably due to the development of adaptive immunity (Okamoto et al. 1992a; Piacentini et al. 1989).

The direct cause of mortality in fish affected by EIBS may vary with the species, intensity of infection and presence of secondary pathogens. For example, in Coho Salmon severely affected by EIBS, the hematocrits may be especially low (e.g. 1-4%) resulting in fish dying of hypoxia after modest exercise induced by grading and pond cleaning or after feeding when dissolved oxygen levels in ponds or raceways are depressed. In more chronic infections, secondary infections may become severe and are likely the proximate cause of mortality.

D. Disease Signs

Diseased fish are usually anemic and lethargic with pale gills (Takahashi et al. 1992b). Pigmentation abnormalities may also be observed and secondary infections are frequently present. Hematocrits are depressed to below normal levels and, in severe cases, below 10% (Leek 1987). Internal tissues are often pale suggesting anemia and splenomegaly is usually apparent (Piacentini et al. 1989). Biochemically, abnormalities in fatty acid metabolism and declines in Na⁺-K⁺ ATPase levels in the kidney have been reported for artificially infected Coho Salmon (Maita et al. 1998).

Histopathologically, there is congestion in the kidney and spleen with hemosiderin deposits in the spleen (Foott et al. 1992). Blood smears reveal anemia with many erythroblasts and a high percentage of leukocytes, due to the loss of erythrocytes or the host response to the secondary infections (Michak et al. 1992; Piacentini et al. 1989). Erythrocytes of diseased fish are more fragile than normal and blood smears show evidence of smudge cells, erythrophagocytosis and the presence of single to multiple cytoplasmic inclusions which range in size from 0.8-2 µm (Figure 1).

E. Disease Diagnostic Procedures

1. Presumptive Diagnosis

The EIBS virus has proven refractory to isolation using a wide range of fish cell cultures (Arakawa et al. 1989; Piacentini et al. 1989). Presumptive diagnosis is based on the presence of anemia and observation of cytoplasmic inclusions in the erythrocytes of blood smears stained with pinacyanol chloride or Leishman-Giemsa (Leek 1987). The diagnosis of EIBS is complicated by the fact that several other morphologically distinct viruses have been reported to cause cytoplasmic inclusion bodies in the erythrocytes of salmonid fish (Landolt et al. 1977, Hedrick et al. 1987, Thorud et al. 1990, Haney et al. 1992, Finstad et al. 2014). Among these, specific molecular tests are available for erythrocytic necrosis virus (ENV; Purcell et al. 2016) and piscine orthoreovirus (PRV; Løvoll et al. 2012). In addition, infections with ENV, but not PRV, are associated with severe anemia. Thus, care is needed in the diagnosis of EIBS to exclude other causes of anemia or erythrocytic inclusion bodies caused by morphologically distinct viruses.

Air dried blood smears are fixed in 100% methanol for 5 minutes and then stained with pinacyanol chloride for 2 minutes. The stain can be prepared by mixing 2.5 g pinacyanol chloride powder with 367.0 mL 95% ethanol and 132.5 mL distilled water (adjust to pH 7.1 – 7.2). Smears are examined at 1000X for characteristic erythrocytic inclusions (Yasutake 1987).

To distinguish inclusions caused by EIBS from ENV, fixed blood smears may be stained using acridine orange and examined by fluorescent microscopy. Methanol/ethanol (1:1) fixed smears are

2.2.2 Erythrocytic Inclusion Body Syndrome - 3

rehydrated in a series of 100%, 70%, and 50% ethanol, stained with 0.1% aqueous acridine orange and washed in phosphate buffered saline. Using fluorescence microscopy at 1000X, inclusions caused by the EIBS virus stain red; those of ENV stain green (Piacentini et al. 1989).

2. **Confirmatory Diagnosis**

Freshly collected heparinized blood cells are processed for transmission electron microscopy by the methods of Leek (1987), Rodger (2007) or Rohovec and Amandi (1981). Blood cells infected with the EIBS virus contain spherical virions approximately 70-80 nm in diameter that appear singly in the cytoplasm or clustered inside membrane-bound organelles or inclusion bodies that contain lamellar structures (Arakawa et al. 1989, Leek 1987; Lunder et al. 1990; Michak et al. 1992; Rodger 2007).

3. **Molecular Assays**

Although there are no molecular assays currently available for the identification of the EIBS virus, PCR assays are available to detect and estimate levels of PRV (Løvoll et al. 2012) or ENV (Purcell et al. 2016). Since its discovery, PRV has been shown to be present in some presumptive cases of EIBS. There is still a degree of uncertainty as to whether PRV has been frequently misidentified as the EIBS virus in the past because both the inclusion bodies observed in stained smears and the virions seen by electron microscopy appear similar. However, experimental challenge studies with PRV have yet to produce the moderate to severe anemia characteristic of EIBS (Garver et al. 2016).

F. Procedures for Detecting Subclinical Infections

There is no practical method to detect subclinical infections. Erythrocytic inclusion bodies can be observed in fish that do not show overt anemia or disease (Rodger 2007). Conversely, at times when anemia is most severe, inclusions may be absent or difficult to find (Piacentini et al. 1989).

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

Because the EIBS virus cannot be grown in cell culture, methods to ensure viability of the pathogen are not required; however, blood for examination should be collected in heparinized tubes and sent to a laboratory on ice. Better, the heparinized blood can be used in the field to prepare air-dried blood smears or placed into fixative for electron microscopy.

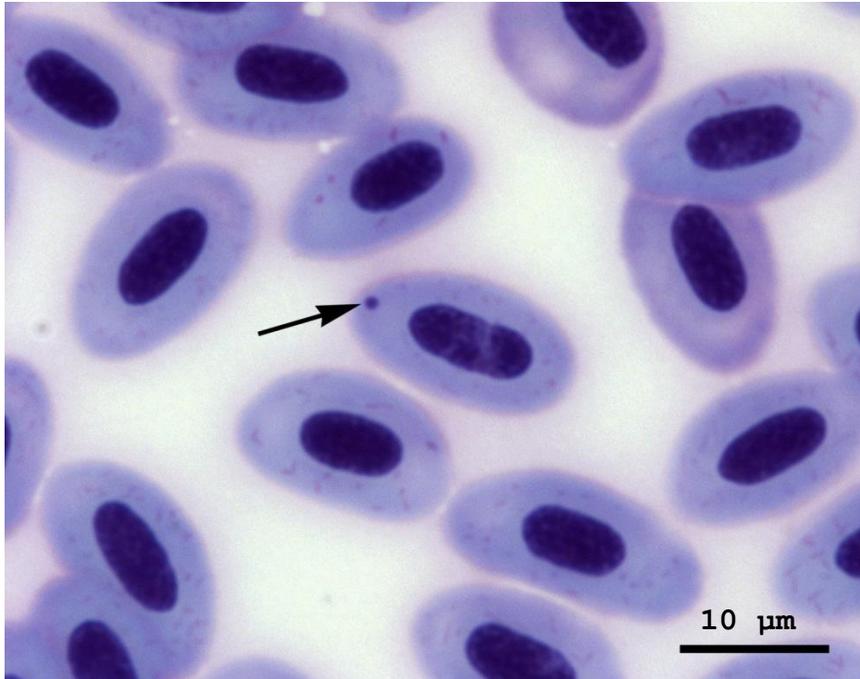


Figure 1. Inclusion body (arrow) in an erythrocyte from Coho Salmon (*Oncorhynchus kisutch*) presumptively diagnosed with EIBS (pinacyanol chloride stain; 1000x magnification). Photo credit Carla Conway.

References

- Arakawa, C. K., D. A. Hursh, C. N. Lannan, J. S. Rohovec, and J. R. Winton. 1989. Preliminary characterization of a virus causing infectious anemia among stocks of salmonid fish in the Western United States. Pages 442-450 in W. Ahne, and E. Kurstak, eds. *Viruses of Lower Vertebrates*. Springer-Verlag, Berlin.
- Finstad, Ø. S., M. K. Dahle, T. H. Lindholm, I. B. Nyman, M. Løvoll, C. Wallace, C. M. Olsen, A. K. Storset, and E. Rimstad. 2014. Piscine orthoreovirus (PRV) infects Atlantic salmon erythrocytes. *Veterinary Research* 45:35.
- Foott, J. S., A. Miller, R. Steiner, and R. P. Hedrick. 1992. Erythrocytic inclusion body syndrome (EIBS) infection of Chinook salmon in Idaho. *Journal of Aquatic Animal Health* 4:306-308.
- Garver, K. A., S. C. Johnson, M. P. Polinski, J. C. Bradshaw, G. D. Marty, H. N. Snyman, D. B. Morrison, and J. Richard. 2016. Piscine orthoreovirus from Western North America is transmissible to Atlantic salmon and sockeye salmon but fails to cause heart and skeletal muscle inflammation. *PLoS ONE* 11(1): e0146229. doi:10.1371/journal.pone.0146229
- Haney, D. C., D. A. Hursh, M. C. Mix, and J. R. Winton. 1992. Physiological and hematological changes in chum salmon artificially infected with erythrocytic necrosis virus. *Journal of Aquatic Animal Health* 4:48-57.

2.2.2 Erythrocytic Inclusion Body Syndrome - 5

- Hedrick, R. P., T. S. McDowell, and J. M. Groff. 1987. Another erythrocytic virus from salmonid fish? Fish Health Section AFS Newsletter 15:5. Available from: <http://www.afs-fhs.org/perch/resources/announcement-of-a-joint-annual-meeting-of-the-fish-health-section-of-the-american-fisheries-society-the-eastern-fish-health-small.pdf>.
- Landolt, M. L., J. R. MacMillan, and M. Patterson. 1977. Detection of an intra-erythrocytic virus in rainbow trout (*Salmo gairdneri*). Fish Health News 6:4-6.
- Leek, S. L. 1987. Viral erythrocytic inclusion body syndrome (EIBS) occurring in juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) reared in fresh water. Canadian Journal of Fisheries and Aquatic Sciences 44:685-688.
- Løvoll M., M. Alarcon, B. B. Jensen, T. Taksdal, A. B. Kristoffersen, and T. Tengs. 2012. Quantification of piscine reovirus (PRV) at different stages of Atlantic salmon *Salmo salar* production. Diseases of Aquatic Organisms 99:7-12.
- Lunder, T. K., K. Thorud, T. T. Poppe, R. A. Holt, and J. S. Rohovec. 1990. Particles similar to the virus of erythrocytic inclusion body syndrome, EIBS, detected in Atlantic salmon (*Salmo salar*) in Norway. Bulletin of the European Association of Fish Pathologists 10:21-23.
- Maita, M., T. Oshima, S. Horikuchi, and N. Okamoto. 1998. Biochemical properties of coho salmon artificially infected with erythrocytic inclusion body syndrome virus. Fish Pathology 33:53-58.
- Michak, P., C. E. Smith, and K. Hopper. 1992. Erythrocytic inclusion body syndrome: a light and electron microscopic study of infected erythrocytes of chinook *Oncorhynchus tshawytscha* and coho *O. kisutch* salmon. Diseases of Aquatic Organisms 12:229-233.
- Okamoto, N., K. Takahashi, A. Kumagai, M. Maita, J. S. Rohovec, and Y. Ikeda. 1992a. Erythrocytic inclusion body syndrome: Resistance to reinfection. Fish Pathology 27:213-216.
- Okamoto, N., K. Takahashi, M. Maita, J. S. Rohovec, and Y. Ikeda. 1992b. Erythrocytic inclusion body syndrome: Susceptibility of selected sizes of coho salmon and of several other species of salmonid fish. Fish Pathology 27:153-156.
- Piacentini, S. C., J. S. Rohovec, and J. L. Fryer. 1989. Epizootiology of erythrocytic inclusion body syndrome. Journal of Aquatic Animal Health 1:173-179.
- Purcell, M.K., S. Pearman-Gillman, R.L. Thompson, J.L. Gregg, L.M. Hart, J.R. Winton, E.J. Emmenegger and P.K. Hershberger. 2016. Identification of the major capsid protein of erythrocytic necrosis virus (ENV) and development of real-time PCR assays for quantification of ENV DNA. Journal of Veterinary Diagnostic Investigation 28:382-391.
- Rodger, H. D. 2007. Erythrocytic inclusion body syndrome virus in wild Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases 30:411-418.
- Rodger, H. D., and R. H. Richards. 1998. Observational study of erythrocytic inclusion bodies in farmed Atlantic salmon, *Salmo salar* L., in the British Isles. Journal of Fish Diseases 21:101-111.
- Rodger, H. D., E. M. Drinan, T. M. Murphy, and T. Lunder. 1991. Observations on erythrocytic inclusion body syndrome in Ireland. Bulletin of the European Association of Fish Pathologists 11:108-111.

2.2.2 Erythrocytic Inclusion Body Syndrome - 6

- Rohovec, J. S., and A. Amandi. 1981. Incidence of viral erythrocytic necrosis among hatchery reared salmonids in Oregon. *Fish Pathology* 15:135-141.
- Takahashi, K., N. Okamoto, A. Kumagai, M. Maita, Y. Ikeda, and J. S. Rohovec. 1992a. Epizootics of erythrocytic inclusion body syndrome in coho salmon cultured in seawater in Japan. *Journal of Aquatic Animal Health* 4:174-181.
- Takahashi, K., N. Okamoto, M. Maita, J. S. Rohovec, and Y. Ikeda. 1992b. Progression of erythrocytic inclusion body syndrome in artificially infected coho salmon. *Fish Pathology* 27:89-95.
- Thorud, K. E., T. Lunder, S. Wiik, and E. Engeland. 1990. Electron microscopic observations of EIBSV-like particles and two intraerythrocytic virus-like particles different from EIBSV in Atlantic salmon, *Salmo salar*, in Norway. *Bulletin of the European Association of Fish Pathologists* 10:95-97.
- Yasutake, W. T. 1987. Standardization of stain used for diagnosing erythrocytic inclusion body syndrome (EIBS). *Fish Health Section AFS Newsletter*. 15:7. Available from: <http://www.afs-fhs.org/perch/resources/announcement-of-a-joint-annual-meeting-of-the-fish-health-section-of-the-american-fisheries-society-the-eastern-fish-health-small.pdf>