

2.2.4 Infectious Hematopoietic Necrosis

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A. Name of Disease and Etiological Agent

Infectious hematopoietic necrosis (IHN) is caused by infectious hematopoietic necrosis virus (IHNV). Synonyms include: sockeye salmon virus disease, Oregon sockeye virus (OSV), Sacramento River Chinook disease (SRCD), and Sacramento River Chinook virus (SRCV) (Figure 1 and Figure 2).

B. Known Geographical Range and Host Species of the Disease

1. Geographical Range

Infectious hematopoietic necrosis virus is considered to be enzootic to areas of the west coast of North America including Alaska, Washington, Idaho, Oregon, and California in the United States, and British Columbia, Canada. The disease has been observed in other areas of the United States including Colorado, Minnesota, Montana, New York, South Dakota, West Virginia, and Virginia but apparently did not become established. Currently, IHNV is considered to have a wide geographic range that includes North America, Europe, and Asia but not the Southern Hemisphere.

2. Host Species

Epizootics of IHN have commonly occurred in juvenile sockeye (kokanee) *Oncorhynchus nerka* and Chinook salmon *O. tshawytscha* and rainbow and steelhead trout *O. mykiss*. The disease has also been reported in Atlantic salmon *Salmo salar*, pink salmon *O. gorbuscha*, chum salmon *O. keta*, and cutthroat trout *O. clarkii* in North America. Brook trout *Salvelinus fontinalis* and brown trout *Salmo trutta* have also been shown to be susceptible. However, recent studies examining the IHNV susceptibility of trout and char in the genus *Salvelinus* have suggested that certain species within this group may be resistant or exhibit intermediate resistance to IHNV. In Japan, epizootics have occurred in amago salmon *O. rhodurus*, yamame salmon *O. masou*, and chum salmon. Coho salmon *O. kisutch* juveniles are considered to be refractory to the disease but the virus has been isolated from juveniles and adults. Non-salmonids including Pacific herring *Clupea pallasii*, cod *Gadus morhua*, white sturgeon *Acipenser transmontanus*, pike *Esox lucius*, shiner perch *Cymatogaster aggregate* and tube snout *Aulorhynchus flavidus* have occasionally been found to be infected in the wild or shown to be somewhat susceptible by experimental infection.

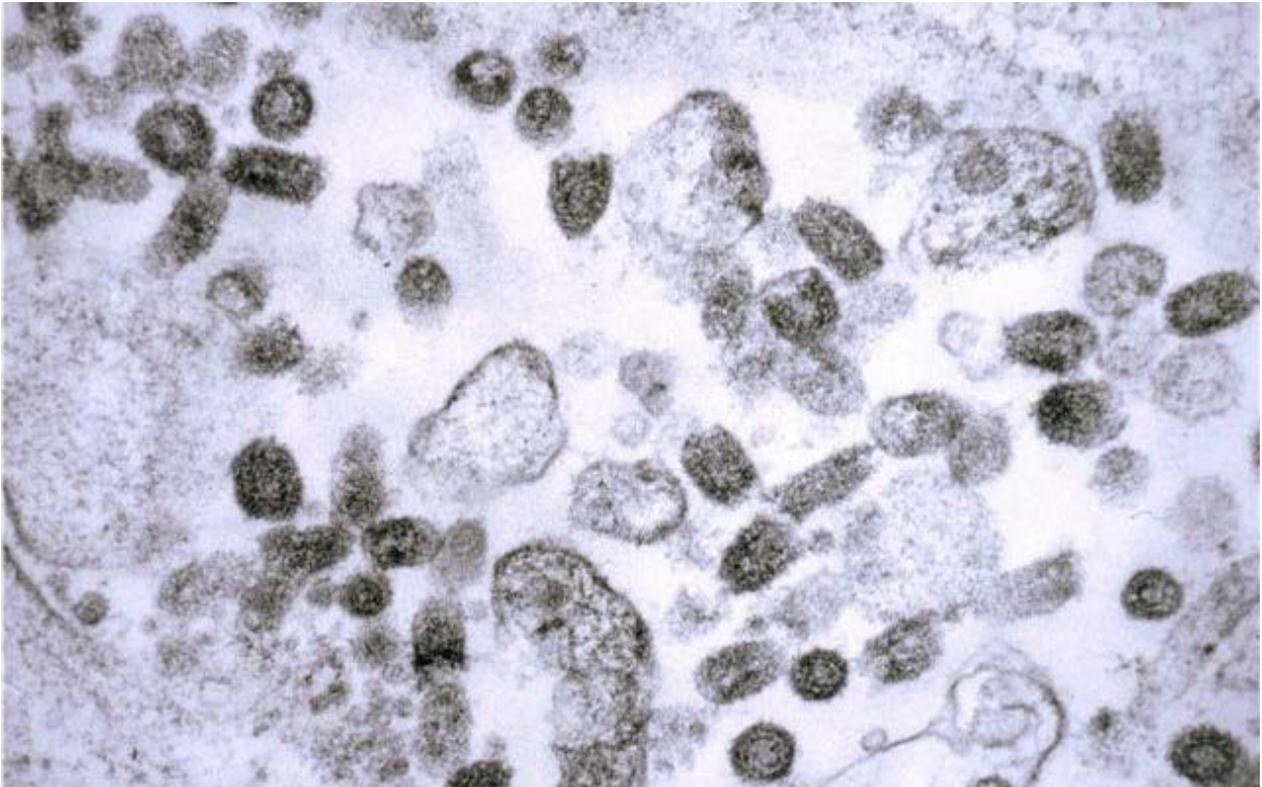


Figure 1. Electronmicrograph image of IHNV in tissue culture. Photo: S.E. LaPatra.



Figure 2. Electronmicrograph image of negative stained IHNV particles. Photo: S.E. LaPatra.

C. Epizootiology

Observation from naturally occurring disease and experimental infections indicate that fish up to two months of age are most susceptible. In recent years, epizootics have also been reported in yearling sockeye and two-year-old kokanee salmon. The disease has been reported in large rainbow trout (100-500g) and yearling Chinook salmon and steelhead. The disease is still less common and more chronic in larger fish than in smaller fish. Most IHN occurs at temperatures of 15°C or less in freshwater.

Beginning in 1992, IHN was diagnosed in netpen reared Atlantic salmon in the marine environment in British Columbia. By 2002 the disease had spread to numerous sites and was reported in fish that ranged in size from 60 g to 6.8 kg and was associated with high rates of mortality independent of fish size.

Horizontal transmission has been demonstrated; waterborne transmission can be accomplished in the laboratory. Clinically infected juvenile salmonids and carrier adults are the reservoirs of virus for waterborne transmission. No other reservoirs of virus have been identified. Evidence for vertical transmission in natural outbreaks is circumstantial and only one report documents such an event under laboratory conditions. A life-long carrier state in a portion of adult rainbow trout that had survived an epizootic as juveniles has been reported. Attempts to confirm this observation in sockeye salmon or steelhead have not been successful, but the numbers of animals examined may not have been sufficient.

The name of this disease, infectious hematopoietic necrosis (IHN), was derived from the clinical manifestation of this disease in very small fish (~0.2 to 8 g) which consisted of the destruction of the blood forming tissues in the anterior kidney and spleen. In this manifestation of IHN a frank viremia occurs and high concentrations of the virus can be detected in all major organs; however, the kidney and spleen generally have the highest concentrations of virus. This has recently been referred to as “hematopoietic” IHN. Another form of the disease which generally manifests in larger fish (>8 g) where the virus can only be isolated from tissues from the brain and is associated with abnormal fish behavior and tetany in a small proportion of the affected animals. This is referred to as “neurotropic” IHN. A third form of IHN occurs in much larger fish (~50 – 100 g) and the virus can generally be isolated from the fin, skin and gills only. This is referred to as “epitheliotropic” IHN. These different manifestations of IHN do not appear to be due to mutations in the surface glycoprotein (G) of the virus but most likely dependent on the life stage and/or the immunological status of the host.

D. Disease Signs

Moribund fish are lethargic, swim high in the water column, and are anorexic. They exhibit exophthalmia, darkening of body color, abdominal distension, pale gills, and hemorrhages at the bases of fins. Fecal casts trailing from the vent have been reported but are not always observed. Swelling of the dorsal cranium has also been observed in some salmon species. Spinal deformities among surviving sockeye salmon and rainbow trout can occur. None of the signs described are considered to be pathognomonic for the disease. Signs of the disease in older fish are different from those observed in young fish. Cutaneous lesions at the bases of fins and on the caudal peduncle often with secondary invasion by fungi and loss of the dorsal fin have been reported. In general, the disease in older fish is more subtle than IHN in smaller fish and different manifestations of the condition are known to occur. Sexually mature adults that are carriers of the virus show no clinical signs.

Gross internal signs include paleness of the viscera (because of anemia) and petechial hemorrhaging in adipose tissue and mesenteries surrounding the viscera. The stomach is devoid of food and contains a milky white exudate. Ascites can be found in the body cavity and petechiation and hemorrhaging is sometimes seen in the swim bladder and lower intestine.

Extensive degeneration and necrosis of the kidney, spleen, liver, and pancreas are observed microscopically. Granular cells of the stomach and intestine may show degenerative changes. Histological changes in the hematopoietic tissue of the anterior kidney are observed during early stages of the disease and may extend throughout the kidney. The tubules and glomeruli are not significantly affected. Necrosis of splenic hematopoietic tissue and of the endocrine and exocrine tissue of the pancreas is diffuse, but the liver can have areas of focal necrosis. Kidney imprints and blood films from infected fish show degenerating leukocytes, large quantities of cellular debris, and macrophages with vacuolated cytoplasm (Figure 3).

Rainbow trout have also been reported with a “neurotropic” form of IHN and exhibited behavioral abnormalities and had high virus concentrations localized in brain tissue (LaPatra et al. 1995) (Figure 4).

E. Disease Diagnostic Procedures

1. Presumptive Diagnosis

To aid in the diagnosis of IHN, certain key features such as life stage and species of fish, water temperature, clinical signs, and disease history of the facility and stock of fish are evaluated. To isolate IHNV, tissue and reproductive fluids are examined by standard cell culture techniques. Processed specimens must be inoculated onto the *Epithelioma papillosum cyprini* (EPC), Chinook salmon embryo (CHSE-214), or fathead minnow (FHM) cell lines. Pretreatment of EPC cell monolayers with a 7% (w/v) solution of polyethylene glycol (PEG; 20,000 MW) has been shown to increase sensitivity of detection and decrease incubation time (Batts and Winton 1990). Cytopathic effect (CPE) includes grape-like clusters of round refractile cells which can be observed 48 to 96 hours post-inoculation depending on the concentration of virus in the inoculum (Figure 5 and Figure 6). Plaque assay procedures similar to those of Burke and Mulcahy (1983) which use a methyl cellulose overlay are also used for isolation and enumeration of IHNV. The SSN-1 cell line derived from striped snakehead has also been evaluated by plaque assay. The results of a preliminary study indicated that the SSN-1 cell line may be more sensitive to IHNV than the EPC line (LaPatra et al. 1999). Clinical signs, microscopic pathology, past history of IHNV, and observation of typical CPE provide the best evidence for a presumptive diagnosis.

2. Confirmatory Diagnosis

Confirmation of IHNV is accomplished by serum neutralization tests with polyclonal rabbit antisera or monoclonal antibodies. Use of a battery of monoclonal antibodies can relate isolates by geographic location (Winton et al. 1988; Huang et al. 1994). Hsu and Leong (1985) described techniques that could be used to detect and differentiate strains of IHNV, but these are not commonly used because radioisotopes are required. An ELISA for IHNV also has been reported (Way and Dixon 1988). A simple immunoblot assay was used to detect IHNV concentrations as low as 100 PFU/mL in cell culture supernatant (McAllister and Schill 1986; Arnzen et al. 1991). A fluorescent antibody test (FAT) for IHNV was developed using either polyclonal or monoclonal reagents (LaPatra et al. 1989a; Arnzen et al. 1991). The FAT was specific and reacted with all isolates of IHNV tested (Figure 7). The test was shown to be equal in sensitivity

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to the plaque assay method but required less time to obtain a definitive diagnosis. Ristow and Arnzen (1989) also described a FAT that was more rapid than previous methods and could be used for serological strain typing of IHNV isolates. Fixed tissue culture cells can also be serologically confirmed to be infected with IHNV by an alkaline phosphatase immunocytochemistry procedure (Drolet et al. 1993). A Polymerase Chain Reaction (PCR) method has been accepted for confirmation of IHNV (Section 2, 4.6.A.2.a “Polymerase Chain Reaction (PCR) Method for Confirmation of IHNV”; Emmenegger et al. 2000). A PCR test has also been developed for fixed and embedded fish tissue (Chiou et al. 1995) along with a biotinylated DNA probe (Deering et al. 1991).

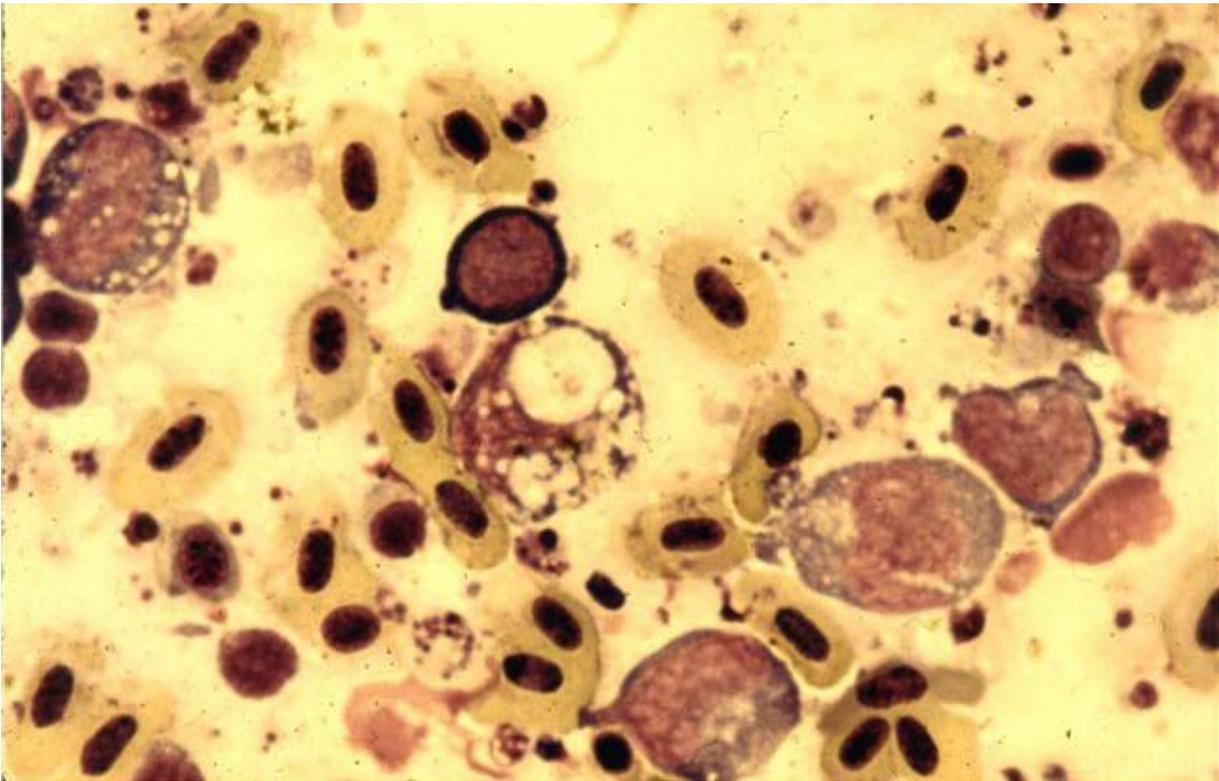


Figure 3. A kidney imprint from an IHN infected rainbow trout stained with Diff Quik®. Photo: S.E. LaPatra.

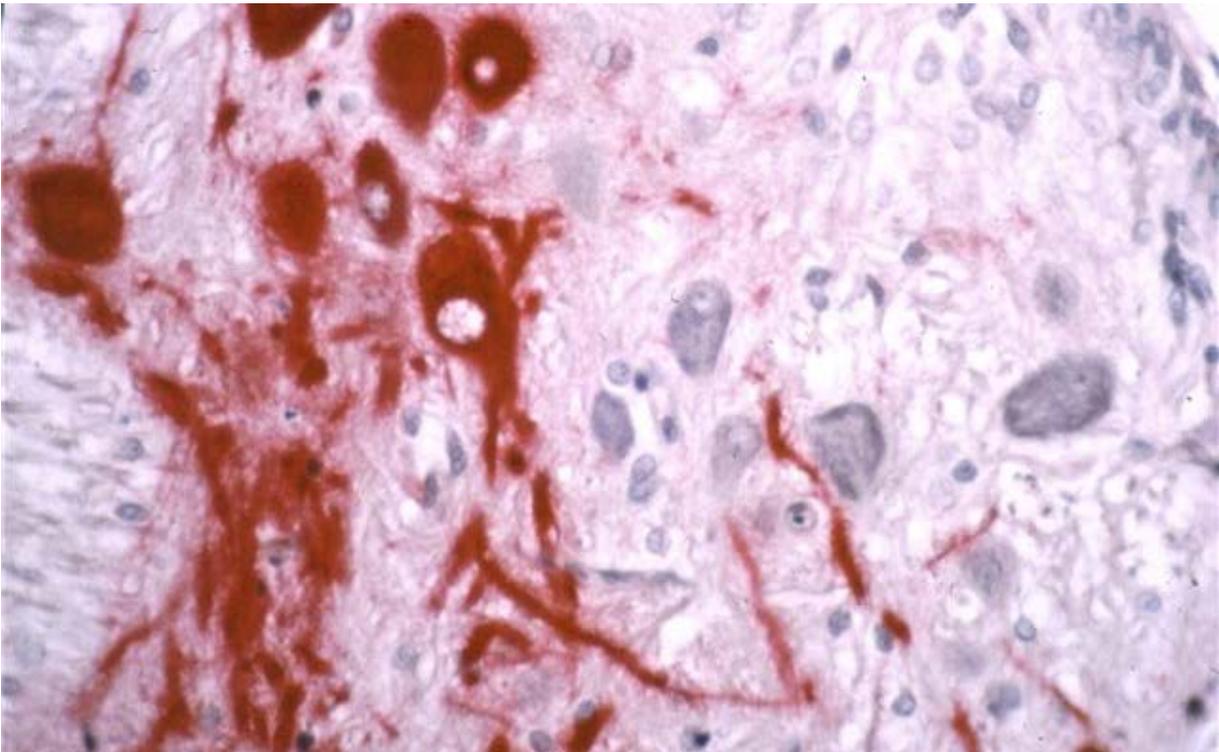


Figure 4. Alkaline phosphatase immunohistochemistry procedure (Drolet et al. 1994) on deparaffinized sections of brain tissue from an effected rainbow trout; the presence of IHNV antigen is indicated by the red staining. Photo: Barb Drolet and S.E. LaPatra.

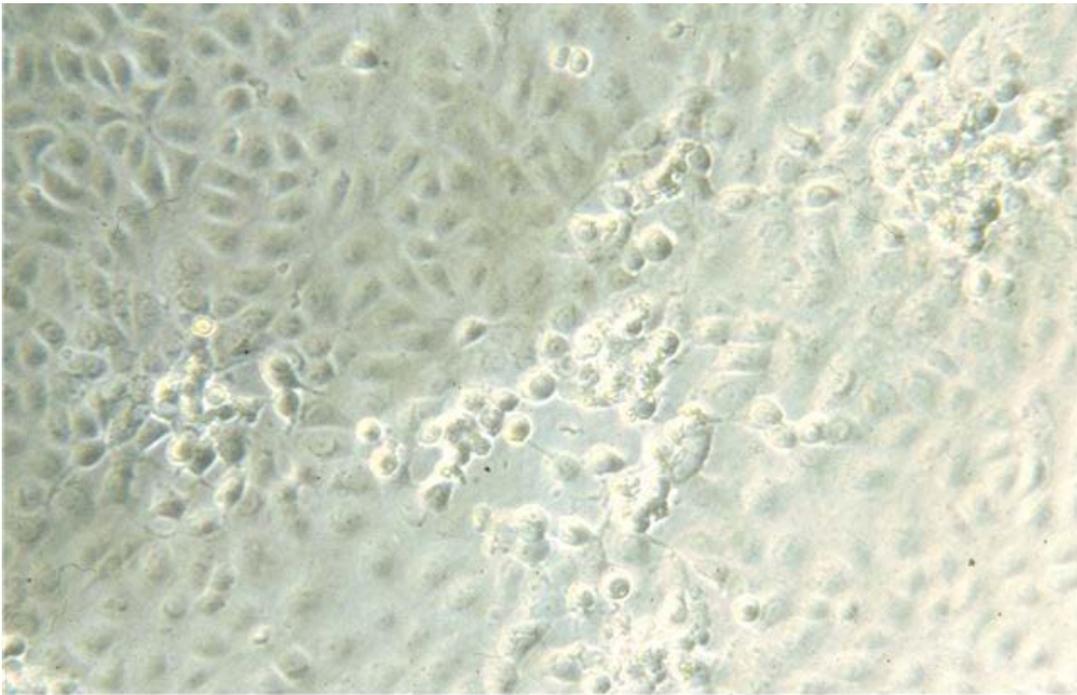


Figure 5. IHNV induced cytopathic effect (CPE) on the CHSE-214 cell line 72 hours post-inoculation. The CPE also can be observed by studying plaque morphology. Photo: S.E. LaPatra.

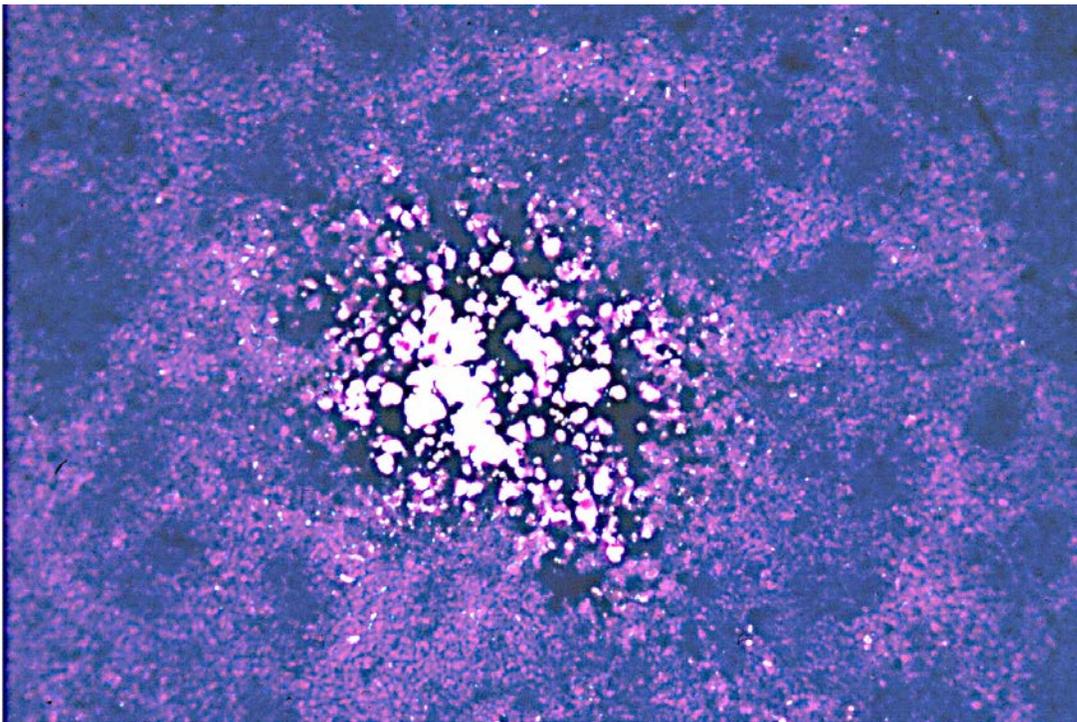


Figure 6. An IHNV plaque on the EPC cell line fixed with formalin and stained with Crystal violet. Photo: S.E. LaPatra.

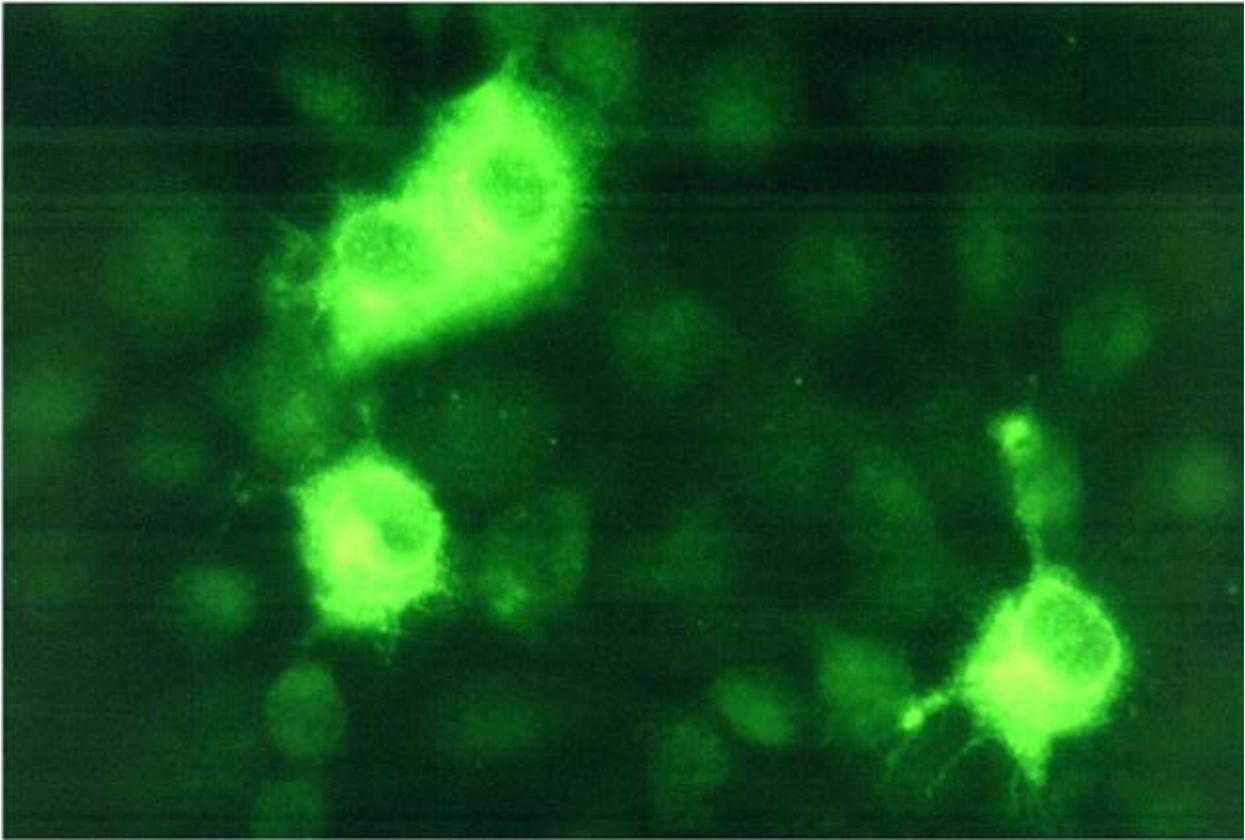


Figure 7. Confirmation of IHNV by FAT on infected cell cultures; the presence of IHNV antigen is indicated by the apple green fluorescence. Photo: S.E. LaPatra.

F. Procedures for Detecting Subclinical Infections

The virus can be isolated readily from specimens obtained from epizootics and can be detected in yearling and adult salmon exhibiting no clinical signs. In adult salmonids infected with IHNV, the highest prevalence of infection may be found in the latest spawning fish and in post-spawned fish. Adult fish from late in the spawning run and gill tissue from older fish should be examined to optimize chances for IHNV detection. IHNV was recently detected in external mucus from naturally and artificially infected juvenile and adult salmonids; mucus may prove to be a useful test sample (LaPatra et al. 1989b).

G. Procedures for Determining Prior Exposure to the Etiological Agent

Hattenberger-Baudouy et al. (1989) found complement dependent neutralizing antibodies in juvenile and adult rainbow trout and suggested that this could be used as a tool for fish health monitoring. Techniques that have been used to detect antibodies to IHNV in fish serum include: neutralization (Hattenberger-Baudouy et al. 1989; Jorgensen, et al. 1991; LaPatra et al. 1993), fluorescent antibody, and ELISA (Jorgensen et al. 1991). In the future, validation of serological techniques for the surveillance and diagnosis for fish virus infections could make fish serology a more acceptable diagnostic technique (LaPatra 1996).

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

See Section 1, 2.1 General Procedures for Virology.

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