2.2.5 Infectious Pancreatic Necrosis

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A. Name of Disease and Etiological Agent

The family Birnaviridae is presently composed of three type genera, *Aquabirnavirus* (aquatic and marine vertebrate and invertebrate hosts), *Avibirnavirus* (bird hosts), and *Entomobirnavirus* (insect hosts). The descriptive term "birna" highlights the bisegmented, double-stranded RNA genome that is the distinctive characteristic of this family of viruses. The birnavirion presents as a non-enveloped, single-layer capsid having icosahedral morphology and measuring about 60 nm in diameter. The complete, infectious virion has a buoyant density of 1.33 gm/cm^3 in CsCl, is stable at pH 3-9, and is resistant to heating at 60C for 1 hr. The major viral structural proteins are VP1 (minor internal protein; RNA-dependent RNA polymerase), VP2 (outer capsid protein; antigen for type-specific character and stimulator of infectivity-neutralizing antibody), and VP3 (internal protein; antigen for group-specific character and stimulator of non-neutralizing antibody) (Delmas et al. 2005).

The type species for the genus *Aquabirnavirus* is infectious pancreatic necrosis virus (IPNV). While IPNV infections were originally associated solely with salmonid fish, IPNV and IPNV-like viruses have been isolated from a variety of nonsalmonid fish and invertebrates from freshwater, estuarine, and marine environments (Wolf 1988, McAllister 1993, Reno 1999). The term "infectious pancreatic necrosis virus" is often used to specifically describe those birnavirus isolates that originate from or cause disease in salmonid fish, while the terms "IPNV-like," "aquatic birnavirus," and "marine birnavirus" are often used to encompass those *Aquabirnavirus* isolates recovered from other fishes and invertebrates and to characterize their respective environments.

The rich immunological and genomic character of the genus *Aquabirnavirus* correlates with the diversity of host species from which the virus has been isolated. Infectivity neutralization assays using polyclonal antibody led to cataloging of isolates into a format where serogroup A contains nine serotypes and serogroup B contains one serotype (Hill and Way 1995). Findings from monoclonal antibody-based epitope characterization (isolate relationships based on reactivity to a panel of monoclonal antibodies) and from genomic characterization (isolate relationships based on gene sequence of conserved and variable regions that code for viral structural proteins) show parallels to the cataloging of isolates based on infectivity neutralization assays (Caswell-Reno et al.1989, Blake et al. 2001).

B. Known Geographical Range and Host Species of the Disease

1. Geographical Range

The genus *Aquabirnavirus* is among the more geographically dispersed groups of viruses. Table 1 provides a list of locations where IPNV isolations have occurred involving cultured and feral species. Information about the distribution of IPNV in feral fishes in the United States can be obtained from the database of the U.S. Fish and Wildlife Service, National Wild Fish Survey (http://www.fws.gov/wildfishsurvey/). For a global perspective, a search on the worldwide web using a descriptor "Aquabirnavirus" or "infectious pancreatic necrosis virus" coupled with the geographical area of concern can provide citations of the worldwide geographical range of the genus.

Table 1. Locations where IPNV isolations have occurred involving natural infections in cultured or feral species.

Continent	**Country**
North America	Canada
	Mexico
	United States
South America	Chile
Europe	Belgium
	Areas of the former Czechoslovakia
	Denmark
	Finland
	France
	Germany
	Italy
	Luxembourg
	Norway
	Republic of Ireland
	Russia
	Spain
	Sweden
	United Kingdom
	Areas of the former Yugoslavia
Asia	Cambodia
	Japan
	Laos
	People's Republic of China
	South Korea
	Taiwan
	Thailand
Oceania	Australia
	New Zealand
Africa	South Africa

2. Host Species
The earliest isolations of IPNV were made from brook trout *Salvelinus fontinalis*, rainbow trout *Oncorhynchus mykiss*, and brown trout *Salmo trutta*; however, IPNV and IPN-like birnaviruses have been isolated from a variety of nonsalmonid fishes and invertebrates from freshwater, estuarine, and marine environments. Table 2 provides a list of families of organisms from which IPNV has been isolated as a naturally occurring infection. An appreciation of the increasing number of host species susceptible to Aquabirnaviruses (species found to be naturally infected or experimentally susceptible) can be accessed by a search on the worldwide web using a descriptor "Aquabirnavirus" or "infectious pancreatic necrosis virus." Information about the distribution of IPNV in wild fishes in the United States can be obtained from the database of the U.S. Fish and Wildlife Service, National Wild Fish Survey (http://www.fws.gov/wildfishsurvey/). The recovery of IPNV under natural and experimentally-induced conditions suggests that avian, mammalian, and invertebrate species might serve as passive mechanical vectors for horizontal transmission of IPNV to fishes.

C. Epidemiology

The clinical presentations in hosts from which Aquabirnaviruses have been isolated are varied. In salmonids, infection with IPNV can occur at any life stage beginning soon after hatching. Clinical signs of infection can occur from about swim up stage through about 5 months of age. Clinical epidemics occur in salmonid fish less than 6 months of age. Disease outbreaks are unusual in older fish, but if they occur, they generally involve virus carriers experiencing a precipitating environmental stressor, such as temperature shift, oxygen depletion, or handling, e.g. spawning practices and transport. Epidemic events can result in cumulative mortality approaching 100% in young fish. The apparent virulence of *Aquabirnavirus* isolates, as judged by virus-induced disease or mortality, varies between and within virus serotype, epitope type, and genetic groupings. Further, the apparent virulence paradigm of an individual isolate can be affected by undefined, intrinsic host-related factors such that an isolate may present a level of virulence in one species and a different level of virulence in another, even closely related, species of fish. Such variations in apparent virulence are evident in isolates of salmonid fish origin. In fish with acute IPNV infection, mortality develops rapidly within a temperature range of 10 to 14C, can be more protracted at temperatures less than 10C, and can develop more slowly and be limited in duration at temperatures greater than 14C (Dorson and Torchy 1981). Salmonid fish of any age, whether cultured or wild, can experience subclinical or inapparent infection with negligible mortality. Antibody response to infection is variable, and environmental conditions and stress factors can affect host immune response to infection. A variety of studies are in progress to develop subunit vaccines for immunization against IPNV infection.

Survivors of exposure to IPNV may become virus carriers, shedding virus for an extended period via feces, urine, and sex products and serving as reservoirs for infection of contemporary and subsequent generations. Horizontal transmission is the mechanism for virus dissemination. Although the virus can be isolated from sex products, IPNV has not been demonstrated to reside within the egg or the sperm. Thus, sex product transmission of IPNV represents a form of horizontal transmission rather than true vertical transmission.

Table 2. Families from which IPNV has been isolated as a naturally occurring infection.

	Family name	**Common name**
Fishes	Acipenseridae	Sturgeons
	Amiidae	Bowfins
	Anguillidae	Eels
	Atherinidae	Silversides
	Bothidaw	Lefteye Flounders
	Carangidae	Jacks
	Catostomidae	Suckers
	Centrarchidae	Sunfishes
	Cichlidae	Cichlids
	Channidae	Snakeheads
	Clupeidae	Herrings
	Cobitidae	Loaches
	Coregonidae	Whitefishes
	Cottidae	Sculpins
	Cyprinidae	Carps and Minnows
	Cyprinodontidae	Killifishes
	Embiotocidae	Surfperches
	Esocidae	Pikes
	Gadidae	Cods
	Gasterosteidae	Stickelbacks
	Gobiidae	Gobies
	Hiodontidae	Mooneyes
	Ictaluridae	Bullhead Catfish
	Lepisosteidae	Gars
	Moronidae	Temperate Basses
	Paralichthyidae	Large-toothed Flounders
	Percichthyidae	Temperate Perches
	Percidae	Perches
	Petromyzontidae	Lampreys
	Pleuronectidae	Righteye Flounders
	Poeciliidae	Livebearers
	Polyodontidae	Paddlefishes
	Salmonidae	Trouts
	Sciaenidae	Drums
	Soleidae	Soles
	Thymallidae	Graylings
	Triglidae	Grunards
	Umbridae	Mudminnows

Table 2 continued. Families from which IPNV has been isolated as a naturally occurring infection.

	Family name	**Common name**
Molluscs	Acmaeidae	Limpets
	Littorinidae	Periwinkle
	Mytilidae	Mussels
	Ostreidae	Oysters
	Pectinidae	Scallops
	Tellinidae	Tellina
	Veneridae	Clams
Crustaceans	Astacidae	European Crayfish
	Daphniidae	Daphnia
	Palaemonidae	Shrimps
	Penaeidae	Prawns
	Portunidae	Crabs
Pseudocoelomates	Branchionidae	Rotifers

Experimental studies show that distribution of IPNV within a susceptible population shows aspects of both propagative and point-source infection models (Bebak et al. 1998, Smith et al. 2000; Bebak-Williams et al. 2002), which may be more indicative of a continuous source infection model. Infection is influenced in the broad sense by the environmental concentration of virus and by host population density and in a more restrictive sense by the character of the virus, by the species, age, and nutritional status of the host, and by ambient environmental factors (McAllister and Owens 1986). The general scenario for IPNV infection in salmonid fish is for fish to become infected by contact with virus laden water. Fish become infectious and begin shedding virus after about 2 days latency and continue shedding virus for about the next 10 days. Those fish that are infectious, but do not develop clinical signs, are candidates to be virus carriers; whereas, those fish that are infectious and develop clinical signs are candidates for death due to IPN. Shedding of virus augments the virus concentration of the ambient environment. An increasing concentration of environmental virus exacerbates the probability for horizontal transmission on a population-wide basis and the probability for subsequent disease and mortality.

D. Clinical Signs

The disease description “infectious pancreatic necrosis” was derived from histopathologic findings in acutely affected young, salmonid fishes. However, animals infected with the IPNV-like aquatic and marine birnaviruses can present with varied clinical signs and pathology. Some isolates of IPN and IPN-like viruses cause acute and chronic disease; whereas, other isolations have been made from ostensibly healthy specimens. Some designations given to Aquabirnavirus infections and diseases in nonsalmonid species are branchionephritis in eels (*Anguilla japonica),* spinning disease in menhaden (*Brevoortia tyrannus*), viral ascites in yellowtail (*Seriola quinqueradiata*), Kumura shrimp disease (*Penaeus japonicus*), and clam gill necrosis (*Meretrix lusoria*) (Reno 1999).

IPNV infection in salmonid fishes can cause a variety of clinical signs and pathologic changes, but none of the presentations truly distinguishes IPN from other fish viral diseases. In addition, some IPNV-infected salmonids develop clinical signs while others appear clinically normal. External clinical signs of IPN can include darkened color (hyperpigmentation), unilateral or bilateral swelling of the eyes (exophthalmia), abdominal (coelomic) distention, presence of a mucoid pseudo-cast extruding from the vent, and hemorrhages at the base and in the fins and on the body surface. Fish often swim erratically, rotating about the long axis or whirling violently. Internal signs can include liver and spleen that are pale compared to normal color and a stomach and intestine that are void of food, but filled with mucoid material that may extrude from the vent. Hemorrhages can occur in the visceral organs.

Marked pathological changes can accompany IPNV infection (Yasutake 1970). Pancreatic tissue can undergo severe necrosis characterized by condensation of chromatin (pyknosis), fragmentation of the nucleus (karyorrhexis), and intracytoplasmic inclusions. The pylorus, pyloric caeca, and anterior intestine can undergo extensive necrosis. Sloughing intestinal epithelium combines with mucus to form a heavy, whitish-colored exudate (catarrah) that may extrude from the vent. Degenerative changes can occur in renal hematopoietic and excretory tissues, liver tissue, and splenic tissue, and demyelinating lesions with inflammation and hemorrhage can occur in the brain. Pancreatic and hepatic tissues can be infiltrated by macrophages and polymorphonuclear leukocytes.

E. Disease Diagnostic Procedures

1. Presumptive Diagnosis

Because clinical signs and pathological changes associated with IPN are variable, they cannot be used for presumptive or definitive diagnosis or to reliably distinguish IPN from other fish viral diseases. In an intensive culture setting, history of the fish populations, the culture station history, clinical signs, and gross necropsy are used to prepare a list of potential diagnoses. The absence of clinical signs does not indicate that the fish are free of IPNV. Virological examination coupled with serological or molecular identification is required for confirmation of clinical or subclinical infection with IPNV.

A variety of cell culture-, immunologic-, and molecular-based assay protocols have been described for detection of IPNV infection. Similarly, a variety of clinical materials have been used, some require sacrifice of fish (internal organs) and others are obtained by non-lethal sampling methods (reproductive fluids, blood fractions, feces, mucus, and external tissues). Although many assay formats and clinical samples have been described, few have undergone rigorous validation by comparison to cell culture assay of internal organ samples. Cell cultures of blue gill fry (BF-2), Chinook salmon embryo (CHSE-214), or rainbow trout gonad (RTG-2) are commonly used for evaluation of clinical materials for detection of IPNV in salmonid fishes. Assays of coelomic (ovarian) fluid for IPNV can provide a level of detection comparable to tissue samples if pellets obtained from centrifugation of coelomic (ovarian) fluid samples are sonicated (10 seconds at 75-100W). However, sonication should not be considered if those same clinical samples are to be used for detection of any of the enveloped fish viruses, e.g. IHNV and VHSV (McAllister et al.1987).

The USFWS/AFS specified format of virological examination for detection of IPNV consists of the assay in cell culture of life stage-appropriate clinical samples of whole fry, internal organs, or reproductive fluids.

For USFWS/AFS specified clinical samples, see Section 2, 2.2, Table 2.1, "Sampling, Virology." (USFWS and AFS-FHS, 2010).

For USFWS/AFS specified sample processing procedures, see Section 2, 4.4, "Virology, Sample Processing Procedures." (USFWS and AFS-FHS, 2010).

For USFWS/AFS specified virus screening procedures, see Section 2, 4.5, "Virology, Screening Method for Viral Isolation." (USFWS and AFS-FHS, 2010).

For USFWS/AFS specified procedures for identification of IPNV, see Section 2, 4.6.B, "Identification of Viruses: infectious pancreatic necrosis virus." (USFWS and AFS-FHS, 2010).

2. Confirmatory Diagnosis

Confirmatory identification of IPNV can be accomplished using immunologic-based or molecular-based assay formats (Dopazo and Barja 2002).

Immunological-based assays include: infectivity neutralization, immunoblot, enzyme-linked immunosorbent assay (ELISA), fluorescent antibody, and immunohistochemical staining. Because several serotypes of IPN virus can be distinguished by infectivity neutralization, use of either an antiserum to each serotype or a polyvalent antiserum preparation is necessary for a competent infectivity neutralization assay. Similarly, even though significant cross-reactivity is apparent using ELISA and other non-infectivity based assay formats, antiserum to each serotype or a polyvalent antiserum is needed for a competent assay.

For USFWS/AFS specified procedures for identification of IPNV by immunologic-based assay, see Section 2, 4.6.B, "Identification of Viruses: infectious pancreatic necrosis virus." (USFWS and AFS-FHS, 2010).

Because of limitations associated with immunological-based confirmatory assays, reverse transcriptase-polymerase chain reaction (RT-PCR) assay is often used to confirm isolations of IPNV in cell culture. More extensive genomic characterization can be applied to develop refined taxonomic and epidemiologic associations.

For USFWS/AFS specified procedures for identification of IPNV by reverse transcriptase polymerase chain reaction (RT-PCR) assay, see Section 2, 4.6.B, "Identification of Viruses: infectious pancreatic necrosis virus." (USFWS and AFS-FHS, 2010).

For USFWS/AFS specified procedures for formulation of reverse transcriptase polymerase chain reaction (RT-PCR) assay, see Section 2, 4.A1.B.2, "Worksheet B.2 – Infectious Pancreatic Necrosis Virus (IPNV)." (USFWS and AFS-FHS, 2010).

F. Procedures for Detecting Subclinical Infections

Subclinical infections can be detected by cell culture assay as described in Section E. "Disease Diagnostic Procedures, Presumptive Diagnosis."

To limit the numbers of fish sacrificed, surveillance and monitoring programs are increasingly using non-lethal sample protocols for collection of clinical materials. However, because these protocols have not been validated by comparative study, the absence of virus detection should be regarded as a provisional observation.

G. Procedures for Determining Prior Exposure to the Etiological Agent

Definitive procedures have not been developed for determining prior exposure to IPNV other than assay for subclinical infection. Export and import facilities should maintain complete records of fish movement and inspection history. Monitoring for IPNV-specific antibody has limited utility because immune response to the virus can be varied and transient. In addition, some IPNV's react with components of normal salmonid serum. Such reactivity can confound assay interpretation (Larsen et al. 2004, Park et al. 2004).

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

See Section 2, 2.2, "Sampling." (USFWS and AFS-FHS, 2010).

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