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INFECTIOUS PANCREATIC NECROSIS (IPN) OF SALMONID FISHES

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INTRODUCTION

Infectious pancreatic necrosis (IPN) is a viral disease principally associated with salmonids, although IPN and IPN-like viruses have been isolated from various nonsalmonid fish and marine invertebrates. Acute infection occurs in 1- to 4-month-old salmonids and can result in cumulative mortality approaching 100%. In contrast, fish 6 months old or older can undergo subclinical or inapparent infection, but experience no significant mortality. Survivors of the disease can become lifelong carriers of the virus. Epizootics of IPN have been reported worldwide, and several virus serotypes are recognized.

The disease known as IPN was probably first described by M'Gonigle (1941) as acute catarrhal enteritis and attributed by him to nutritional factors. The viral etiology of IPN was established by Wolf and co-workers (1960). Comprehensive reviews of the disease and the virus have been made by Dorson (1982), McAllister (1979), Munro and Duncan (1977), Pilcher and Fryer (1980), Roberts (1978), and Wolf (1972, 1976).

ETIOLOGY

The biochemical, biophysical, and biological properties of IPN virus have been intensively investigated (McAllister 1979; Pilcher and Fryer 1980; Wolf 1976). The IPN virion is an unenveloped icosahedral particle measuring about 60 nm in diameter and containing three or four structural proteins and two segments of double-stranded RNA. These characteristics suggest that IPN and IPN-like viruses represent a new taxonomic group of RNA viruses--for which the designation birnaviruses has been proposed.

Infectivity associated with whole fish, tissue homogenates, and cell culture medium can be preserved for years by freezing at -20°C or lower, storage at 4°C in 50% glycerol, or by lyophilization. Some IPN virus isolates, however, are sensitive to freezing and thawing unless the pH of the preparation is slightly acidic. The virus is not affected by exposure to ether, chloroform, or glycerol, but is rapidly inactivated by chlorine, iodophors, or ultraviolet irradiation. Infectivity is progressively inactivated by exposure to heat (56-60°C), ozone, formalin, drying, and extremes of pH (pH 2.0 or 12.0).

The serological relations among the IPN and IPN-like virus isolates are complex and multiple cross-reacting serotypes occur among isolates from salmonid and nonsalmonid fishes and marine invertebrates. The basic salmonid serotypes are the North American VR-299 and the European Ab and Sp--also identified as serotypes 1, 2, and 3, respectively (Macdonald and Gower 1981). Infectious pancreatic necrosis and IPN-like virus isolates from nonsalmonid fish and from marine mollusks and crustaceans

are categorized in the Ab, Sp, and He serotypes and sero group 2 (Dorson 1982). Within each of the serotypes there are a variety of cross-reacting isolates. Polyvalent antisera available for diagnostic use may be a composite of antisera to as many as seven different virus isolates. The IPN and IPN-like viruses are exceptionally good antigens, and antisera have been prepared in rabbits, mice, guinea pigs, and fish. Most immunization schedules specify the use of a single intramuscular injection of purified virus in Freund's complete adjuvant, followed at 2-week intervals by multiple intravenous injections of purified virus.

The virus replicates to a maximum titer of 10^8-10^9 PFU/mL in a wide range of cell cultures. Replication occurs rapidly at 10-26°C, slowly at 4°C, and not at all at 30°C, and is sensitive to interferon produced in fish cell culture. High multiplicity of infection promotes production of autointerfering virus, which has been implicated for the persistence of virus in the in vivo and in vitro carrier states.

The genetics of IPN virus has been superficially investigated. Variant or mutant strains have been implicated in alterations in host range, virulence, plaque size, and sensitivity to neutralization by normal trout serum.

CLINICAL SIGNS AND HISTOPATHOLOGY

A variety of external and internal clinical manifestations can become evident during the course of the disease, but none distinguish IPN from other salmonid virus diseases (Snieszko et al. 1959; Wolf 1966; Yasutake 1970). Affected fry and fingerlings are darkly pigmented, exhibit exophthalmia and abdominal distension, and trail mucoid pseudocasts from the vent. Although infrequent, petechial hemorrhages can occur at the bases of the pectoral and pelvic fins. The behavior of infected fish varies from quiescence with weak respiration to periods of sporadic hyperactivity during which fish swim in a corkscrew manner, rotating about their long axis or whirling violently.

Internally, the liver and spleen are pale, and the stomach and intestine are devoid of food but filled with mucoid material. Petechial hemorrhages are evident throughout the pyloric ceca and pancreatic tissues, and hematocrit values are depressed. During an acute epizootic, severe mortality--as great as 100%--can occur in fish 1 to 4 months old. Growth may be retarded among survivors.

Marked pathologic changes occur in the pancreatic, renal, and hepatic tissues, but none are distinctive for IPN (McKnight and Roberts 1976; Yasutake 1970). Pancreatic acinar cells, and occasionally islet tissue, undergo severe necrosis characterized by pyknosis, karyorrhexis, and intracytoplasmic inclusions. The pylorus, pyloric ceca, and anterior intestine show extensive necrosis, and sloughing intestinal epithelium

combines with mucus to form a whitish catarrhal exudate. Degenerative changes are sometimes evident in renal hematopoietic and excretory tissues and in liver tissue, and demyelinating lesions with inflammation and hemorrhage can occur in the brain. In addition, pancreatic and hepatic tissues are infiltrated by macrophages and polymorphonuclear leucocytes. Electron microscopy reveals virus particles in pancreatic, hepatic, renal, and splenic tissue.

DIAGNOSIS

The standard method for diagnosis consists of isolation of the virus in cell culture and serological identification by specific immune serum (Kelly et al. 1978; McDaniel 1979; Stevenson 1977; Wolf and Quimby 1971, 1973). A variety of fish cell lines and experimental conditions have been used to isolate the virus. The cell lines BF-2, CHSE-214, and RTG-2, and incubation temperatures of 15-20°C, are used most often. The FHM cell line is not well suited for primary isolation of IPN virus because the sensitivity of FHM cells to different serotypes of the virus varies (Jørgensen 1971). Cytopathology is generally evident in 48 h. Prolonged incubation and blind passage might be required if the initial virus titer is low. In addition, autointerference can affect virus detection and isolation at low dilutions (Nicholson and Dexter 1975). Virus can be recovered from selected internal organs more frequently than from feces, sex products, or peritoneal washings. Although the success of virus isolation varies, the posterior kidney tissues yield virus more consistently than do other tissues (Frantsi and Savan 1971*a*; Yamamoto 1974). The probability of recovering virus is increased if fish are physiologically stressed. There is some evidence that in older carriers the virus can be isolated only from the brain. The virus has been recovered from fish concurrently suffering from bacterial kidney disease, bacterial gill disease, or *Pseudomonas fluorescens* infection, or from infectious hematopoietic necrosis (IHN) or viral hemorrhagic septicemia (VHS) virus infection.

Confirmed identification of IPN virus requires serological reactivity in a neutralization, fluorescent antibody, immunoperoxidase, complement fixation, or enzyme-linked immunosorbent assay. Rapid, sensitive serological techniques used in human and veterinary viral diagnostics will undoubtedly soon be applied to fish viral diagnostics.

SOURCE AND RESERVOIR OF INFECTION

The reservoir of infection is carrier fish, which shed virus and infect their contemporaries or subsequent generations. The carrier incidence varies, but can exceed 90% of the survivors of an epizootic. There is no indication of sex-related differences in carrier incidence. The carrier state can be maintained for many years in both hatchery and

natural environments, but some surveys of carrier populations suggest that the incidence decreases with time (Yamamoto 1975*a*, 1975*b*; Yamamoto and Kilistoff 1979). The host immune response seems to affect the carrier state because lower levels of virus occur in fish with circulating virus-neutralizing antibody. Variations in the carrier state have been observed with different species of fish. Rainbow trout (*Salmo gairdneri*) appear to have a lower carrier incidence and a carrier state of shorter duration than do brook trout (*Salvelinus fontinalis*). The low incidence of virus isolation from the progeny of wild carrier fish and from virus-free fish introduced into wild carrier populations suggests that carrier-mediated transmission may not be as significant in nature as in the hatchery. Regular IPN epizootics occur in hatcheries that obtain eggs from carrier brood stock, or use surface water supplies inhabited by carrier fish.

TRANSMISSION

Horizontal transmission of IPN virus occurs as a consequence of virus shed with feces and urine (Billi and Wolf 1969; Wolf et al. 1968). Under epizootic conditions hatchery water may contain 10^5 or more $TCID_{50}$ of virus per milliliter. The virus probably gains access to the host by contact with gills, ingestion with food, or passage through sensory pores of the lateral line system. In 1- to 4-month-old salmonids, patent infection occurs with overt clinical signs and generally a high level of mortality. The virus can be transmitted to fish 6 months old or older, but the infection is generally inapparent and seldom results in mortality. Epizootics with high levels of mortality have recurred in older fish after sudden stress (Roberts and McKnight 1976).

Egg-associated transmission occurs as a consequence of carrier fish shedding virus at spawning (Wolf et al. 1963). The virus has been isolated from iodophor treated eggs and from eyed embryos, suggesting that the virus is inaccessible to routinely used chemical disinfectants (Bullock et al. 1976; Fijan and Giorgetti 1978).

Experimentally, IPN virus has been transmitted by injection, immersion, hyperosmotic infiltration, and feeding. The observed virulence varies with the method of exposure and the species or strain of fish, and also with the virus serotype and virus repeatedly passaged in cell culture. Homeotherms, poikilotherms, and fomites can serve as mechanical vectors. Infectious IPN virus can be recovered from the feces of inoculated chicken, owl, gull, and mink, and infectivity persisted in two species of protozoans fed virus-infected cell cultures. In addition, the virus has been recovered from several species of naturally or experimentally exposed nonsalmonid fish. Of particular significance is the dissemination of virus by improper disinfection of nets, tanks, equipment, and apparel.

INCUBATION PERIOD

The time required for mortality to occur from infection with IPN virus varies with the concentration of virus, the method of exposure, the temperature, and the age, species, and physiological condition of fish (Dorson and Torchy 1981; Frantsi and Savan 1971*b*). Fish exposed by immersion die in 6 to 20 days and fish exposed by injection die in 3 to 10 days. Incubation periods are short at water temperatures of 10-16°C and high concentrations of virus (10^4-10^5 PFU/mL of water for immersion; 10^5-10^6 PFU for intraperitoneal injection). Incubation periods lengthen at colder water temperatures (4-6°C) or lower concentrations of virus. Mortality due to IPN is greatest at 10-13°C and is relatively low at 4-6°C and at 16°C and above. The duration of an epizootic varies with temperature. Cumulative mortality peaks in 3 to 6 weeks at 10-16°C, but an epizootic may persist for 3 to 4 months at 4-6°C. Stressful rearing conditions enhance the probability of disease outbreaks and exacerbate their occurrence.

PERIOD OF COMMUNICABILITY

The stage of life at which fish become susceptible to IPN is poorly defined. Virus has been isolated from eyed embryos, and yolk sac fry are reportedly susceptible to experimental infection. Epizootics generally occur in feeding fish, sometimes beginning when the fish are only 3 to 4 weeks old. The infection is communicable, with consequent mortality, until the fish are 5 to 6 months old (Dorson and Torchy 1981; Frantsi and Savan 1971*b*). If older fish become infected, they generally suffer inapparent infection and have no significant mortality.

SUSCEPTIBILITY AND RESISTANCE

Infectious pancreatic necrosis virus has been recovered from a wide range of salmonid and nonsalmonid fish. Some isolations were made during natural epizootics or following experimental inoculation while others were made from healthy appearing fish (Adair and Ferguson 1981; Ahne 1978; Castric and Chastel 1980; Dorson 1982; McAllister et al. 1982; Munro and Duncan 1977; Sano et al. 1981; Seeley et al. 1977; Stephens et al. 1980). The following species are susceptible to infection:

Salmonids	
brown trout	*Salmo trutta*
cutthroat trout	*S. clarki*
rainbow trout	*S. gairdneri*
Atlantic salmon	*S. salar*
brook trout	*Salvelinus fontinalis*
lake trout	*S. namaycush*
arctic char	*S. alpinus*

amago trout	*Oncorhynchus rhodurus*
chinook salmon	*O. tshawytscha*
coho salmon	*O. kisutch*
pink salmon	*O. gorbuscha*
sockeye salmon	*O. nerka*
yamame salmon	*O. masou*
huchen	*Hucho hucho*
grayling	*Thymallus thymallus*
Nonsalmonids	
Atlantic menhaden	*Brevoortia tyrannus*
Atlantic shad	*Alosa sapidissima*
barbel	*Barbus barbus*
bream	*Abramis brama*
common carp	*Cyprinus carpio*
discus fish	*Symphysodon discus*
European eel	*Anguilla anguilla*
goldfish	*Carassius auratus*
Japanese eel	*Anguilla japonica*
northern pike	*Esox lucius*
roach	*Rutilus rutilus*
southern flounder	*Paralichthys lethostigma*
walleye	*Stizostedion vitreum vitreum*
zebra danio	*Brachydanio rerio*

Isolation of IPN virus from white sucker (*Catostomus commersoni*), lamprey (*Lampetra fluviatilus*), minnow (*Phoxinus phoxinus*), and perch (*Perca fluviatilis*) seems to be the result of fish ingesting virus with infected waste from nearby salmonid hatcheries, rather than overt virus infection (Bucke et al. 1979; Munro et al. 1976). Viruses resembling that of IPN have been isolated from several species of marine mollusks and crustaceans (Underwood et al. 1977).

Stress-mediated outbreaks and recurrence of IPN epizootics have been reported. Resistance to infection increases with age and might correlate with developing immunocompetence. Virus-neutralizing antibody has been demonstrated in naturally infected fish 3.5 months old.

The carrier state can be affected by the host immune response because lower levels of virus occur in fish with circulating virus-neutralizing antibody.

RANGE

Infectious pancreatic necrosis occurs in Canada and the United States, Japan, Chile, and most of eastern and western Europe. Essentially, IPN virus is attaining worldwide distribution.

CONTROL

Infectious pancreatic necrosis is most effectively controlled by preventing contact between host and virus; there is no treatment for the virus infection. If the rearing facility is contaminated, ponds, raceways, and hatchery implements can be disinfected by treatment with chlorine (200 mg/L for 1 h). The treatment should be monitored by periodic chlorine quantitation because the available chlorine concentration is affected by the amount of organic matter present and by pH (Desautels and MacKelvie 1975; Elliott and Amend 1978). The water supply should be protected and controlled to the maximum extent possible by using enclosed spring and well water supplies. Unfortunately, open streams are often used and thus hatchery populations can be exposed to any infectious disease carried by resident fish. Laboratory studies indicate that ozone and ultraviolet irradiation have potential application for decontamination of large volumes of water (MacKelvie and Desautels 1975; Wedemeyer et al. 1979). Not only should the rearing facility and its water supply be free of virus, but the fish introduced should be certified as specific-pathogen-free. Eggs should originate from certified brood stock because IPN virus appears to be transmitted with eggs in spite of iodophor treatment (Wolf et al. 1968).

Effective vaccines are only in the developmental stages. At present, inactivated IPN virus vaccines elicit a protective response when administered by injection but not by immersion, hyperosmotic infiltration, or feeding. Efforts to develop an attenuated IPN virus vaccine and to identify potential avirulent vaccine strains of IPN virus from nonsalmonid fish continue.

There is no evidence of maternally transferred immunity, but susceptible fry can be protected by passive transfer of antibody or interferon. Substantive data indicate that resistance to IPN virus infection is inheritable and can be enhanced by selective breeding.

There is evidence suggesting that IPN virus mortality can be moderated by rearing fish at 4-6°C or at 16°C or above, but confirming data are needed. The incidence of acute IPN can be reduced if factors that promote physiological stress and hence lower resistance to disease are moderated. The sudden onset of stressful conditions has caused the recurrence of an epizootic in older fish.

There are no effective chemotherapeutics for treatment of IPN. The antiviral agent virazole, a synthetic nucleoside, inhibited IPN virus replication in cell culture and somewhat reduced the mortality in rainbow trout fry infected with IPN virus (Savan and Dobos 1980). However, because virazole is a reversible inhibitor, effectiveness decreases with time and thus repeated treatment is necessary. A cost analysis showed antiviral therapy to be economically prohibitive. The interferon inducer

tilorone was tested but no protection was observed. Chemotherapy with polyvinlypyrrolidone-iodine, ε-aminocaproic acid, tranexamic acid, and benzydamine appear to reduce IPN virus mortality, but there has been no virological evaluation of these compounds or assessments of mode of action or field applicability (Economon 1973; McAllister 1981).

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