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Viral Hemorrhagic Septicemia of Fishes

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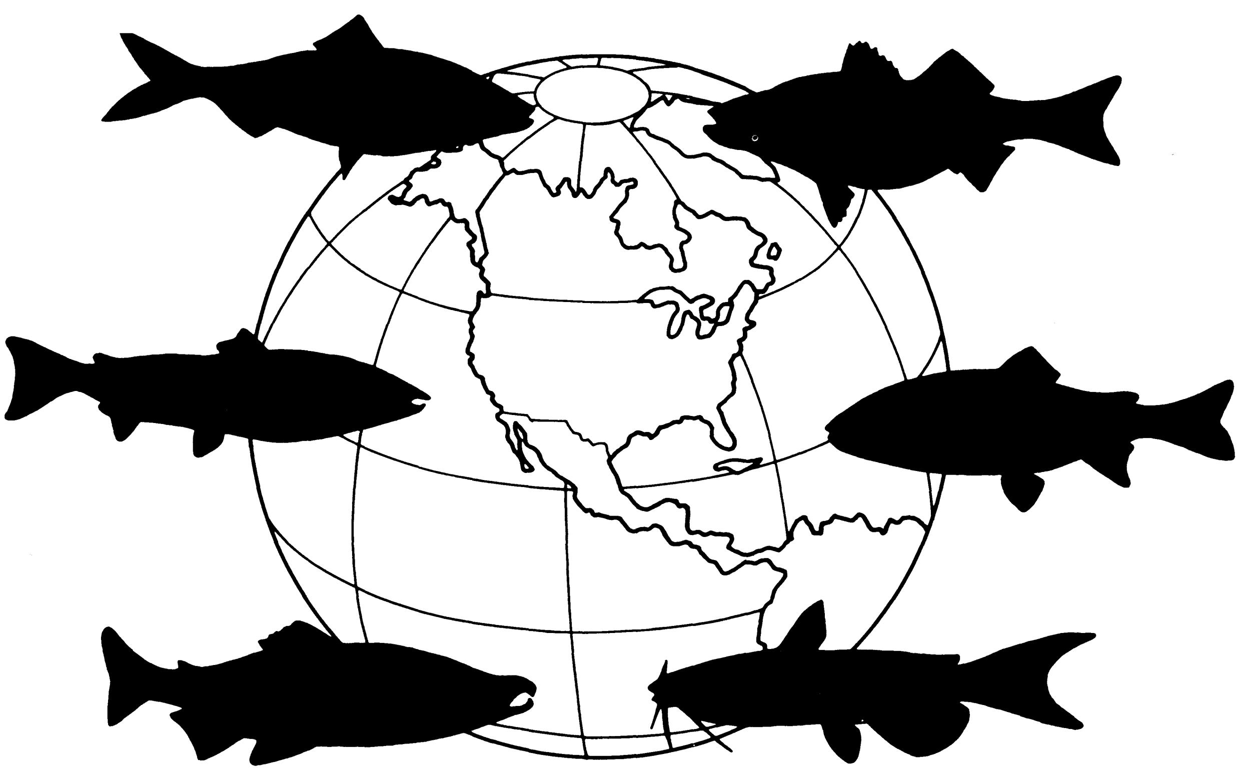

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Introduction

Viral hemorrhagic septicemia (VHS), a systemic infection of various salmonid and a few nonsalmonid fishes, is caused by a rhabdovirus designated as the viral hemorrhagic septicemia virus (Office International des Epizooties 1963). The virus infection occurs in salmonids and certain other fishes of any age and may result in significant cumulative mortality. Fish that survive may become carriers. Viral hemorrhagic septicemia has been reported under various names, of which "Egtved disease" and "Infektiose Nierenschwellung und Leberdegeneration" are the best known. The viral etiology of the disease was established by Jensen (1965), and several serotypes of the virus (also known as "Egtved virus") are currently recognized.

Comprehensive reviews of the disease and the virus have been published by de Kinkelin (1983), McAllister (1979), Pilcher and Fryer (1980), Roberts (1978), and Wolf (1988).

Geographical Distribution

Viral hemorrhagic septicemia is enzootic in most countries of continental Eastern and Western Europe, and the virus has been isolated in the Puget Sound area of Washington in the United States. No outbreaks of VHS or isolations of VHS virus have been reported elsewhere.

Host Susceptibility

In Europe, epizootics of VHS occur primarily in rainbow trout, *Oncorhynchus mykiss*; brown trout, *Salmo trutta*; and to a lesser extent in northern pike, *Esox lucius* (Jorgensen 1980; Meier and Jorgensen 1980). Natural infections have also occurred in grayling, *Thymallus thymallus*, and whitefish *Coregonus* sp. (Wizigmann et al. 1980; Ahne and Thomsen 1985; Meier et al. 1986). Outbreaks of VHS have been suspected in pollan, *Coregonus lavaretus*, and lake trout, *Salvelinus namaycush*. In the United States, natural infections have been diagnosed in chinook salmon, *O. tshawytscha*; coho salmon, *O. kisutch*; and steelhead (sea-run rainbow trout).

Fish shown by experimental challenge to be susceptible to VHS virus infection are Atlantic salmon, *Salmo salar*; brook trout, *Salvelinus fontinalis*; golden trout, *O. aguabonita*; rainbow trout × coho salmon hybrids; giebel, *Carassius auratus gibelio*; sea bass, *Dicentrarchus labrax*; and turbot, *Scophthalmus maximus* (de Kinkelin and Castric 1982; Castric and de Kinkelin 1984; Wolf 1988). Fish shown by experimental challenges to be refractory to VHS virus infection are common carp, *Cyprinus carpio*; chub, *Leuciscus cephalus*; Eurasian perch, *Perca fluviatilis*; roach, *L. rutilus*; and tench, *Tinca tinca*.

Clinical and Histopathologic Signs of Disease

A variety of clinical signs and histopathologic changes may be apparent in fish infected with VHS virus. Some fish show frank clinical manifestations of disease, whereas others look normal. Historically, clinical and pathologic signs of VHS have been catalogued into acute, chronic, and latent forms. Such descriptions represent degrees of severity rather than progressive stages of the disease.

Clinical Signs

The clinical signs of VHS vary with the severity of infection (Yasutake 1970; Wolf 1988). Acute signs are typically accompanied by a rapid onset of heavy mortality. Fish are lethargic, dark in color, exophthalmic, and anemic. Hemorrhages are evident in the eyes, skin, and gills and at the bases of the fins. Internally, punctiform hemorrhages are evident in periocular tissues, skeletal muscle, and viscera; the liver appears mottled and hyperemic and the kidneys are red and thin. In chronically infected fish, significant cumulative mortality occurs, but is protracted. Fish are lethargic, dark in color, exophthalmic, and severely anemic, but not grossly hemorrhagic. The abdomen is markedly distended due to edema of the liver, kidneys, and spleen. The liver appears pale and petechiated, and the kidneys are ashen. In a latent infection, mortality is low, and the fish seem nearly normal, but may be hyperactive. Inapparent virus carriers show no clinical signs of VHS.

Histopathological Changes

Histopathologic changes are generally confined to the liver, kidneys, spleen, and skeletal muscle (Ghittino 1965; Yasutake 1970; Amlacher et al. 1980; Wolf 1988). In acutely affected fish, the liver sinusoids are engorged with blood, and hepatocytes show focal to extensive necrobiotic changes—cytoplasmic vacuoles, pyknosis, karyolysis, lymphocytic invasion, and occasionally intracytoplasmic and intranuclear inclusions. Similar changes occur in the spleen and in the hematopoietic and renal elements of the kidneys. In skeletal muscle, erythrocytes sometimes accumulate in muscle bundles and fibers, but little tissue damage occurs. In chronically infected fish, liver sinusoids remain enlarged and contain coagulated plasma, and kidney and splenic hematopoietic tissues and mononuclear lymphoid cells are hyperplastic. No remarkable histopathologic changes have been reported in inapparent virus carriers.

Etiology

The VHS rhabdovirion is an enveloped, bullet-shaped particle about 180 nm long and 60 nm in diameter. Arrayed over the surface of the virion envelope are peplomers 5 to 15 nm long. The envelope is acquired as the virus matures, by budding from cell surface membranes or into cytoplasmic vacuoles. The intact virion, which sediments to a density of 1.14–1.15 g/cm^3, is composed of five structural proteins and contains one segment of single-stranded RNA (de Kinkelin and Scherrer 1970; McAllister 1979; de Kinkelin 1983).

The virus is moderately stable in cell culture medium and can be preserved for years by freezing at −20° C or lower and by lyophilization. It is inactivated by exposure to ether, chloroform, glycerol, formalin, sodium hypochlorite, iodophors, ultraviolet irradiation, or heat (56–60° C). The virus is stable at pH 5.0–10.4, but is inactivated at pH 3.5 (McAllister 1979; de Kinkelin 1983).

In cell culture, the VHS virus replicates to a titer of 10^7–10^8 plaque-forming units per milliliter. The optimum temperature for virus replication is 14–15° C; virus yield is reduced at 6° C, and little replication occurs above 20° C (de Kinkelin and Scherrer 1970). Optimum virus replication in cell culture requires that the pH of the medium be maintained in the range of pH 7.4–7.8 (Campbell and Wolf 1969). Interferon can be produced in cell cultures infected by VHS virus, and nascent or exogenous interferon reduces virus yield (de Kinkelin and Le Berre 1977).

At least three VHS virus serotypes (designated F1, F2, 23.75) can be distinguished by infectivity neutralization assays (Le Berre et al. 1977; de Kinkelin 1983). Hyperimmune sera for virus identification are generally prepared in rabbits, but virus-neutralizing antibody titers are generally low because VHS virus is a weak immunogen (Ahne 1981; Hill et al. 1981). Hybridoma cell lines have been developed that secrete binding and neutralizing monoclonal antibody specific to VHS virus (Lorenzen et al. 1988; P. de Kinkelin, unpublished data; P. E. McAllister, unpublished data).

Virus Detection and Identification

The VHS virus can be recovered from homogenates of internal organs, sex products, or urine. Concentrations of virus are higher in the anterior kidney and spleen than in liver, heart, or muscle (de Kinkelin 1983). Brain samples should be included when one is assaying for inapparent virus carriers (Castric and de Kinkelin 1980). Little virus can be recovered from feces. Although infected fish can develop an immune response to VHS virus, the detection of anti-VHS antibody is not a reliable indicator of the presence or absence of current infection (Dorson and Torchy 1979; Jorgensen 1982a, b). Nevertheless, the detection of VHS virus-specific neutralizing antibody in fish can be a useful tool for VHS surveillance (Olesen and Vestergard Jorgensen 1986). Mixed virus infections have been reported in which VHS virus was isolated from fish concurrently infected with infectious pancreatic necrosis virus.

The isolation of VHS virus in cell culture is the standard for diagnosis. The virus replicates in a variety of piscine cell lines—for example, BF-2, CaPi, CHSE-214, EPC, FHM, PG, RTG-2, RTM, STE, and sea bass—as well as in primary cultures of tench, carp ovary, and goldfish, *Carassius auratus* (McAllister 1979; de Kinkelin 1983). In addition, VHS virus replicates in cell lines of mammalian (BHK-21 and WI-38) and reptilian (GL1 and TH-1) origin. The optimum temperature for virus replication in cell culture is 14–15° C (de Kinkelin and Scherrer 1970). Replication is greatly influenced by hydrogen ion concentration; a pH of 7.4 to 7.8 must be maintained in diagnostic assays (Campbell and Wolf 1969; Wolf and Quimby 1973). The detection of VHS virus in cell culture can be enhanced by pretreating cells with a 7% solution of polyethylene glycol (molecular weight = 20,000) or by including DEAE-dextran (50 μg/mL) or polyethylene glycol with the sample during virus adsorption (Campbell and Wolf 1969; W. N. Batts and J. R. Winton, unpublished data). Detection of virus in tissue sections by fluorescent antibody staining is less sensitive than virus isolation in cell culture (Jorgensen and Meyling 1972).

A variety of serological techniques can be used to identify VHS virus: infectivity neutralization, fluorescent antibody, immunoperoxidase, complement fixation, immune precipitation, counter-current immunoelectophoresis, and immunoblot and plate enzyme-linked immunosorbent assays (Meier and Vestergard Jorgensen 1975; Ahne 1981; de Kinkelin 1983; McAllister and Schill 1986; Way and Dixon 1988). The three currently recognized serotypes of VHS virus (F1, F2, and 23.75) are distinguished by infectivity neutralization; therefore, three antisera (or a composite polyvalent antiserum) are used for virus identification by infectivity neutralization assay. Viral antigen can be detected in inoculated cell cultures by indirect fluorescent antibody and immunoperoxidase staining (Meier and Jorgensen 1975; Faisal and Ahne

1980), and immunoblot and plate ELISA systems are used for detection and identification of VHS virus in cell culture fluids (McAllister and Schill 1986; Way and Dixon 1988).

Cross-reactivity is seen among the VHS virus serotypes in indirect fluorescent antibody assays and in some instances in infectivity neutralization assays (Jorgensen 1972, 1980; Meier and Jorgensen 1980). Similarly, one can identify all three VHS virus serotypes by immunoblot ELISA, using antiserum solely to VHS serotype F1 (McAllister and Owens 1987).

Epizootiology

Transmission

The VHS virus is readily transmissible to fish of all ages, and survivors of infection can become lifelong carriers that shed virus with urine and sex products. The virus ostensibly gains access to the fish through the secondary gill lamellae. Virus shed with sex products appears to be solely a surface contaminant of the egg and is readily dissipated. Although virus can be isolated from eggs for 3 to 4 h after spawning, true vertical transmission has not been demonstrated. Experimentally, fish can be infected by cohabitation, immersion, intraperitoneal and intramuscular injection, brushing virus on the gills, and feeding (Jorgensen 1980; de Kinkelin and Castric 1982; de Kinkelin 1983; Castric and de Kinkelin 1984).

In Europe, the gray heron, *Ardea cinerea*, is known to be a mechanical vector of VHS virus (Olesen and Jorgensen 1982; Peters and Neukirch 1986), but the virus is inactivated in the gastrointestional tract of birds (Eskildsen and Jorgensen 1973). The virus is apparently not transmitted by parasitic vectors and, as judged from a study with the fruit fly *Drosophila melanogaster*, it does not replicate in insects (Bussereau et al. 1975).

In the hatchery environment, mechanical transfer of VHS virus on the surface of animate or inanimate objects presents a substantial hazard. The virus has been isolated from feral fish in waters receiving hatchery effluent, and can persist in water for several days (de Kinkelin and Scherrer 1970).

Factors Affecting the Disease

Fish of any age are susceptible to infection, although sac fry and fish older than 6 months are sometimes resistant (Ghittino 1965). Epizootic losses occur at temperatures of 3° to 12° C (mortality is greatest at 3° to 5° C); mortality and the proportion of virus carriers decrease at higher temperatures (de Kinkelin 1983). Deaths from VHS rarely occur at temperatures above 15° C.

Infected fish mount a strong interferon response, but may ultimately succumb to infection (Dorson et al. 1975; de Kinkelin 1983). Increased interferon synthesis may play a role in transiently mitigating the effects of VHS at relatively high temperatures (de Kinkelin et al. 1982). Virus-neutralizing antibody has also been demonstrated in infected fish, but the antibody response in survivors of epizootic and inapparent infections varies with the fish and the season of the year (Jorgensen 1971; Dorson and Torchy 1979; Dorson et al. 1979).

In captive fish, culture and environmental stress seemingly increase susceptibility and recurrence of infection (Ghittino 1965; de Kinkelin 1983). In feral fish, inapparent infection is more common than disease (Jorgensen 1982b).

Disease Control

Prevention of contact between the virus and the host is the most effective method for controlling VHS. A systematic program of hatchery disinfection, combined with restocking with specific-pathogen-free fish and eggs, has been used successfully (Kehlet 1973; Jorgensen 1974a, 1980). Eggs used for restocking are decontaminated by iodophor treatment. The water supply should ideally be controlled and virus-free, although ultraviolet irradiation has been used to inactivate VHS virus in the water supply (Maisse et al. 1980). Conditions that promote physiological stress should be alleviated. Although VHS rarely occurs above 15° C, disease control by temperature manipulation has not been described. Selective breeding to increase host resistance to VHS has not been successful.

The potential for vaccinating fish against VHS has been demonstrated, and several avirulent VHS virus vaccines are under development (Jorgensen 1976; de Kinkelin and Le Berre 1977). One vaccine candidate, a VHS virus variant of low pathogenicity (F25), was selected by serial passage in EPC cells at progressively increasing temperature. This F25 variant replicates at 25°C, but the wild-type virus does not (de Kinkelin et al. 1980; de Kinkelin and Bearzotti 1981). A second vaccine candidate, a low-pathogenicity virus strain (Reva), was selected by serial passage in RTG-2 cells (Jorgensen 1982b). Although both the F25 and the Reva strains induced a protective response, they also retained residual pathogenicity. The mechanism of the protective response is unclear. Some mechanism occurring within 48 h after immunization, such as interferon stimulation, might be responsible for early protection, whereas the antibody response occurs later (Jorgensen 1982b; Bernard et al. 1985). Fry can be protected by injection of interferon (de Kinkelin et al. 1982). Although the protective effects of avirulent vaccines have been demonstrated in the laboratory, their efficacy under production conditions has not been proven, and their value for controlling VHS in healthy fish populations has been questioned (Enzmann 1983).

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