Epizootic Hematopoietic Necrosis

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Importance

Epizootic hematopoietic necrosis (EHN) is a systemic iridoviral disease of fish. The epizootic hematopoietic necrosis virus (EHNV) causes EHN in redfin perch and rainbow trout. This disease is highly fatal in redfin perch; affected farms usually have serious economic losses, and severe declines can occur in wild populations. Rainbow trout are less likely to be infected, and the cumulative mortality rate is usually low. Closely related viruses cause serious outbreaks of epizootic hematopoietic necrosis in catfish and sheatfish. Currently, the transmission of EHN viruses is incompletely understood. Their control is also complicated by their prolonged survival in the environment and resistance to disinfectants.

Etiology

Epizootic hematopoietic necrosis is a systemic disease characterized by necrosis of the liver, spleen and hematopoietic tissues within the kidney. This disease is caused by viruses of the epizootic hematopoietic necrosis group in the genus *Ranavirus* and family Iridoviridae. To date, the causative viruses include EHNV in redfin perch and rainbow trout, and European sheatfish iridovirus (ESV) and European catfish iridovirus (ECV) in catfish and sheatfish. Genetic studies suggest that ECV and ESV are isolates of the same virus; EHNV is a different virus. Other iridoviruses causing systemic necrotizing syndromes may also occur in fish.

Species Affected

In nature, EHNV has been reported only in redfin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*). Species that have been infected experimentally include Macquarie perch (*Macquaria australasica*), mosquito fish (*Gambusia affinis*), silver perch (*Bidyanus bidyanus*), mountain galaxias (*Galaxias olidus*), Murray cod (*Maccullochella peelii peelii*), and Atlantic salmon (*Salmo salar*). Other species may also be susceptible. ESV has been reported from sheatfish/ wels catfish (*Silurus glanis*), while ECV can infect European catfish (*Ictalurus melas*), channel catfish (*Ictalurus punctatus*), goldfish (*Carassius auratus*) and short finned eels (*Anguilla australis*).

Systemic necrotizing iridovirus syndromes have also been reported in other species of fish including turbot (*Scophthalmus maximus*) in Denmark.

Geographic Distribution

EHNV is endemic only in Australia. Within Australia, infected rainbow trout have been reported only from fish farms in the Murrumbidgee and Shoalhaven river catchments of New South Wales, while infected redfin perch occur in many locations in southern Australia. Outbreaks of EHN have occasionally been reported in other countries including Kuwait, Pakistan and Peru. ECV/ ESV is endemic in Europe.

Transmission

Understanding of EHN transmission is still incomplete; however, fish can be infected by bath inoculation, and spread through the water is likely. Oral transmission may occur; naturally infected fish have gastrointestinal lesions that are not reported after intraperitoneal inoculation. Infection through the gills or skin has also been proposed. Asymptomatically infected fish have been reported, but it is controversial whether these are true carriers. Vertical (egg-associated) transmission has not yet been seen.

The EHN group of viruses can be transmitted on fomites, and birds may act as mechanical vectors. EHNV can survive in the avian digestive tract for a few hours, and might be transmitted in regurgitated food. It could also be carried on the feathers, feet and bill. EHNV is highly resistant to drying. This virus can remain infective for more than 97 days in the water and for at least 113 days in dried fish tissues. It can also survive for more than 300 days in cell cultures at 4°C (39°F), and for two years in fish tissues stored at –20°C (-4°F).

Incubation Period

The incubation period for experimentally infected rainbow trout is 3-10 days in water temperatures of 19-21°C (66-70°F), and 14-32 days in water temperatures of

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8-10°C (46-50°F). In experimentally infected redfin perch, the incubation period is 10-11 days at 19-21°C, and 10-28 days at 12-18°C (54-64°F).

Clinical Signs

The clinical signs are nonspecific. In perch, sudden death is the most common sign. Darkening of the body surface, ataxia, lethargy and erythema around the nostrils and brain region have also been seen. Hemorrhages may occur in the gills and at the base of the fins.

Symptoms reported in experimentally infected rainbow trout include darkening of the body surface, lethargy, inappetence, abdominal distension and loss of equilibrium. Skin ulcers, flared opercula and reddening at the base of the fins have also been reported in outbreaks; however, these lesions may be due to concurrent infections, suboptimal water quality and other husbandry problems, which are commonly associated with clinical EHN in this species.

Deaths have also been reported in experimentally infected Macquarie perch, silver perch, mosquito fish and mountain galaxias.

Post Mortem Lesions

In redfin perch, there may be swelling of the kidney, spleen and/ or liver, hemorrhages at the base of the fins and focal hemorrhages in the gills. The spleen is often swollen, but it is occasionally pale and shrunken. Petechiae may be found on the viscera. Multiple white to yellow areas of focal necrosis are sometimes found in the liver.

In rainbow trout, the lesions may include abdominal distension with serosanguineous ascitic fluid, and swelling of the spleen or kidney. Petechial hemorrhages have been seen on the viscera in a few fish. Focal necrosis in the liver is rare. The gross lesions may be minimal in this species.

Morbidity and Mortality

EHN epizootics in redfin perch are most common in the spring and summer. In this species, disease has not been reported at water temperatures below 12°C (54°F). In rainbow trout, outbreaks have been reported in water temperatures between 11°C (52°F) and 17°C (63°F), and fish can be infected experimentally between 8°C (46°F) and 21°C (70°F).

Redfin perch are highly susceptible to epizootic hematopoietic necrosis. The morbidity rate is very high in this species, and most infected fish die; however, any survivors appear to be resistant to re-infection. During an epidemic in a naïve population, the mortality rate is high in both adult and juvenile fish. In areas where this virus has become endemic, most infections occur in fingerlings and juveniles.

Rainbow trout are relatively resistant to EHN; although the case fatality rate is high, the morbidity rate in this species is usually low Outbreaks in rainbow trout appear to be related to poor water quality, and are often accompanied by protozoal or fungal skin diseases, systemic bacterial infections and external parasites. Although disease can occur in trout of all ages, it is most common in young fingerlings up to 125 mm fork-length. The daily mortality rate in these fingerlings is less than 0.2%. The cumulative mortality rate is usually 3-4% or less

ESV/ ECV can cause high morbidity and mortality rates in susceptible species. ESV outbreaks have been associated with mortality rates up to 100% in sheatfish.

Diagnosis

Clinical

Epizootic hematopoietic necrosis should be suspected in redfin perch when an epidemic is characterized by sudden high mortality and histological evidence of necrosis in the renal hematopoietic tissue, spleen and liver. During outbreaks in rainbow trout, far fewer fish are usually affected and there may be evidence of poor husbandry. EHN can be difficult to recognize in this species, and may be dismissed as normal losses.

Laboratory tests

Epizootic hematopoietic necrosis can be diagnosed by isolating EHNV or ECV/ ESV in cell cultures; many fish cell lines including CHSE-214 (Chinook salmon embryo), FHM (fathead minnow), EPC (epithelioma papulosum cyprini), and BF-2 (bluegill fry) cells can be used. The identity of the virus can be confirmed by immunostaining, enzyme-linked immunosorbent assay (ELISA), immunoelectron microscopy, polymerase chain reaction (PCR) or other methods. Cross-reactions occur between EHNV, ECV/ ESV and other fish and amphibian ranaviruses when antibody-dependent techniques are used. PCR combined with either restriction endonuclease analysis (REA) or sequence analysis can differentiate these viruses. Real-time PCR assays to distinguish closely related ranaviruses have recently been published.

Viral antigens can also be identified directly in tissues by immunostaining methods or ELISA. Immunoblotting (Western blotting) may also be used. Nucleic acids can be identified by PCR. Electron microscopy or immunoelectron microscopy may also be helpful.

Serology may become effective in screening fish populations, but it has not yet been validated for routine diagnosis. Although an ELISA has been described in redfin perch and rainbow trout, this test has not yet been standardized, and interpretation of the results may be difficult. Viruses in the EHN group do not induce neutralizing antibodies.

Samples to collect

Before collecting or sending any samples from animals with a suspected foreign animal disease, the proper authorities should be contacted. Samples should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease.

The target organs in infected fish include the liver, kidney, spleen and other parenchymal tissues. Whether EHNV or ECV/ ESV can be found in ovarian fluid, gonadal tissues or milt is currently unknown.

For general disease diagnosis in symptomatic fish, the OIE recommends collecting whole fish less than or equal to 4 cm; the viscera including the kidney from fish 4 to 6 cm long; and the kidney, spleen and liver from larger fish. Generally, OIE recommended samples from asymptomatic animals include the kidney, liver, spleen, and heart; however, EHNV is usually found only in symptomatic fish or fish that have died from this disease.

Samples should be taken from ten diseased fish and combined to form pools with approximately 1.5 g of material (no more than five fish per pool). The pools of organs or ovarian fluids should be placed in sterile vials. The samples may also be sent in cell culture medium or Hanks' balanced salt solution with antibiotics. They should be kept cold [4°C (40°F)] but not frozen. If the shipping time is expected to be longer than 12 hours, serum or albumen (5-10%) may be added to stabilize the virus. Ideally, virus isolation should be done within 24 hours after fish sampling.

Recommended actions if epizootic hematopoietic necrosis is suspected

Notification of authorities

Epizootic hematopoietic necrosis should be reported to state or federal authorities immediately upon diagnosis or suspicion of the disease.

Federal: Area Veterinarians in Charge (AVIC): https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/contact-us

State Animal Health Officials:

http://www.usaha.org/federal-and-state-animal-health

Control

In areas where epizootic hematopoietic necrosis is not endemic, it is controlled by culling, disinfection, quarantines and other measures.

In endemic regions, good biosecurity and sanitation are necessary to prevent the virus from entering a farm. Whether carriers occur is controversial, but EHNV has been reported in asymptomatic fish and can be introduced in these animals. Due to their resistance to inactivation, viruses in the EHN group are presumed to persist for months or years on infected farms in the water, pond sediments, plants and equipment. Other methods of spread are also likely; EHNV has occurred in widely separated river systems and impoundments, and it is able to spread upstream. Outbreaks in rainbow trout are usually related to poor husbandry. In this species, good management (low stocking rates; adequate water quality) can reduce the risk of disease. In redfin perch, high morbidity and mortality rates can be expected in an outbreak, regardless of husbandry conditions. Vaccines are not available for any species.

EHNV is highly resistant to drying and disinfection. In dried surface films, this virus can be destroyed by 70% ethanol for two hours but it is resistant to sodium hypochlorite. In liquid suspension, EHNV can be destroyed by sodium hypochlorite, heating to 60°C (140°F) for 15 minutes, or pH of 4.0 or 12.0. Farm equipment should be scrubbed to remove dried films, then disinfected with sodium hypochlorite. Lime may be effective in earthen ponds.

Public Health

There is no indication that epizootic hematopoietic necrosis is a threat to human health.

Internet Resources

USDA APHIS Aquaculture Disease Information
http://www.aphis.usda.gov/animal_health/animal_dis_spec/aquaculture/

World Organization for Animal Health (OIE)
http://www.oie.int

OIE Manual of Diagnostic Tests for Aquatic Animals
http://www.oie.int/international-standard-setting/aquatic-manual/access-online/

OIE Aquatic Animal Health Code
http://www.oie.int/international-standard-setting/aquatic-code/access-online/

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