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Viral nervous necrosis —an emerging disease in finfish aquaculture

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Viral nervous necrosis (VNN) caused by fish nodaviruses, is a serious disease of finfish affecting over 40 fish species from tropical to temperate areas across the world. Majority of the reports have been among larval and juvenile fishes from aquaculture farms reaching mortality up to 100%, although disease in adult fish has been reported. Fish surviving infection can become sub-clinical carriers. The disease can be vertically transmitted by carrier broodstock, or horizontally transmitted from sub-clinically infected fish to other susceptible fish.

VIRAL nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) is one of the major constraints on culture of a number of marine fish species across the world. The etiological agent of this disease is piscine nodavirus called Betanodaviruses. Betanodaviruses, members of the family *Nodaviridae*, cause highly destructive disease in hatchery reared larvae and juveniles of a wide variety of marine fish species in both temperate and tropical climates. The epidemic spread and devastating impacts of VNN in marine fish has been well recognized in many Asiatic countries. In India, the first report on the occurrence of betanodavirus outbreak was reported among a few batches of seabass larvae with 80-90% in 2003. Subsequently, a few more cases of suspected nodavirus infections have been recorded among seabass broodstock in some hatcheries. Studies indicate that sub-clinical infection of nodavirus does exist in many species of cultured and wild marine fishes in India.

Etiology

Betanodaviruses are small (25-34 nm), non-enveloped, icosahedral viruses with bipartite positive sense RNA genome. The larger genomic segment RNA1 (3.1 kb) encodes RNA dependant RNA polymerase (so called protein A). The smaller genomic segment RNA2 (1.4 kb) encodes coat protein (CP). A small subgenomic RNA, RNA3, is synthesized from RNA1 which encode for one or two non-structural protein called B1 and B2. Betanodaviruses are classified into four genotypes based on a phylogenetic analysis of the partial nucleotide sequences of the coat protein gene RNA2. These are striped jack nervous necrosis virus (SJNNV), redspotted grouper nervous necrosis virus (RGNNV), tiger puffer nervous necrosis virus (TPNNV) and barfin flounder nervous necrosis virus (BFNNV). The sequence similarities between these genotypes were 75.8% or greater at nucleotide level and 80.9% at the

Seabass (*Lates calcarifer*) – healthy and clinically infected fish with betanodavirus

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amino acid level. Natural infections of nodaviruses in marine fish occur within a wide range of water temperatures. SJNNV and TPNNV cause disease only in the striped jack (*Pseudocaranx dentex*) and the tiger puffer (*Takifugu rubripes*), respectively. Whereas, BFNNV has been isolated from some cold water fish species such as bar fin flounder, the turbot and Atlantic halibut. RGNNV has the broad host range and cause disease in a variety of warm water fish species particularly groupers and seabass. But recently, some studies indicated that nodaviruses strains/species do not exhibit strict host specificity nor are some hosts only susceptible to one strain/species. Among seabass and groupers there appears to be an association between high water temperatures and clinical symptoms; a positive correlation between increasing water temperature and virulence.

Transmission

The disease was first described in Japanese parrotfish, *Oplegnathus fasciatus* in Japan and barramundi, *Lates calcarifer* in Australia in 1990. Thereafter, the disease was reported in variety of marine fish species, including 40 species or more belonging to 16 families, from the Indo-pacific region, Mediterranean and Scandinavian countries, and North America. While nodaviruses have been regarded as pathogens of marine fish, several instances of natural infection in fishes such as seabass (*Lates calcarifer*), European eel (*Anguilla anguilla*), Tilapia (*Oreochromis niloticus*) cultured in freshwater facility and in aquarium fish (marine and freshwater) and invertebrates have been diagnosed in some countries like India, Singapore, Taiwan, Korea, Japan, France and Australia (Table 1).

There is a lack of information on natural routes of transmission of betanodaviruses. Although horizontal transmission represents the most common route, vertical transmission has also been highly suspected in fishes of aquaculture importance. While vertical transmission through spawners has been proven for some species, it is only speculation for others. Multiple spawning is likely to increase disease transmission. The disease had been reported to transmit from one species to another by cohabitation and waterborne challenges. The exact mode of horizontal transmission and the possibility of inapparent carriers shedding virus in natural conditions are yet to be revealed. As betanodaviruses are quite resistant to environmental conditions it is possible that they are readily translocated by commercial activities via influent water, juvenile fish held on the same site and carriage on utensils, vehicles, etc. Many issues are still to be resolved particularly the mortality associated with betanodaviruses in adult fish, latent infection among wild fishes and their spread in natural environments.

Clinical signs

Affected fish may show varying clinical signs depending on species, age, and the environmental conditions. Acute and sub-acute forms are characterized by varying symptoms and mortality rate. The major clinical signs of VNN are characterized by common behavioural changes such as lack of appetite, erratic, spiral or belly-up swimming and dark coloration body accompanied with cellular necrosis and vacuolation in the central nervous system (brain and spinal cord) and retina on histopathology. Basophilic intracytoplasmic inclusions are also found in the affected organs due to the multiplication of virus. It also multiplies in the gonad, liver, kidney, stomach and intestine in advanced stage. The disease affects mainly larval and juvenile fish leading to mortality reaching up to 100%. Adult fish often act as subclinical carriers.

Diagnosis

Viral nervous necrosis can be diagnosed at least presumptively by demonstrating characteristic lesions in the brain and/or retina by light microscopy. Detection of virions by EM, viral antigens or antibodies by serological methods (IFAT, IHC, and ELISA) or viral nucleotides by molecular techniques (RT-PCR) and tissue culture of virus are other methods available. However, the test systems developed to date have shown variable specificity but molecular techniques, particularly PCR amplification have become the main diagnostic method for fish nodaviruses. The mainstay of this approach has been the PCR for a target sequence of about 430 bases of SJNNV RNA2. More recently, it was reported that nested PCR was 10-100 folds more sensitive than the RT-PCR and permitted diagnosis by using blood, sperm, as well as nervous and ovarian tissues. Antibody based and genome based kits are commercially available in international market.

The difficulties in finding cell lines that supported betanodavirus replication delayed isolation of the virus and limited full understanding of the mechanism of infection and epidemiology of the disease. The culture of betanodaviruses has proved complex until the successful isolation of a nodaviruses from European seabass, *Dicentrarchus labrax* using SSN-1 cell lines derived from striped snakehead (*Ophicephalus striatus*). Subsequently several cell lines; GF-1 from grouper *E. coioides*, E-11 (a cloned cell line from SSN-1), SAF-1 (derived from gilthead seabream, *Sparus auratus*) permissive to nodavirus has been reported by various workers for isolation and quantification based on the cytopathic effect (CPE). At least some of these permissive cell lines showed highly reproducible form of CPE and formed the basis for a successful virus titration system for all strains of nodaviruses and opened a new phase in virological and molecular biological studies on piscine nodaviruses. Viral titration using the E-11 cell line clearly revealed differences in the optimal growth temperature among

the four genotypes: 25 to 30°C for RGNNV, 20 to 25°C for SJNNV, 20°C for TPNNV, and 15-20°C for BFNNV. Cell lines were also developed from groupers, *E. coioides* and *E. awoara*, which support grouper nervous necrosis viruses. Permissive cell lines have also been developed from barramundi/Asian seabass tissues in various countries including India.

Table 1. Clinical cases of viral nervous necrosis (VNN) reported among selected fish species* from different geographical locations

Fish species	Common name	Geographical area/Country
Lates calcarifer	Asian seabass	Australia, China, India, Indonesia, Israel, Malaysia, Philippines, Singapore, Tahiti, Taiwan, Thailand
Epinephelus spp.	Grouper	China, India+, Israel, Japan, Korea, Malaysia, Mediterranean, Philippines, Singapore, Taiwan, Thailand
Mugil cephalus	Grey mullet/flat head	India+, Israel, Italy, Japan
Liza spp.	Mullet	Iran
Chanos chanos	Milk fish	India+, Japan
Rachycentron canadum	Cobia	Taiwan
Oreochromis sp.	Tilapia	France

* Only potential cultivable marine / brackishwater fish species in India is listed.
+ Unpublished data

Prevention and control

There is no successful treatment for viral diseases in fish. No commercial vaccines are yet available. The virus carrying broodstock were considered the most important inoculums source of the virus to their larvae. RT-PCR assays with or without nested PCR have been developed as a powerful diagnostic tool. These protocols have greatly improved test sensitivity, allowing better control of VNN infection through identification and stamping out of infected spawners. This finding led to the successful control of VNN of larval striped jack, where elimination of virus-carrying broodstock by RT-PCR and disinfection of fertilized eggs by ozone were applied. To shut out the horizontal transmission of the virus via contaminated rearing water and utensils disinfection is found to be important in prevention of the disease. Strict hygiene within hatcheries such as: (a) disinfection of eggs (iodine or ozone) and materials (chlorine); (b) rearing of each batch of larvae/juveniles in separate tanks supplied with sterilized (UV or ozone) seawater; and (c) rigorous separation of larval and juvenile striped jack from brood fish proved useful in many countries. The growing concern in finfish aquaculture sector is the occurrence of betanodavirus carriers in the form of adult marine, brackishwater and freshwater fishes and many invertebrates as the potential source of infection. Further, the combination of stress like spawning, transportation, temperatures etc. are known to express the infection and entail economic losses. It is also likely that, sub-clinically infected samples may constitute a persistent potential source of nodavirus from one species another susceptible fish species. Much remains to be learnt about the epidemiology of VNN especially on natural conditions to identify possible risk factors associated with translocation of species for aquaculture or stocking purposes. Salinity tolerance of betanodaviruses is also important in the context of culture of many marine/brackishwater fish fishes in low saline/freshwater environments. The problems are further compounded by the reported susceptibility of some freshwater fishes including aquarium fishes.

A significant feature of commercial hatchery is the movement of live animals between production sites at one location to culture sites at a different location with potential for transmission of pathogens *in situ*, facilitating the spread to a relatively naive host and/or environment. Hence there is a need to be more cautious in movement of fish from one location to another. However, surveillance of fish seed and quarantine measures within the country are almost non existent. Not much less important is the need for screening broodstock fish to ensure disease free seeds for aquaculture. Epidemiology of betanodavirus infection in fish and the development of specific and sensitive diagnostic tool for selecting viral-free broodstock for hatcheries and disease free larvae/juveniles for farming are being investigated.

SUMMARY

Research on marine and brackishwater finfish diseases received less attention in India as compared to shrimp diseases. Sporadic occurrence of infectious diseases due to viral nervous necrosis in hatcheries and farms get unnoticed due to the less knowledge on viral diseases and inadequate diagnostic capability. With the growing importance on culture of marine/brackishwater finfish in the country, VNN is likely to be emerged as a disease of significance in India as is the case with many Asian countries. Surveillance of important viral diseases of fin fishes is needed, as there is a potential to spread viruses as a consequence of aquaculture. Hence, screening of broodstock fish should be implemented advantageously with the enhanced knowledge on molecular based diagnostics to rule out the carrier status. Much more is to be done to study the host-pathogen interaction of the virus, transmission between farmed and wild population of fish to assess the risk of possible vertical transmission and the movement of live seeds from one geographical region to another for aquaculture. ■