Sub-clinical betanodavirus infection in freshwater and marine fishes
Need for surveillance in Indian aquaculture

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Focal Points at a Glance

Pointing out that the viral diseases among finfish do not receive the needed attention the authors tell us that Surveillance of important viral diseases of finfish is needed, as there is a potential to spread viruses with frequent disease occurrences as a consequence of aquaculture activities. 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, and 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.

Introduction

Viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) is one of the major disease constraints in the aquaculture of a number of marine fish species across the world[1]. The etiological agent is a piscine nodavirus, which is member of the family Nodaviridae that causes a highly destructive disease with casualties reaching upto 100% in hatchery reared larvae and juveniles. The family Nodaviridae comprises two genera, Alphanodavirus (type species-*Nodamura virus*, NOV), primarily infecting insects, and Betanodavirus (type species-Striped jack nervous necrosis virus, SJNNV), members of which infect fish. Since its first description[2] in Japanese parrotfish, *Oplegnathus fasciatus*, in Japan, the disease has been reported from over 40 fish species under 16 families in the Indo-pacific region, Mediterranean & Scandinavia and North America[3]. The present communication focuses on the growing importance of this viral infection in marine fish aquaculture in India as an emerging potential disease.

Pathogens and strains

Betanodaviruses are small (25-34 nm), icosahedral and non-enveloped viruses with bipartite positive sense RNA genome. The larger genomic segment RNA1 (3.1 kb) encodes RNA dependant RNA polymerase (protein A) and the smaller genomic segment RNA2 (1.4 kb) encodes coat protein. A small subgenomic RNA, RNA3, synthesised from RNA1 during viral propagation encodes for one or two non-structural proteins called B1 and B2. Currently there are four species within the genus betanodavirus based on partial nucleotide sequences of the coat protein gene (RNA2), though few more genotypes are reported[4]. These are striped jack nervous necrosis virus (SJNNV), redspotted grouper nervous necrosis virus (RGNNV), tiger puffer nervous necrosis virus (TPNNV) and bar-fin flounder nervous necrosis virus (BFNNV).

Clinical signs

The major clinical signs of VNN are characterised by cellular necrosis and vacuolation in the central nervous system (brain and spinal cord) and retina accompanied with common behavioural changes such as lack of appetite, erratic, spiral or belly-up swimming and dark colouration of body. There are considerable variations in the age at which disease is first noted and the period over which mortality occured. In general, the earlier the signs of disease occur, the greater is the rate of mortality. In the case of seabass, the earliest onset of clinical signs of the disease is during 18-21 day post hatch. Recently, the disease is observed in fishes irrespective of their age. Instances of asymptomatic / sub-clinical infection in which the fishes do not show any clinical signs of disease in wild may possibly act as potential carriers. ☞

Transmission

There is a lack of information on natural routes of transmission of betanodaviruses. While nodaviruses have been regarded as pathogens of marine fish, natural development of disease has been reported from fishes of low saline and freshwater environments including freshwater aquarium fishes. Salinity tolerance of betanodaviruses is also important in the context of culture of many marine / brackishwater fishes in low saline environments since many nodavirus isolates from marine fishes are able to infect their freshwater counterparts or other fishes. Although horizontal transmission represents the most common route, vertical transmission has also been highly suspected in fishes of aquaculture importance. 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 studied. 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. Possible risk factors associated with translocation of species for aquaculture or stocking purpose from one location to another is yet to be studied. The inherent potential for transmission of pathogens *in situ via* fish seed facilitates the spread to a relatively naive host and/or environment. Latent infection among wild fishes and their spread in natural environment are some of the other issues to be resolved.

Epidemiology

VNN has been reported from more than 40 fish species across the world including marine, brackishwater and freshwater ones. Betanodaviruses have marked host specificity, although the primary structure of the viral RNAs and encoded proteins are similar among betanodaviruses. 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 coldwater fish species such as barfin flounder, turbot and Atlantic halibut. RGNNV has the broadest host range and cause disease in a variety of warm water fish species particularly groupers and seabass. Recently, some studies indicated that nodaviruses strains/genotypes do not exhibit strict host specificity nor some hosts only are susceptible to one strain / genotypes. This suggests that a classification based on host species is not appropriate and a classification based on molecular characteristics have been mooted. Natural infections of nodaviruses in marine fish occur within a wide range of water temperatures. Among seabass and groupers there appears to be an association between high water temperatures and clinical symptoms; a positive correlation between water temperature and virulence. However, the mechanism underlying the host specificity and virulence is unknown.

Status in India

The first observation of VNN in India was made in a batch of hatchery produced seabass larvae in 2003 accounting for 80-90% mortality[5,6]. Detailed investigation followed later on to confirm by histopathology, immunohistochemistry (IHC), immunofluorescent antibody test (FAT), electron microscopy and nested RT-PCR[7,8]. Subsequently, a few more cases of nodavirus infections have been recorded in seabass hatcheries[9] and ornamental fish-breeding centres[10]. The susceptible ornamental fishes included a batch of freshwater aquarium fish, gold fish and rainbow shark[10]. Detection of betanodavirus in a batch of newly hatched gold fish larvae (TL, 0.8 cm & BW 10mg) in an indoor aquarium indicates the possibility of parental transmission. Most of these cases have been diagnosed based on conventional histopathological and/or RT-PCR techniques. One of the serious limitations of these studies is failure to diagnose the live virus in the fish by means of cell culture and quantification. Recently, a cell line, SISK, isolated from sea bass kidney permissive to betanodaviruses has been established in India for the first time[11]. This achievement would be useful in studying the pathogen and diseases progression in fish.

A wide range of fish species might be susceptible to nodavirus infection and sub-clinically infected wild fish population might be a major source of infection to the farmed fish. In India, betanodavirus infection has been observed in cultured and wild population of brackishwater / marine fish species such as *Lates calcarifer, Mugil cephalus, Chanos chanos, Epinephelus tauvina, Sardinella longiceps, Amblygaster clupeoides, Thrissocles dussumieri, Leiognathus splendens, Upeneus sulphureus, Mystus gulio,* etc. The infection occurred in clinical form only under hatchery / farm conditions as is the case with Asian seabass (*Lates calcarifer*). The VNN infection in low value trash fishes raise concerns over the probable horizontal transmission of the virus into the cultured fishes since most of these fishes are used as feed in finfish aquaculture.

There is no data available on carrier status of broodstock fishes for nervous necrosis virus (NNV) at the time of collection from the wild and during its captive maintenance for breeding purpose. No specific screening for NNV is being carried out for spawners / brooders during a breeding schedule. Various aspects of their occurrence, biology of infection, etc. is poorly understood in finfishes. Lack of knowledge on epidemiology and pathogenesis of nodavirus infection makes control measures difficult and losses continue on a regular basis. The first step in combating an infectious disease is detection and identification of the causative agent using a reliable diagnostic method followed by better surveillance techniques.

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 electron microscopy, viral antigens or antibodies by serological methods (indirect fluorescent antibody test, IHC, enzyme linked immunosorbent assay) or viral nucleotides by molecular techniques and tissue culture of virus are other methods available. However, the test system developed to date has shown variable specificity towards nodavirus detection. RT-PCR is the most sensitive among them and has become the main diagnostic method for fish nodaviruses[12,13]. The mainstay of this approach has been the PCR for a target sequence of coat protein gene. 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 nerves related and ovarian tissues.

The difficulties in finding cell lines that supported betanodaviruses 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 nodavirus from European seabass, *Dicentrarchus labrax* using SSN-1 cell lines derived from striped snakehead, *Ophicephalus striatus* (Bloch). Subsequently several cell lines; GF-1 from grouper, *E. coioides*, E-11 (a cloned cell line from SSN-1), SAF-1 from gilthead seabream, *Sparus auratus* L. permissive to nodavirus has been reported by various workers for isolation and quantification based on the cytopathic effect (CPE). At least some of these conducive 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.

Control measures

Natural infections of nodaviruses in marine fish occur within a wide range of water temperatures. Issues of concerns 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-oriented repeated spawning, transportation, elevated temperatures, salinity variations 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 exporting countries for susceptible fish species elsewhere. Diagnostic assays for VNN may be conveniently used not only to eliminate virus-positive brooder fish, but also to check the fish status during on-growing, especially in floating or submersible cages when contacts between wild and cultured fish are most likely to occur.

Broodfish act as the most important source of the virus to their larvae by vertical transmission. This finding led to the successful control of VNN of larval fish, by eliminating virus-carrying broodstock by RT-PCR screening and disinfection of fertilised eggs with iodine or ozone or others in many Asian countries. Strict hygiene within hatcheries assisted in the control of VNN. Disinfection of hatchery/farm materials with chlorine; rearing of each batch of larvae/juveniles in separate tanks supplied with sterilised (UV or ozone) seawater; and rigorous separation of larval and juvenile fish from brood fish were also found useful.

Conclusions

Research on marine and brackishwater finfish diseases receive less of 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 emerge as a disease of significance in India, as is the case with many Asian countries. Surveillance of important viral diseases of finfish is needed, as there is a potential to spread viruses with frequent disease occurrences as a consequence of aquaculture activities. 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, and 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.

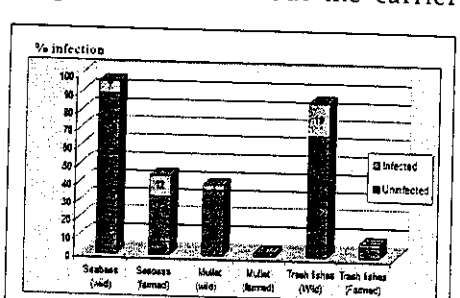

Fig. 1: Prevalence of betanodavirus infection by RT-PCR in fishes

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Table 1: Detection of betanodavirus infection in freshwater ornamental, brackishwater and marine fishes in India by RT-PCR

Ecosystem	Sources	Fish species (common name)	No of positive sample/ No of fish examined
Freshwater	Aquarium fish breeding centre	*Carassius auratus* (Gold fish)	10/10
Freshwater	Aquarium fish breeding centre	*Epalzeorhynchos frenatum* (Rainbow shark)	4/6
Brackishwater	Hatchery/farm/wild	*Lates calcarifer* (Asian seabass)	6/71
Brackishwater	Farm/wild	*Mugil cephalus* (Mullet)	3/36
		Chanos chanos (Milk fish)	3/6
		Mystus gulio	1/1
Marine	Wild caught	*Epinephelus tauvina* (Grouper)	1/3
		*Sardinella longiceps**	2/5
		*Amblygaster clupeoides**	1/3
		*Thrissocles dussumieri**	3/4
		*Leiognathus splendens**	2/4
		*Upeneus sulphureus**	2/5
		Total	**38/ 154**

*Low value fishes: being used as trash fish in fish hatchery/farm

Hilsa fish price escalates as catch dwindled

Every year, as the monsoon clouds gather, an average Bengali becomes restive and the season's first shower prompts him to rush to the fish stalls in the nearest market place in search of Hilsa, locally known as Ilish, the iconic fish of the Bengal delta and a delicacy. The picture is the same in both West Bengal and Bangladesh. Hilsa truly transcends border, religion and culture.

The availability of the fish in Bangladesh is much better, in terms of quality and quantity, thanks to a series of conservation measures enforced by the authorities concerned in the past few years, says a report. The annual production comes to about three lakh tonnes.

In West Bengal, the production of the fish in the Ganga-Bhagirathi-Hooghly river system, the main breeding ground of the fish, has dropped from 60,000 tonnes in 2010-11 to 20,000 tonnes in 2011-12 and 10,000 tonnes 2012-13.

The Hilsa price in West Bengal is now skyrocketing. More than 70 per cent of the catch weighs 500 gm or less, each a sure recipe for disaster from conservation point of view.

Only in April this year, did the West Bengal Government come out with certain notifications restricting irresponsible and unsustainable harvesting of the fish. Creating awareness about the need for maintaining restraint in certain months of the year is critically important. But supply management is not enough. Equally important is the demand management, i.e., persuading Ilish lovers to refrain from buying any fish that weighs 500 gm or less.

Also, the five-star hotels must desist from holding the so-called Hilsa Festivals a report says. Monitoring, control and surveillance, which presupposes willing participation of various stakeholders is another task. There are other issues such as how to tackle the problem of siltation of the river system and discharge of industrial wastes and effluents into it, how to arrange for livelihood of poor fishermen during the ban period, and the list can be long.

Hilsa, a marine species, migrates to estuaries and inland waters for spawning purpose. Unless it gets favourable water conditions for resting and breeding, its migration gets impaired, it is observed.

There is also an explanation as to why more Hilsas migrate to Padma river in Bangladesh and Irrawady in Myanmar in preference to Ganga-Bhagirathi-Hooghly river system.

At a workshop on Hilsa conservation, organised by the International Union for Conservation of Nature (IUCN) held recently, it was revealed that the crisis in West Bengal started from the 1990s ever since the mechanised boats in large number in large numbers were deployed for fishing. The nets used by the trawlers for catching prawns and shrimps were also used for catching Hilsa with the result small sized ones were getting caught.

The political will has to be strong enough to enforce conservation. It is stated that, in Bangladesh, Hilsa, being a major foreign exchange earner and accounting for six per cent of GDP, plays a critical role in the national economy. So the awareness there about conservation is much higher, says a report.