Betanodavirus of Marine and Freshwater Fish: Distribution, Genomic Organization, Diagnosis and Control Measures

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Abstract The family *Nodaviridae* include the genera *Alphanodavirus* and the *Betanodavirus* which are non-enveloped, single stranded RNA viruses. Alphanodavirus include the insect viruses while betanodavirus include species that are responsible for causing disease outbreaks in hatchery-reared larvae and juveniles of a wide variety of marine and freshwater fish throughout the world and has impacted fish culture over the last decade. According to International Committee on Taxonomy of Viruses, the genus *Betanodavirus* comprises four recognized species viz barfin flounder nervous necrosis virus, red-spotted grouper nervous necrosis virus (RGNNV), striped jack nervous necrosis virus and tiger puffer nervous necrosis virus with the RGNNV being the most common. The viruses are distributed worldwide having been recorded in Southeast Asia, Mediterranean countries, United Kingdom, North America and Australia. The disease has been reported by different names such as viral nervous necrosis, fish encephalitis, viral encephalopathy and retinopathy by various investigators. The virus is composed of two segments designated RNA1 and RNA2 and sometimes possesses an additional segment designated RNA3. However, genome arrangement of the virus can vary from strain to strain. The virus is diagnosed by microscopy and other rapid and sensitive molecular methods as well as immunological assays. Several cell lines have been developed for the virus propagation and study of infection mechanism. Control of

nodavirus infection is a serious issue in aquaculture industry since it is responsible for huge economic losses. In combination with other management practices, vaccination of fish would be a useful strategy to control the disease.

Keywords Betanodavirus · Fish · Genome arrangement · Diagnosis · Disease control · Vaccination

Introduction

Aquaculture is recognized as one of the fastest growing industry among the food producing industries and is expected to contribute to the reduction in gap between demand and supply of fishery products [33]. The marine aquaculture fish production has increased rapidly during the past several years due to their higher market demand and economic value. However, rapid expansion and intensification of aquaculture has led to disease outbreaks. Among the infectious diseases, viral diseases are the most serious since they cause severe losses to fish aquaculture production. Many viral diseases of fish have been reported worldwide [66, 92, 108] of which infection caused by betanodavirus is a major concern. The virus has emerged as a major constraint to fish culture and is responsible for catastrophic losses reported worldwide. The disease is associated with high mortalities (up to 100 %) particularly in larvae and juvenile fish species in various part of the world including Europe, North America, Asia, Japan and Australia [107].

Betanodavirus infection was first reported in *Lates calcarifer* (Barramundi) in Australia [41] and an identical disease was also observed later in European seabass, *Dicentrarchus labrax* (L.) in Caribbean [11]. The infections are also reported in hatchery-reared Japanese

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parrotfish, *Oplegnathus fasciatus* in Japan [122] and Barramundi larvae (*L. calcarifer*) in Australia [42]. Several reports are also documented from turbot *Scophthalmus maximus* [12], European seabass *D. labrax* [13] redspotted grouper *Epinephelus akaara* [80], and striped jack *Pseudocaranx dentex* [81]. The infections can occur in a variety of cultured warm water and cold water marine fish species [83, 86] as well as some fresh water fish [52, 53]. Presently ~40 species of fish species are known to be affected with betanodavirus infection [28, 83] with the most recent one being freshwater guppy [23, 52, 53] and aquarium fish such as gold fish and rainbow shark [62]. Therefore, it is very necessary to detect the virus before any clinical signs appear. In addition to microscopic analysis, many sensitive and rapid molecular techniques are available [36, 64, 89, 113, 117]. Paying attention to the control measures such as the new generation vaccine strategies is important together with adoption of better management approaches. Current review will describe about this virus infection, diagnosis of the disease and the control measures.

Aetiology

Betanodavirus, a single stranded RNA (ssRNA) virus, is responsible for serious problem in cultured fish. Huge mass mortality due to this virus has been recognized since 1980s around the world. The virus in particular affects larval and juvenile stages of fish. This is a non-enveloped virus with an icosahedral capsid (triangulation number = 3) ranging from 25 to 34 nm in diameter. The capsid consists of 32 capsomeres. The virus is mainly composed of two segments (RNA1 and RNA2). The RNA1 segment encodes two non-structural viral replicase proteins, while the RNA2 encodes the structural capsid protein. The disease has been reported by a variety of names such as viral nerve necrosis (VNN) [122], fish encephalitis virus [12] and viral encephalopathy and retinopathy [83, 93]. The virus is found to be affecting both cold water and warm water fishes and reported throughout the world [83]. It was initially believed that the virus was a parvovirus or a picornavirus. By 1990 the nomenclature of the virus was confirmed and was placed in the family *Nodaviridae*, genus *Betanodavirus*. Betanodavirus is a distinct group from the insect nodaviruses (*Alphanodaviruses*) [90] in the 7th report of the International Committee on Taxonomy of Viruses [8, 86]. The recognized members are: barfin flounder nervous necrosis virus (BFNNV), red-spotted grouper nervous necrosis virus (RGNNV), striped jack nervous necrosis virus (SJNNV) and tiger puffer nervous necrosis virus (TPNNV) and RGNNV is most widely distributed [64, 90, 91, 107].

Occurrence and Distribution

Betanodavirus infections have been reported in all continents except South America [65, 83, 86] especially in regions where intensive culture of marine species is rampant. These include, south and east Asia (Japan, Korea, Taiwan, China, Philippines, Thailand, Vietnam, Malaysia, Singapore, Indonesia, Brunei, India, China), Oceania (Australia, Tahiti), the Mediterranean (Israel, Croatia, Bosnia, Greece, Malta, Italy, France, Spain, Portugal, Tunisia), the UK, Scandinavia (Norway), and North America (USA, Canada) [9, 21, 25, 30, 86, 94, 127]. Betanodavirus infection is one of the most devastating diseases of marine aquaculture and also represents a threat to wild fish populations because of its high infectivity and broad host range [94]. Initially, the infection is observed in 19 fish species (belonging to 10 families, 3 orders), including Japanese parrotfish, redspotted grouper, striped jack, Barramundi, turbot, and European seabass [82]. Later, 32 more species (16 families, 5 orders) is listed which have been recorded as hosts for this virus [83].

More recently, around 40 host species have been reported (22 families, 8 orders) which includes Atlantic cod (*Gadus morhua*); thread-sail filefish (*Stephanolepis cirrhifer*); spotted wolf fish (*Anarhichas minor*) sturgeon *Acipenser* sp., turbot (*S. maximus*); Chinese catfish (*Parasilurus asotus*), guppy (*Poecilia reticulata*), dragon grouper (*Epinephelus lanceolatus*), Japanese tilefish (*Branchiostegus japonicus*), firespot snapper (*Lutjanus erythropterus*), bluefin tuna (*Thunnus thynnus*); sevenband grouper (*Epinephelus septemfasciatus*), Golden pompano, (*Trachinotus blochii*) (*E. septemfasciatus*) and redspotted grouper (*E. akaara*) [46, 54, 69, 86, 98, 100, 103]. Larval or juvenile stages are more prone to this viral infection, but significant mortality can also occur in older fish up to production-size as in European seabass, sevenband grouper, humpback grouper *Cromileptes altivelis* and Atlantic halibut *Hippoglossus hippoglossus* [4, 37, 71, 83, 86, 125]. Infections are also reported in freshwater fish, such as Chinese catfish (*P. asotus*); Australia catfish (*Tandanus tandanus*); Barramundi (*L. calcarifer*); Medaka (*Oryzias latipes*); guppy (*Poicelia reticulata*) and zebrafish (*Danio rerio*) [5, 22, 52, 53, 62, 63, 76]. Several experimental infection studies have demonstrated that both marine and fresh water finfish are susceptible to betanodavirus infection [23, 38, 39, 52, 53, 63]. It has been seen that Asian seabass juveniles are more susceptible to betanodavirus from 10 day onwards with high mortalities of 80 % and smaller sized fish are more prone to the virus than bigger sized fish in the cages. This could be due to attributed also to various stress factors like overcrowding in the cages, cannibalistic nature of seabass, size grading procedure applied in cage culture system etc. (Shetty et al.

unpublished data). Among the betanodavirus genotypes, host range of SJNNV and TPNNV are limited to striped jack (*P. dentex*) and tiger puffer (*Takifugu rubripes*), respectively whereas BFNNV genotype has been isolated from cold water species, such as Barfin flounder (*Verasper moseri*) and Pacific cod (*Gadus macrocephalus*). RGNNV genotype was found to have a broad host range causing disease among a variety of warm water fish species, particularly groupers and seabass. This has led to discussions on possible host specificity and temperature dependence in betanodavirus strains [4, 22].

Clinical Signs

Classical signs of disease are most commonly observed in larval and juvenile fishes since they are commonly affected [82] and sometimes in adult fish [37, 71, 103]. Diseased fish show various clinical symptoms which include reduced appetite, emaciation, colour change (darkening), abnormal (whirling) swimming pattern, neurological dysfunction, exophthalmia, swim bladder hyperinflation, floating belly up with inflation of swim bladder, anorexia and gas accumulation and extensive mortality (Fig. 1) [6, 37, 38, 49, 64, 77, 85, 97, 102, 117, 126], (Shetty et al. unpublished data). Gross lesions are uncommon, but overinflation of the swimbladder has been observed [37, 92]. Several investigators have confirmed the presence of betanodavirus in fish without evidence of any disease signs as in brown meagre (*Sciaena umbra*) and Atlantic salmon (*Salmo salar*) [9, 29, 43, 48].

Genome Organization

The betanodavirus genome is organized as a bisegmented positive sense ssRNA. Recent complete genome sequence of the virus reveals that the genome size is 4.5 kb in which the larger segment, RNA1 (3,103 nt) with a G+C content of 49.6 %, encodes the RNA-dependent RNA polymerase

(protein A) flanked by a 78 nt 5'-nontranslated regions (NTR) and a 77 nt 3'-NTR region. The open reading frame (ORF) contains a 982-amino-acid with a calculated molecular mass of 110.74 kDa. On the other hand, the smaller segment, RNA2 (1433 nt) having a G+C content of 53.24 % contains a 338-amino-acid major ORF which encodes the coat protein with a calculated molecular mass of 37.059 kDa flanked by 26 nt 5'-NTR and a 390 nt 3'-NTR [19, 75, 107, 118]. RNA2 forms a major ORF of capsid protein and consists of a highly conserved region and a variable region. In addition to this, RNA3, a sub genomic RNA (371 nt) has a 62 % G+C composition, containing an ORF encoding a peptide of 75 amino-acids corresponding to a hypothetical B2 protein which is a non-structural protein having a suppressor function for post-transcriptional gene silencing [19, 34, 61, 110]. RNA3 is formed only in infected cells and is not packaged into the viral particles (not in chronically infected fish) [79, 90, 110] and is transcribed from the 3' end of RNA1. However, the genome arrangement of the virus can vary from strain to strain. To substantiate and understand this, phylogenetic analysis was carried out based on the gene encoding viral coat protein for various viral strains [16, 45, 90, 91]. For example, the sequence similarities among SJNNV and the coat protein gene from different fish VNN viruses was found to be 75.8 % or greater at the nucleotide level and 80.9 % or greater at the amino-acid level [90]. In the fish betanodaviruses, there is a highly conserved region of 134 amino-acids with a sequence similarity of 92.5 % or greater which is not found in the coat protein of insect nodaviruses [90].

More recently, phylogenetic analysis based on the sequence of both RNA1 and RNA2 from 120 viral strains from different countries of Europe was performed. Viruses sampled from individual countries tended to cluster together thus indicating a major geographic subdivision among betanodaviruses [94]. Our results on the analysis of the sequence of T4 region of the nodaviral RNA2 coat protein of Indian strain from Asian seabass (*L. calcarifer*) show high similarity (>90 %) with the other Asian strains and belongs to RGNNV which is the widely distributed genotype of all the known genotypes of betanodavirus (Fig. 2) (Shetty et al. unpublished data). Analysis also suggest that strain variation of the virus is not related to the host-dependent evolution and/or geography. Betanodavirus appear to adapt easily to other fish species [16].

Diagnosis of Betanodavirus

Clinical signs of betanodavirus infection are characteristic in larvae and fry and the indication of disease onset. However, it is important to diagnose the preclinical state before disease occurs so that necessary good health management measures can be undertaken to avoid the viral

Fig. 1 Asian seabass (*L. calcarifer*) juveniles infected with nodavirus. A: Arrow shows dark coloration on the body surface. B & C: Highly emaciated infected fish

Fig. 2 Phylogenetic tree constructed from the variable region of coat protein sequences of betanodaviruses using MegAlign program (Windows version 5.05, DNASTAR, USA). Accession numbers: HM017076, HM017077 [Asian seabass (*L. calcarifer*), India] are the coat protein sequences generated in our lab and shown in rectangular box (Shetty et al. unpublished data); EU380202, FJ617262, GQ120525: [Asian seabass (*L. calcarifer*), Malaysia]; AF245004: [Giant Grouper (*E. lanceolatus*), Taiwan]; EF591371 and EF591372: [Barramundi (*L. calcarifer*), Australia]; D38636: [Redspotted grouper (*E. akaara*), Japan]; EF558369: [Redspotted grouper (*E. akaara*),

China]; AF499774: [Guppy (*P. reticulata*), Singapore]; AF175518: [Brownspotted Grouper (*E. malabaricus*), Thailand]; AF175516: [Barramundi (*L. calcarifer*), Singapore]; AF318942: [Greasy grouper (*E. tauvina*), Singapore]; D38637 and EU236149: [Tiger puffer (*Takifugu rubripes*), Japan]; D38635, EU236147 and EU826138: [Barfin flounder (*V. moseri*), Japan]; AF175519, D30814 and AB056572: [Striped jack (*P. dentex*), Japan]. The distance between sequences is represented by the length of each pair of branch and the unit below the tree indicates the number of substitution events

disease outbreak. Diagnostic assays for betanodavirus are important to identify any outbreaks of infection and to screening of the broodstock that may be act as carriers [113]. Ideally, the methods for diagnosis should be rapid, sensitive, specific and reliable. Various methods are available for betanodavirus diagnosis such as microscopy, molecular, immunological and cell culture methods.

Microscopy

Microscopic analysis of any lesion sites produced by betanodavirus provides useful information in order to understand the nature of infection. Although the virus is neurotropic, it may replicate in other tissues such as the liver and spleen. Typical histopathological lesions include severe widespread degeneration and vacuolation throughout the central nervous system (CNS) of the fish and all retinal layers. The bipolar and ganglion cell retinal layers exhibit the most obvious vacuolation. Gliosis is also a common finding in the CNS. Vacuolation is usually greater in the grey matter of the optic tectum and cerebellum and there is often involvement of Purkinje cells. Vacuolation can also be seen in the white matter, adjacent to the ventricles [112]. Immunohistochemistry study reveals the location of immunopositive cells and shows that the virus favours neural cells which are in the early stages of

proliferation. This indicates that the virus enters the CNS along nerves and blood vessels during the viraemic stage of the disease [64, 71]. The virus also can be detected by immunofluorescence microscopy by using monoclonal antibody [101]. Virions of the appropriate size and shape can be clearly and rapidly demonstrated using electron microscopy. Analysis of electron micrographs reveals that the cell clusters have large macrophage like cells with multiple large membranes bound inclusions filled with virus particles. The virus particles sometimes form membrane-bound necklace-like arrangements [6, 48, 82].

Reverse Transcriptase PCR

Reverse transcription (RT) followed by polymerase chain reaction (PCR) is an important technique for amplification of RNA [120]. Reverse transcriptase PCR has been applied to amplify a portion of the coat protein gene (RNA2) of betanodavirus and is a powerful and sensitive method for detecting the infection [6, 10, 50, 90]. Till date, PCR is the main diagnostic test that is being used by most of laboratories. The sensitivity of this test depends on the strain of betanodavirus which is being targeted; hence specific primers are continuing to be developed [40]. Nested RT-PCR has been found to be 10–100 times more sensitive than the previously reported RT-PCR methods [29, 117].

Fig. 3 RT-PCR detection of betanodavirus in Asian seabass (*L. calcarifer*) (Shetty et al. unpublished data) using T4NV-F2/R primer set [89]. M: 100 bp DNA ladder, Genei Bangalore, Lane 1 positive control. Lane 2 Negative control. Lane 3–6 positive samples

The method can be applied to screen the virus from cultured and wild fish even when there are no clinical signs [43, 44]. Fish betanodavirus and its association with mass mortality of Asian seabass, (*L. calcarifer*) is detected by using RT-PCR (Fig. 3) [6, 97, 102], (Shetty et al. unpublished data). Real-time PCR also is sometimes used for rapid and sensitive detection of this virus. As nodavirus is very stable and can survive in seawater for a long time [36], it has been possible to detect and quantify the virus in seawater from rearing facilities for Atlantic halibut *H. hippoglossus* larvae [87]. Real-time PCR assay has been useful to study the transmission and development of this viral infection in juvenile [56].

Another useful method is the nucleic acid sequence based amplification (NASBA) [27] which is an isothermal method for nucleic acid amplification that is particularly suited to RNA targets [68]. The method amplifies a target-specific product through oligonucleotide primers and the co-ordinated activity of 3 enzymes: reverse transcriptase, RNase H, and T7 RNA polymerase. Real-time detection in NASBA is performed by applying molecular beacons, which are incorporated directly into amplification reactions [72]. The real-time NASBA procedure for the detection of betanodavirus has been developed and sensitivity of this assay was compared to a conventional single-tube RT-PCR assay [113].

Immunoassays

Immunological based assays which include enzyme-linked immunosorbent assay (ELISA) is the commonly used techniques for the detection of betanodavirus. The test involves the detection of specific nodavirus antibodies in blood and other body fluids. ELISA was one of the first immunological methods to be developed [2, 24] to detect SJNNV but was not very sensitive. It is commonly used since a large number and a variety of samples can be screened rapidly by these methods. Since the sensitivity of the technique may vary, it is more useful for epidemiological studies and outbreaks rather than for diagnostic purposes of detecting subclinical levels of infection.

A convenient test to confirm the presence of betanodavirus infection is by immunofluorescent antibody testing using polyclonal and monoclonal antibody [82]. It is both rapid and economical and the polyclonal anti serum helps the detection of the full range of betanodavirus strains known. However, greater tissue quantities are required than for the ELISA test.

Cell Lines

For virus propagation, characterization and studies on infection mechanism, cell lines are important. The tissue culture method for isolates of fish betanodavirus was established in 1999 when the snakehead cell line (SSN-1) cell line derived from striped snakehead (*Ophicephalus striatus*) was developed to isolate and propagate betanodavirus from diseased seabass juveniles [35]. The virus from farmed Atlantic halibut *H. hippoglossus* is found to be stable without losing or changing the virulence after repeated cell culture [31]; however permissiveness is temperature dependent. SSN-1 cell line is useful for propagating and differentiating genotypic variants of piscine nodavirus [59]. The cell line has been cloned and since it shows stable cytopathic effect expression, it can be used for qualitative and quantitative analyses of piscine nodaviruses rather than the SSN-1 cell line [60]. Yet another cell line derived from the brain tissue of Barramundi *L. calcarifer* was developed which was used to study the mechanisms of NNV-persistent infection in vitro and in vivo [24]. A tropical marine fish cell line from the spleen of orange spotted grouper, *Epinephelus coioides* is developed and characterized which is found to be useful as a tool for transgenic and genetic manipulation studies [101]. India's first marine fish cell line was developed and characterized from the kidney of Asian seabass (*L. calcarifer*) and consisted predominantly of epithelial-like cells [104]. A spleen cell line from Asian seabass (SISS) [95] and two other cell lines, SIMH and SIGE were derived from the heart of milkfish (*Chanos chanos*), a euryhaline teleost, and from the eye of grouper (*E. coioides*), respectively [96].

Epidemiology

Betanodavirus is an acute infectious disease of primarily finfish larvae and fry. To control the infections, it is important to understand the epidemiology and pathogenic mechanisms of the virus. Betanodavirus infection can be influenced by host factors such as age [1, 3], environmental factors such as water temperature [106, 115, 125] and other stress factors such as suboptimal feed, suboptimal water quality, crowding, transport and repeated spawning of broodstock [55, 64, 84, 115]. The virus can be transmitted

both horizontally [15, 49, 71, 99] and vertically [2, 7, 14, 49, 83, 84, 92, 102, 119]. It can persist in the host for a long time sub-clinically and may cause severe mortality under extreme environmental conditions [105]. The horizontal transmission may occur from infected fish, feed, contaminated trash fish/carrier animals and contaminated water supply [18, 44, 47, 69, 87, 119]. The cannibalistic nature of fish such as Asian seabass and brown-marbled grouper fingerlings may also enhance the horizontal transmission of the virus [78]. Feeding of trash fish to cultured fish is also found to be a source of infection [47]. Some of the betanodavirus infected larval fish can survive and act as a carrier for the next generation [87]. Several authors studied the horizontal transmission of this virus during outbreaks and confirmed it by various experimental studies [3, 15, 88]. Arimoto et al. [2] was probably the first to demonstrate vertical transmission by detecting the virus in the fertilized eggs and also in 65 % of the striped jack brood fish by antibody-based ELISA technique. Vertical transmission has also been demonstrated in European seabass [26], Japanese flounder, barfin flounder [123], Atlantic halibut [51] and Asian seabass (*L. calcarifer*) [7].

Disease Control Strategies

During the last decade and continuing to the present, betanodavirus has been one of the major limiting factors in the culture of fish all over the world. In intensive aquaculture, where single or multiple species are reared at high densities, infectious disease agents are easily transmitted between individuals. First step to control the betanodavirus infection would be the development of location specific better management practices (BMPs) which are scientific management procedures. Significant benefits could be achieved in farming systems by adopting these BMPs. The BMP approach includes the use of specific-pathogen-free broodstock, quality feeding, improved husbandry practices, good sanitation, use of probiotics and immunostimulants etc.

Since the epidemiological knowledge of betanodavirus disease is limited, it important to follow combination of measures to reduce the risk factors. Betanodavirus is shed from broodstock during spawning and probably adheres to the egg surface thus infecting larvae when hatching takes place. The stress that a seropositive broodstock encounters needs to be addressed for reducing the shedding. It would be ideal to reduce the stocking density. To prevent the vertical transmission of the virus to the offspring, the eggs can be washed in ozonated water for inactivating the virus which may be found on the surface. Horizontal transmission can be reduced by avoiding extensive mixing of batches of larvae/juveniles [105]. Other methods that can be considered include the ozonation of influent water,

maintaining quarantine of ill and introduced fish, maintaining biosecurity between different parts of the facility and disinfecting tanks between batches [83].

It has been observed that betanodavirus strains tend to be geographically related rather than to the fish species from which it has been isolated [40]. To ensure that the fish are as healthy as possible, a useful and practical approach would be vaccination of fish and boosting their immune system. Various types of vaccines have been developed such as formalin-inactivated vaccine, subunit vaccine with recombinant coat protein of betanodavirus and nodavirus-like particle vaccine [58, 74, 111, 116, 118, 121, 124]. Vaccination trials have shown protection efficacy against betanodavirus infection. For example, recombinant partial capsid protein of SJNNV [58] and AHNV [111] vaccinated turbot and Atlantic halibut fish are protected significantly against nodavirus challenge. Additionally, use of DNA vaccine based on the gene encoding for G protein of VHSV offers high but short term protection against the nodavirus infection [109]. Vaccination against betanodavirus of larvae or juvenile fish is a difficult process because the immune system of fish is not well developed at this stage and the most effective vaccination method which is delivering the vaccine candidate by injection is impractical due to small size of the fish. Brood fish, on the other hand can be immunized before spawning to minimize the risk of vertical transmission. Oral vaccination can be standardized to prevent disease occurring at the early larval stage. For example, Artemia-encapsulated recombinant *Escherichia coli* expressing the NNV capsid protein gene delivered through oral route showed a certain degree of protection after challenge with NNV (relative percentage survival up to 69.5 %) [73]. The protection efficacy can be improved by manipulating the expression in the host. Antigen expressed in *Vibrio anguillarum*, a common marine bacterium with immune-stimulatory capability followed by oral vaccination can enhance the efficacy in a shorter incubation period and can reduce the risk of NNV infection at early stage [17]. Some natural products from plants are found to be effective against viruses and can be used as antiviral drugs [57]. Many antiviral drugs have been synthesized in the recent past and researches on development of potent antiviral agents are continuing to address the issue of viral diseases [32, 70]. Several antiviral compounds, like tilapia hepcidin 1-5 (TH1-5), cyclic shrimp anti-lipopolysaccharide factor [20], furan-2yl-acetate [114], gymnemagenol [67] and Dasyscyphin C (C28H40O8) [70] are also reported to be active against fish betanodavirus.

Concluding Remarks

Betanodavirus infection in freshwater and in marine fish species is a serious issue. The past few decades have

witnessed a lot of interest in addressing disease problems due to nodavirus and many studies on the various aspects of this virus have been carried out on its occurrence, distribution, genomics, pathogenesis, pathogenicity and protection strategies. Several rapid and sensitive diagnostics have been developed to identify the risk of presence of the virus in brood fish and to enable stocking of virus free larvae and juveniles. However, further studies are required to identify infected broodstock and development of a commercial vaccine that would be useful to the industry. Research on the molecular characteristics of the virus, interactions between host and the pathogen, route of transmission in aquaculture and survival of the virus in the natural environment is in progress. Studies are required to analyze and compare the betanodavirus types as they continue to be reported from various regions. To reduce the severe economic losses to the aquaculture industry, the control of disease is very important. A combination of good management practices together with vaccination of adult and/or young fry would be ideal. Discovery of new generation recombinant vaccines with appropriate practical delivery method such as the oral route using nanoparticles and boosting the immunogenicity of antigen with adjuvant or any immune component would be the ideal means of controlling the disease.

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