

13. Microbial Diseases in Shrimp Aquaculture

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Introduction

Aquaculture is one of the fastest growing food production sectors in the world (Subasinghe et al. 1998). According to FAO statistics, over 80% of fish produced by aquaculture comes from Asia, with the production valued at \$ 38.855 billion (FAO, 1996). However, disease outbreaks have caused serious economic losses in several countries. According to a World Bank Report, global losses due to shrimp diseases are around US \$ 3,000 million (Lundin, 1996). Thus, health management is of major importance in aquaculture. Diseases caused by microorganisms are most devastating and, this chapter discusses various microbial diseases of shrimp and strategies for management of microbial diseases.

Bacterial diseases

Bacterial diseases may cause a range of problems ranging from mass mortalities to growth retardation and sporadic mortalities. *Vibrio* spp are the most important bacterial pathogens of shrimp. *Vibrio* spp are aquatic bacteria that are widely distributed in fresh water, estuarine and marine environments. Over 20 species are recognized, some of these are human pathogens (eg. *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*) while some species are pathogens of aquatic animals including shrimp (eg. *V. harveyi*, *V. splendidus*, *V. penaeicida*, *V. anguillarum*, *V. parahaemolyticus*, *V. vulnificus*). *Vibrio* spp are commonly observed in shrimp hatcheries, grow-out ponds and sediments (Otta et al. 1999a, 2001).

Though most *Vibrio* spp are regarded as opportunistic pathogens, some like *V. harveyi* could be primary pathogens. *V. harveyi* are luminous bacteria that are found in coastal and marine waters, in association with surface and gut of marine and estuarine organisms and also in shrimp pond water and sediment (Ruby and Neilson 1978, Yetinson and Shilo 1979, Orndorff and Colwell 1980, Otta et al. 1999a, 2001). However, they can cause serious mass

mortalities in shrimp hatcheries in Asia (Sunaryanto and Mariam 1986, Tansutapanit and Ruangpan 1987, Lavilla-Pitogo et al. 1990, Karunasagar et al. 1994). Karunasagar et al. (1994) noted that certain strains of *V. harveyi* isolated from sea water had high LD₅₀ for *P. monodon* larvae, while isolates from moribund larvae had a low LD₅₀ suggesting that *V. harveyi* strains may vary in virulence. Pizzutto and Hirst (1995) reported that strains of *V. harveyi* virulent to *P. monodon* formed a separate cluster in protein profile and M13 DNA fingerprinting. So far, no virulence factors have been definitely established in this species, though a number of suggestions have been made eg. extracellular products including proteases, hemolysins and cytotoxins (Liu et al. 1996 a, b), low molecular weight lipopolysaccharide lethal toxin (Monterio and Austin 1999) and protein toxins T1 and T2 (Harris and Owens 1999).

Association between *V. harveyi* and bacteriophages was reported by Pasharawipas et al. (1998) in the brown gill syndrome (TBGS) in *P. monodon*. They further noted that lysogenic *V. harveyi* itself could not induce TBGS and luminescence was not critical for shrimp pathogenicity. Oakey and Owens (2000) noted that one of the toxin producing strains of *V. harveyi* (VH642) was lysogenic and carried a myovirus like phage (VHML). This phage has been completely sequenced and a putative virulence gene coding for an ADP-ribosylating toxin has been

identified. However, the phage genome did not code for protein toxins (T1 and T2) described by Harris and Owens (1999). Thus, further studies are required to confirm the role of bacteriophage in the virulence of *V. harveyi*.

Filamentous bacteria such as *Leucothrix mucor*, *Thiothrix* sp, *Flexibacter* sp, *Flavobacterium*, *Cytophaga* sp may cause infection in penaeid shrimp larvae. Discolouration of gills, low growth and feeding, increased mortality and lethargy are common signs of the disease. The disease is associated with poor water quality. Higher degree of infection may lead to necrosis in gill tissue. The disease can be diagnosed by microscopic examination of gills.

Viral diseases

About 20 viruses have been recognized as causative agents of diseases in shrimp. These include members of Parvoviruses, Baculoviruses, Picornaviruses, Toga-like viruses and some of the newly identified viral families. Some of the important viral pathogens affecting Asian shrimp are discussed below:

Whitespot syndrome virus (WSSV)

WSSV continues to be one of the most serious disease problems faced by the shrimp farming industry worldwide (Takahashi et al. 1994, Chou et al. 1995, Wongteerasupaya et al. 1995, Lo et al. 1996a, b, Flegel 1997, Karunasagar et al. 1997, Hsu et al. 1999). This virus was first reported in 1982 in *P. japonicus* cultured in northeastern Taiwan (Chou et al. 1995). Since then, WSSV has caused mortalities and consequent serious damage to the shrimp industry (Inouye et al. 1994, Chou et al. 1995, Wongteerasupaya et al. 1995, Lo et al. 1996a, b, Karunasagar et al. 1997, Hossain et al. 2001a, b). Considering its virulent nature, wide host range, wide geographic distribution, high mortality, catastrophic economic losses, WSSV has become the single most-dangerous virus to the penaeid shrimp farming industry.

WSSV is known to affect most commercially important species of penaeid shrimp including *P. monodon*, *P. japonicus*, *P. indicus*, *P. chinensis*, *P. merguensis*, *P. aztecus*, *P. stylirostris*, *P. vannamei*, *P. duorarum* and *P. setiferus* (Lightner 1996a). Wild marine shrimp such as *P. semisulcatus*, *Metapenaeus dobsoni*, *M. monoceros*, *M. elegans*, *Heterocarpus* sp., *Aristeus* sp., *Parapanaeopsis styliifera*, *Solenocera indica*, *Squilla mantis* and fresh water cultured species *Macrobrachium rosenbergii* have also been found to harbor this virus (Lo et al. 1996a, Hossain et al. 2001a; Chakraborty et al. 2002). This virus has also been detected in many captured and cultured crustaceans and other arthropods including crabs (*Charybdis feriatius*, *C. annulata*, *C. lucifera*, *C. cruciata*, *Macrophthalmus sulcatus*, *Gelasimus marionis*, *Metapograpsus messor*, *Scylla serrata*, *Sesarma oceanica*, *Matuta planipes*, *Helice tridens*, *Pseudograpsus intermedius*), pest prawn *Acetes* sp., small pest palaemonid prawn, larvae of Ephydriidae insect and *Artemia* (Lo et al. 1996a, Maeda et al. 1998, Otta et al. 1999, Chen et al. 2000, Hossain et al. 2001a, b, Chakraborty et al. 2002).

WSSV infects most tissues originating from both ectoderm and mesoderm. These include subcuticular epithelium, gills, lymphoid organs, antennal gland, hematopoietic tissues, connective tissue, ovary and the ventral nerve cord (Wongteerasupaya et al. 1995, Wang et al. 1999). The principal clinical signs of the

syndrome is the presence of white spots on the exoskeleton and epidermis of the diseased shrimp, ranging from 0.5 to 2.0 mm in diameter (Takahashi et al. 1994, Chou et al. 1995, Wang, et al. 1995, Lo et al. 1996a). The other signs of the disease include rapid reduction in food consumption, lethargy, anorexia, loose cuticle and often, a generalized reddish to pink discoloration (Nakano et al. 1994, Durand et al. 1997, Karunasagar et al. 1997, Otta et al. 1999).

WSSV is a tailed, rod-shaped, double stranded DNA virus (Wongteerasupaya et al. 1995, Durand et al. 1996) with a very large genome in the order of 300kb (Van Hulten et al. 2001). Sequence analysis of WSSV genomic DNA and the comparison of sequence data has shown WSSV to be unique not showing any homology with any known virus (Lo et al. 1997). Now WSSV has been assigned to a new viral family (Nimaviridae) and a new viral genus (Whispovirus; Van Hulten et al. 2001). WSSV from China, Taiwan and Thailand have been completely sequenced and variation in nucleotide sequence between viruses have been reported.

The current diagnostic methods for WSSV include conventional methods such as microscopy and histopathology and rapid molecular methods such as gene probes (Durand et al. 1996, Lo et al. 1996a, Wongteerasupaya et al. 1996, Nunan and Lightner 1997) and polymerase chain reaction (Takahashi et al. 1996, Lo et al. 1996b).

WSSV infections could be in acute phase in which clinical signs and histopathological changes are observed or in chronic phase in which clinical signs and histopathological changes are not seen and the virus is detectable only by sensitive diagnostic methods like PCR (Lo et al. 1996 a, b, 1997, Hossain et al. 2001a, b). Nested PCR has been reported to increase sensitivity of detection by 10^3 - 10^4 times (Lo et al. 1996b) and sensitivity increases as the amplicon size decreases (Hossain et al. 2004). It is common to observe WSSV by nested PCR in apparently healthy *P. monodon* from farms that go through a normal crop (Tsai et al. 1999).

Since WSSV can infect ovary, vertical transmission of the virus from brood stock to eggs has been demonstrated (Lo et al. 1997) and presence of WSSV in wild brood stock by a number of investigators (Lo et al. 1996a, Otta et al. 1999). Lightly infected brood stock (nested PCR positive) may produce either infected or uninfected larvae. PCR is being commonly used in Asia to screen *P. monodon* larvae before stocking in ponds and the risk of crop loss has been reported to be high when larvae positive for WSSV by non-nested PCR are stocked (Umsha et al. in press). Using nested PCR, the prevalence of WSSV in hatchery-reared post-larvae in India has been

reported to be 75 % (Otta et al. 2003). Simultaneous presence of WSSV, with other viruses such as monodon baculovirus (MBV) and hepatopancreatic parvovirus (HPV) has been reported from India (Otta et al. 2003, Umesha et al. 2003). Other molecular and immunological methods such as *in-situ* hybridization (Durand et al. 1996, Wongteerasupaya et al. 1996, Lightner 1997, Wang et al. 1998, Chen et al. 2000), dot blot nitrocellulose enzyme immunoassay (Nadala and Loh, 2000), ELISA (Nadala et al. 1997, Sahul Hameed et al. 1998) and western blotting (Nadala et al. 1997, Magbanua et al. 2000) have also been applied to detect WSSV in shrimp and carrier species.

Monodon Baculovirus (MBV)

MBV is the first reported virus of *P. monodon* and the second virus of penaeid shrimp (Lightner and Redman 1981). It is a nuclear polyhedrosis virus (NPV) of the family Baculoviridae (Lightner and Redman 1981). As with all NPVs, it has a double stranded circular DNA genome of 80-100 x 10⁶ Da within a rod shaped, enveloped particle often found occluded within proteinaceous bodies (Rohrmann 1986).

MBV has been identified and reported in *P. monodon*, *P. merguensis*, *P. semisulcatus*, *P. kerathurus*, *P. vannamei*, *P. esculentus*, *P. penicillatus*, *P. indicus*, *Metapenaeus ensis* (Johnson and Lightner 1988, Lightner 1988, Chen et al. 1989a, Ramasamy et al. 1995, Vijayan et al. 1995, Karunasagar et al. 1998b). The occurrence of MBV with WSSV and HPV in hatchery reared *P. monodon* post larvae was reported for the first time in India by Manivannan et al. (2002).

MBV is a common, widespread pathogen and despite its wide distribution, is not a highly virulent pathogen of *P. monodon* (Nash et al. 1988). However, occasionally, severe mortalities may be observed in post-larval (over 90%) and juvenile stages (70%). Shrimps infected with MBV show a significant growth retardation and the hepatopancreas of affected shrimps become generally pale yellow to brownish referred to as "white turbid liver" instead of normal grayish green color (Lightner et al. 1983b, Chang and Chen 1994). MBV is generally found in mixed infection with other pathogens including viruses (IHHNV, HPV, WSSV), bacteria (*Vibrio* spp, *Pseudomonas* spp), parasites (*Zoothamnium* spp, *Epistylis* spp.; Anderson et al. 1987, Lightner et al. 1987, Chen et al. 1989a, Manohar et al. 1996, Karunasagar et al. 1998a, Umesha et al. 2003). The target organs of MBV are hepatopancreas and anterior midgut (Lightner et al. 1983b).

The principal diagnostic feature of MBV infection is the presence of hypertrophied nuclei with single or multiple spherical occlusion bodies (Lightner et al.

1983b, Fegan et al. 1991). Direct staining with malachite green and conventional histopathology was used initially to detect MBV (Lightner and Redman, 1991). However, rapid and sensitive molecular techniques like PCR (Vickers et al. 1992, Chang et al. 1993, Lu et al. 1993) and genomic probes for its detection have been developed (Vickers et al. 1993, Poulos et al. 1994b). ELISA has also been developed to detect MBV (Hsu et al. 2000). A nested PCR detection has also been described (Belcher and Young 1998, Otta et al. 2003).

Transmission of MBV occurs only horizontally through fecal oral route. MBV in the fecal matter of brood stock, infect eggs and larvae in hatcheries. The prevalence of MBV in the hatchery can be substantially reduced by washing the eggs or nauplii before they are transferred to rearing tanks (Chen et al. 1992, Natividad and Lightner 1992). The best way to eliminate MBV from hatchery is to identify carrier broodstock or to spawn females individually and discard contaminated batches of larvae (Flegel et al. 1995a). MBV is relatively well tolerated by *P. monodon* so long as rearing conditions are optimal (Fegan et al. 1991).

Hepatopancreatic Parvovirus (HPV)

The HPV was first reported by Lightner and Redman (1985) in postlarvae of *Penaeus chinensis*. In Thailand, HPV in the black tiger shrimp, *P. monodon*, was first reported in 1992 by Flegel and Sriurairatana (1993, 1994). Presence of HPV in *P. monodon* postlarvae in India was reported by Manivannan et al. (2002) and Umesha et al. (2003). This virus infects several penaeid species and is widely distributed in many parts of the world, including Asia, Africa, Australia and north and South America (Paynter et al. 1985, Colorni et al. 1987, Brock and Lightner 1990, Fulks and Main 1992, Lightner and Redman 1992, Lightner, 1996a).

Shrimps affected by HPV usually show non-specific gross signs, including atrophy of the hepatopancreas, anorexia, poor growth rate, reduced preening activities and as a consequence increased tendency for surface and gill fouling by epicomensal organisms (Lightner and Redman 1985, Chen 1992, Lightner et al. 1992, Sukhumsirichart et al. 1999). A number of cultured and wild penaeids have been reported as hosts for HPV (Flegel et al. 1992b, Bower et al. 1994, Turnbull et al. 1994, Manjanaik et al. [in press]). High levels of HPV infection have been reported especially in early juvenile stages (Flegel et al. 1995, Lightner, 1996a) and the transmission of HPV is believed to be both vertical and horizontal (Lightner and Redman, 1992).

HPV has been isolated and characterized from *P. chinensis* (HPV chin) from Korea (Bonami et al. 1995b) and from *P. monodon* (HPVmon) from Thailand (Sukhumsirichart et al. 1999). Both viruses comprise unenveloped, icosahedral particles of approximately 22 to 24 nm diameter as seen by negative staining by TEM. The nucleic acid of both is single stranded DNA, although the genome size of HPV chin was reported to be 4 to 4.3 kb while that of HPV mon was reported to be 5.8 kb. Based on genome size it appears that HPV chin and HPV mon are quite different.

HPV can be diagnosed histologically (Lightner and Redman, 1985, 1992). A rapid field test using Giemsa stained smears of hepatopancreas has been reported, but the sensitivity and accuracy of detection is low (Lightner 1996a). Gene probes (Bonami et al. 1995b, Mari et al. 1995) and sensitive PCR assay (Sukhumsirichart et al. 1999, Pantoja and Lightner 2000, Phromjai et al. 2002) have also been developed for diagnosis of HPV. Due to difference in the genome of HPVchin and HPVmon, primers described by Pantoja and Lightner (2000) do not produce amplicons with HPVmon. Using nested PCR, HPVmon has been detected in number of wild shrimp species including *P. monodon* (Manjanaik et al. in press)

Infectious hypodermal hematopoietic necrosis virus (IHHNV)

IHHNV was first detected in juvenile *Penaeus stylirostris* from Hawaii in 1981 (Lightner et al. 1983a). The virus has since been detected in a number of other penaeid species and from stocks around the world, including the Americas, Oceania and Asia (Lightner 1996a,b, Flegel 1997).

IHHNV is a small, icosahedral non-enveloped virus containing a single stranded linear DNA genome approximately 4.1 kb in length (Bonami et al. 1990, Mari et al. 1993).

Nearly 100% of the IHHNV genome has been sequenced (Nunan et al. 2000, Shike et al. 2000). It contains 3 large open reading frames (ORF1, 2 and 3). ORF1 comprises approximately 50% of the genome encoding a polypeptide of 666 amino acids, which is predicted to be a non-structural protein 1 (NS1) based upon its degree of homology with 2 mosquito brevidensovirus (Shike et al. 2000). ORF2 encodes a 343 amino acids putative non-structural protein 2 (NS2). ORF 3 encodes a 329 amino acids polypeptide.

Effects of this virus vary among penaeid species. For example, shrimps such as *P. vannamei* and *P. monodon* infected with IHHNV do not show mortality. However, infection by this virus results in a disease called runt–deformity syndrome (RDS) in both species

(Bell and Lightner 1984, Kalagayan et al. 1991, Primavera and Qunitio 2000), and this can also cause substantial economic losses (Wyban et al. 1992).

Detection of this virus is usually done by histopathology (Lightner et al. 1983a, Lightner 1996a). In-situ hybridization and polymerase chain reaction (PCR) provide the highest available detection sensitivity for IHHNV (Lightner et al. 1992a, 1994). A real-time PCR method using a fluorogenic 5' nuclease assay detector has been developed to detect this virus in penaeid shrimps (Tang and Lightner 2001). The infected shrimp show a reduced food consumption, cannibalism, lethargy and increased mortality. Affected animals show an opaque abdominal musculature and numerous focal melanised areas.

Yellow Head Virus (YHV)

YHV was first detected in central Thailand in 1990 in pond reared black tiger prawns *Penaeus monodon*. It is a positive–sense, single stranded RNA virus (genus Okavirus) in a new family Roniviridae of the order Nidovirales (Cowley et al. 2000, Mayo 2002). Yellow head disease is usually characterized by light yellow coloration of the dorsal cephalothorax area and generally pale or bleached appearance of affected shrimp. The yellow color in the cephalothorax region results from the underlying yellow hepatopancreas visible through the translucent carapace in moribund shrimp (Chantanachookin et al. 1993).

YHV is widespread in cultured stocks of *P. monodon* in Thailand. Shrimps infected with YHV die within a few hours of developing color and the whole crop can be lost within 3-5 days after the first appearance of affected shrimp (Flegel et al. 1995b). YHV has also been shown to infect and cause disease in *P. vannamei* and *P. stylirostris* (Lu et al. 1994). *Palaemon sylliferus* and *Acetes* sp. have been recorded as carriers of YHV (Flegel et al. 1995b).

YHV is known to exist as at least 3 different genotypic clades (Walker et al. 2001). The original YHV clade reported from Thailand differs from gill associated virus (GAV) clade of Australia by approximately 15% in nucleic acid sequence (Cowley et al. 1999). Third intermediate clade has been found in Thailand and Vietnam (Walker Per. Comm.). These viruses have been included in a YHV-complex (Walker et al. 2001).

YHV infections can be diagnosed histologically in infected shrimp by the appearance of massive systemic necrosis and basophilic cytoplasmic inclusions in tissues of ectodermal and mesodermal origin (Boonyaratpalin et al. 1993, Chantachookin et al. 1993). For the detection of YHV, a number of rapid diagnostic procedures like simple staining

(Flegel and Sriurairatana, 1993, 1994) dot blot nitrocellulose enzyme immunoassay (Lu et al. 1996, Nadala and Lo 2000), western blot technique (Nadala et al. 1997), reverse transcriptase PCR (Wongteerasupaya et al. 1997) and gene probe (Tang and Lightner, 1999) have been developed. Immunological techniques can also be used for detection of infection. Antibodies against YHV were produced in Thailand (Sithigorngul et al. 2000, 2002) to assist in histopathological diagnosis of YHV and in the development of rapid test for field use.

Gill-associated virus (GAV)

Gill associated virus (GAV) was first noticed in Queensland, Australia where it has caused significant mortalities in juvenile, adult cultured and wild spawners of *P. monodon* since 1996 (Spann and Lester 1997). GAV is a rod-shaped, enveloped (+) RNA nidovirus closely related to the YHV from Thailand in genome sequence and organization (Cowley et al. 1999, Cowley and Walker 2002). *P. monodon* infected with GAV displays pink to red coloration of the body and appendages, and pink to yellow coloration of the gills. Other signs of disease include lethargy, lack of appetite, secondary fouling and tail rot (Spann et al. 1997). Nucleotide sequence comparison of regions in the putative polymerase genes of multiple GAV and LOV isolates has indicated that they are genetically indistinguishable populations (Cowley et al. 2000b). Screening of wild and cultured penaeids using the sensitive RT-nested PCR test has indicated that *P. monodon* is the only known natural host of GAV in Queensland (Cowley et al. 2000b). Spann et al. (2000) have shown that *P. monodon*, *P. esculentus*, *P. japonicus* and *P. merguensis* are susceptible to GAV infection and develop disease.

Baculoviral midgut gland necrosis virus (BMNV)

BMNV was first reported by Sano et al. (1981). It is a type C baculovirus of penaeid shrimp and is distinguished from Type A baculovirus (BP and MBV) by its inability to produce an occlusion body in the nuclei of infected cells (Mathews 1982, Johnson and Lightner 1988). BMNV has become a serious problem to hatchery-reared larvae of *P. japonicus* in southern Japan (Sano et al. 1981, 1984, 1985, Sano and Fukuda 1987). A sudden onset and a high mortality rate characterize BMN disease in the larval stages. The disease is most severe in the PL stages up to about PL-9 or PL-10, by which time cumulative mortalities typically reach up to 98% of affected population and to decrease rapidly by PL-20. The typical signs of this disease is white turbid midgut gland that shows remarkable cell necrosis and no inclusion body in the section, rod shaped particles,

resembling baculovirus are found in the affected nuclei under the electron microscope (Takahashi et al. 1998). Diagnosis may be confirmed by histological demonstration of the characteristic pathology of BMN. Hepatopancreatic tubercle epithelial cells undergoing necrosis possess markedly hypertrophied nuclei that are characterized by marginated chromatin, diminished nuclear chromatin, nuclear dissociation, and the absence of occlusion bodies (Sano et al. 1981, 1984, 1985, Momoyama 1993, Sano and Fukuda 1987). A fluorescent antibody technique has been developed in Japan for rapid detection of the virus (Sano et al. 1984, Momoyama 1988). Sano and Momoyama (1992) have developed a technique for rinsing eggs to prevent the shrimp larvae from becoming infected with BMNV.

Baculovirus penaei (BP)

The BP was first detected in 1974 in the pink shrimp, *Penaeus duorarum* (Couch 1974a, b). BP has been reported to cause significant mortalities in the larval, post-larval and early juvenile stages of *P. aztecus*, *P. stylirostris*, *P. vannamei* and *P. penicillatus* (Couch 1991, Lightner and Redman 1991, 1992).

BP infects only the hepatopancreas and midgut epithelial cells, and it is transmitted from shrimp to shrimp (Lightner and Redman 1998b). The typical route of infection of shrimp larvae is via fecal contamination of spawned eggs from BP infected adult spawners (Johnson and Lightner 1988, Lightner 1996a) fecal oral contamination through feces from infected larvae or from cannibalism of diseased larvae (Overstreet et al. 1988, LeBlanc and Overstreet 1990, 1991).

Detection of BP is done by examining fresh squash preparation of the hepatopancreas or feces for the presence of tetrahedral occlusion bodies by light microscope (Overstreet et al. 1988, Lightner and Redman 1992). Advanced techniques like gene probes and immunodiagnosics like ELISA have been developed to detect this virus (Lewis 1986, Lightner et al. 1992a, Bruce et al. 1994a). A PCR based detection procedure has also been developed (Wang et al. 1996).

Taura syndrome virus (TSV)

Taura syndrome was reported as a new disease in 1992 in commercial penaeid shrimp farms located near the mouth of River Taura in the Gulf of Guayaquil, Ecuador (Jimenez 1992). Since its discovery, this lethal shrimp disease has spread into major shrimp growing region in the Americas by mid 1996 (Lightner 1996 a,b, Lightner et al. 1997). This disease represents a serious problem in the culture of *P. vannamei* due to

the high level of mortality and the economic losses (Lightner et al. 1997). TSV causes 3 distinct disease phases in infected shrimp. The peracute/acute phase of the disease is characterized by moribund shrimp displaying an overall pale reddish coloration caused by the expansion of the red chromatophores. Shrimp in this phase usually die during the process of moulting. If the shrimp survive through the peracute/acute phase, the recovery phase begins. Multifocal, melanized cuticular lesions are the major distinguishing characteristics of the recovery phase (Lightner 1996a,b). In the chronic phase of TSV infection, infected shrimp appear and behave normally, but remain persistently infected perhaps for life (Hasson et al. 1997b).

The initial pointers to the cause of TS were two commonly used banana fungicides Tilt and Calixin and the condition was thought to be toxicity syndrome (Jimenez, 1992). Later transmission electron microscopy studies of infected shrimp demonstrated the presence of putative cytoplasmic virus particles named TSV (Brock et al. 1995).

Rapid spread of the TSV in pond populations occurs through cannibalization of infected moribund and dead shrimp by healthy members of the same population (Brock et al. 1995, Hasson et al. 1995). TSV infected shrimp display histological lesions characteristic of the disease, which are necrosis, and nuclear pyknosis of the cuticular epithelium of the general body surface, appendages, gills, mouth, esophagus, stomach and hindgut (Brock et al. 1995, Lightner, 1996b). The lesion is characterized by the presence of inclusion bodies that give TSV lesion a “peppered” or “buckshot” appearance which is considered to be pathognomonic for the disease (Brock et al. 1995, Hasson et al. 1997). For the detection of TSV, gene probes (Lightner, 1996a, Hasson et al. 1997, 1999, Mari et al. 1998) and RT-PCR (Nunan et al. 1998) have been developed.

Lymphoid organ Parvo-like virus (LOPV)

LOPV was first detected by Owens et al. (1991) in cultured *P.monodon*, *P.merguensis* and *P.esculentus* in Australia. Affected shrimps exhibit multinucleated giant cell formation in their hypertrophied lymphoid organs (Owens et al. 1991). Cells making up the “giant cells” in the Australian shrimp displayed mild nuclear hypertrophy and marginated chromatin and found discrete, often fibrocyte encapsulated spherical structures identical to the lymphoid organ spheroids described in *P.monodon* from Taiwan (Lightner et al. 1987a). Basophilic intranuclear inclusion bodies are commonly found in giant cells and these were found to contain DNA by acridine orange staining and fluorescent microscopy (Owens et al. 1991).

LOPV is somewhat similar to IHHNV with rare cowdry type A inclusion bodies (Owens and Hall – Mendelin, 1990). In the hybrid animals of *P.monodon* and *P.esculentus*, a low grade mortality was observed from the time the shrimps were 3-4 g body weight (Munday and Owens, 1998). LOPV is still a poorly understood viral disease of penaeid shrimps.

Lymphoid organ vacuolization virus (LOVV)

LOVV is a rod shaped enveloped RNA virus (Toga-like virus) which is endemic in healthy wild and cultured *P.monodon* in Queensland (Spann et al. 1995). It may occur as a common, if not a significant pathogen in Asian and Australian penaeid shrimps (Bower et al. 1994). The histological picture is very similar to that found in the YHV infections. However, transmission electron microscopy showed that the inclusions were cytoplasmic and consisted of amorphous material next to hypertrophied nuclei (Flegel et al. 1995). Histological changes include highly vacuolated cytoplasm and intracytoplasmic inclusion bodies that range from Fuelgen positive basophilic and discrete bodies in the cytoplasm of lymphoid organ cells. The nuclei of affected cells are slightly hypertrophied with marginated chromatin, in some foci, affected cells form spheroids that lack a central vessel (Bonami et al. 1992). Not much is known about the prevalence and pathogenicity of LOVV in wild or cultured penaeid shrimp.

Spawner –isolated mortality virus (SMV)

Fraser and Owens (1996) reported SMV for the first time in *P.monodon* at a research facility in Townsville, northern Queensland, and Australia in 1993. SMV has been associated with mortalities in broodstock of *P.monodon* and with mud –crop mortality syndrome on grow-out ponds. The affected animals exhibited lethargy, failure to feed, redness of the carapace and pleopods and an increased mortality rate. Experimental infection has produced 100% mortality. Excretion of red feces is a characteristic feature of this disease. The small (20 nm), icosahedral virions were observed in gut cells with transmission electron microscopy and partial characterization indicated that it was a non-enveloped DNA virus, similar to parvovirus (Fraser and Owens 1996). This virus is similar or identical to mid-crop mortality syndrome (MCMS) viral agent (Owens et al. 1998). Natural infection of the red-claw crayfish *Cheax quadricarinatus* with SMV is recorded in Australia, but it is not known whether the SMV is transferred from shrimp to crayfish or from crayfish to shrimp (Owens and McFlena, 2000).

Owens et al. (1998) reported that, gastrointestinal tract is the first tissue to be infected by SMV. Target organs of SMV include the hepatopancreas, midgut caecae,

midgut and to a lesser extent, the hindgut caecae. In a heavy infection, the virus would break through the lamina propria of the gut and become systemic, localizing in the lymphoid organ, gonads and heart. Therefore, the most likely route of SMV would be through the gut, voided with the feces (Owens et al. 2003).

Rhabdovirus of penaeid shrimp (RPS)

RPS was reported from an IHNV infected *P. stylirostris* (Lu et al. 1991). It is a bullet shaped ssRNA virus. This virus has been isolated from the American penaeid shrimp, *P. vannamei* and *P. stylirostris* but not in other species (Lightner et al. 1996a). When the blue shrimp *P. stylirostris* was infected experimentally, it did not develop any clinical signs and mortality. A streptavidin-biotin-enhanced nitrocellulose enzyme immunoassay was developed for detection of rhabdovirus of penaeid shrimp in the tissue of infected animals (Nadala et al. 1992).

Management of viral diseases

Since there is no known treatment for viral diseases, the major strategy for disease management is avoidance. However, in practice this is very difficult. In Asia, the devastating mortalities caused by WSSV have led to development of strategies such as PCR screening of brood stock before spawning and PCR screening of larvae before stocking to avoid the entry of virus into aquaculture system. But, this has led to only partial success because WSSV has a broad host range (Lo et al. 1996a, Hossain et al. 2001a, 2001b) and can survive in a number of carrier animals that can be a source of virus for the ponds. Further, WSSV survives in water up to 20 days and therefore, in areas with high density of farms, discharged water from heavily infected farms could be taken up by uninfected farms and thus virus could spread into ponds stocked with PCR negative seeds. Thus, PCR screening and stocking virus free seeds could only reduce the risk of crop loss but cannot guarantee against WSSV infection during culture period.

PCR monitoring of culture ponds show long-term presence of WSSV in apparently healthy animals from ponds going through a normal crop. This shows that though animals are infected, they do not come down with the disease often mortalities are precipitated by sudden stress such as salinity stress (sudden rain) or temperature stress (winter). Treatment of *P. monodon* with immunostimulants has been shown to help overcome WSSV infection (Karunasagar et al. 1996, Sano et al. 2003). Though there is no evidence of specific immune response in shrimps, there is

evidence to show that non-specific immune response can be stimulated by immunostimulants (Devaraja et al. 1998).

Fungal diseases

Fungi occur in aquatic environment and about 500 fungal species have been isolated from marine and estuarine environment. Some of the aquatic fungi are opportunistic pathogens of shrimp. Mostly larval stages are affected and the common causative agents are *Lagenidium callinectes* and *Serolpidium* spp. The protozoa and mysis stages are generally affected with clinical signs such as lethargy and mortality. Fungal spores and mycelia are observed in affected tissue, particularly gill and appendages. Larval mycosis is a problem in many hatcheries in India. Gopalan et al. (1980) reported *Lagenidium marina* and *Serolpidium parasitica* infection in *P. monodon*. Ramasamy et al. (1996) reported mortalities in *P. monodon* larvae at nauplii, zoea and mysis stages. Fusariosis and black gill disease caused by *Fusarium* spp may affect all developmental stages of penaeid shrimp. *Fusarium* spp (*F. solani*, *F. moniliformae*) are opportunistic pathogens that may lead to high mortalities (90%). Disease is noticed in ponds where water quality management is poor. Fungal hyphae can be detected in affected animal tissue using light microscopy.

Parasitic diseases

A number of parasites, particularly protozoa, may affect shrimp at different developmental stages. Epi and edocommusal protozoa may be found adhering to gills, cephalothorax, periopod and other appendices and also internal organs. At high levels of infection, these protozoa may induce gill obstruction (brown gill) leading to anorexia, reduced growth, locomotion and increased susceptibility to infection by opportunistic pathogens. Protozoa such as *Zoothamnium*, *Epistylis*, *Vorticella*, *Anophrys*, *Acineta* sp, *Lagenophrys* and *Ephelota* may be encountered as external parasites.

Ciliates such as *Paranophrys* spp and *Parauronema* sp may cause mortalities in larvae and juveniles. Ciliates may enter shrimp body through wounds and invade hemolymph and gills. This may lead to mass mortalities, particularly in conjunction with other parasitic flagellates such as *Leptomonas* sp. Diagnosis can be achieved through examination of hemolymph which appears turbid, does not clot and shows reduced hemocyte count and numerous ciliates.

Gregarians are endoparasitic protozoa infecting shrimp. These parasites generally have two hosts – usually a mollusk or an annelid worm and crustaceans. Gregarians found in shrimp include *Nematopsis* spp,

N. litopenaeus, *Paraphioidina scolecoide*, *Cephalobolus litopenaeus*, *C. petiti* and *Cephaloidophoridae stenai*. Trophozoites and gametocytes may be found attached to the intestinal wall or may occur in the lumen. The parasites may cause reduced absorption of food from the gut and occasionally intestinal blockage, but these diseases seem to have little impact on aquaculture.

Microsporidia such as *Agmasomasp*, *Microsporidium* sp may invade the muscle, heart, gonads, gills or hepatopancreas. The common microsporidian disease is cotton shrimp disease. The infection leads to opacity of the affected tissue and shrimp may appear crooked. Though infection may not be lethal, the appearance affects marketability. The diagnosis can be established by demonstrating microsporidian spores in the affected muscle tissue.

Other diseases

Swollen hind gut syndrome (SHG)

The SHG was first reported by Lavilla Pitogo et al (2002) in *P.monodon* post larvae. SHG mainly affected the hindgut and to some extent, the posterior midgut. PL infected with SHG shows enlargement and distension of the hind-gut folds and its junction with the midgut. In some cases, swollen midgut was also

noticed. SHG causes gradual mortality in affected postlarvae, but larvae shows no abnormal swimming behaviour.

SHG has direct impacts on the hatchery and may also affect growout systems (Lavilla-Pitogo *et al.*, 2002). It has been suspected that natural food and artificial feed quality, husbandry practices, water quality and presence of toxic substances from chemical prophylactics are responsible for SHG, but no specific cause has been pinpointed so far (Lavilla-Pitogo *et al.*, 2002). Direct microscopic examination of PL is a must for the detection of SHG.

SHG can be controlled by good water quality and feed management. Use of newly-hatched batches of brine shrimp should be maximized to avoid left over on sanitary procedures for nauplii production and enrichment should be employed (Dhont *et al.*, 1993).

Management of shrimp diseases

Effective management of the health of shrimp requires consideration of the fact that there is a delicate balance between the host, pathogen and environment. Most often pathogens are present in association/ in the environment and shrimp are apparently healthy and show normal growth. Often conditions such as high stocking density, poor water quality, sudden changes

Table 1. Examples of bacterial species used as probiotics in aquaculture.

Probiotic	Application	Suggested mode of action
<i>Vibrio</i> and <i>Lactobacillus plantarum</i>	Addition to water in halibut larval tanks	Immunostimulation
<i>Bacillus</i> spp	Addition to rotifer diet before feeding turbot larvae	Antagonism and/ or improved nutrition
<i>Lactobacillus</i> or <i>Carnobacterium</i>	Enrichment of rotifers used for feeding turbot larvae	Antagonism and/ or improved nutrition
<i>Vibrio alginolyticus</i> like bacteria	Enrichment of rotifers used for feeding turbot larvae	Antagonism and/ or improved nutrition
<i>Carnobacterium divergens</i>	Addition to diet of Atlantic cod fry	Antagonism
<i>Pseudomonas fluorescens</i>	Addition to culture water in rainbow trout culture	Competition for iron
<i>Vibrio alginolyticus</i>	Bathing shrimp larvae in bacterial suspension	Antagonism
<i>Bacillus</i> strain S11	Addition shrimp larval diet	Antagonism
<i>Vibrio alginolyticus</i>	Addition to hatchery water with <i>P. vannami</i> postlarvae	Antagonism
<i>Bacillus</i>	Addition to pond water in <i>P. monodon</i> culture	Antagonism

in environmental factors precipitate disease. Some of the important strategies for health management have been outlined below:

Avoidance of pathogens: This can be done through selection of specific pathogen free broodstock, exclusion of carrier animals in culture systems, filtration and sanitisation of water before intake.

Improving host conditions through good nutrition and immunostimulation: A number of microbial molecules such as β 1,3 glucans, peptidoglycans, polysaccharides have been shown to stimulate the non-specific immune mechanisms in shrimp (Itami et al. 1994, Sung et al. 1996, Karunasagar et al. 1996, Devaraja et al. 1998).

Improving environmental conditions: Most disease problems are triggered by deterioration of water quality. It has been estimated that less than 20% of nitrogen and 10% of phosphorus input into shrimp pond gets incorporated into animals (Funge –Smith and Briggs 1998), the rest ends up in the environment. Degradation of accumulated wastes by bacteria producing toxic gases like ammonia and hydrogen sulphide could lead to blackening of the pond sediment and affect the health of animals. Microorganisms are metabolically versatile and certain autotrophic bacteria such as *Nitrosomonas* and *Nitrobacter* can oxidize ammonia to nitrate and nitrite. Sulphide oxidising bacteria can oxidize sulphides to sulphates. Rao et al. (2000) demonstrated that bacteria capable of oxidizing ammonia and nitrite are generally found in shrimp culture ponds but on their levels could be low at certain occasions, particularly at the later part of the culture period. This suggests that supplementation of bacteria capable of oxidizing toxic wastes (bioaugmentation) could be useful in improving water quality in shrimp culture ponds.

There is increasing interest in using probiotics in aquaculture. Traditionally, the term probiotics has been used for “live microbial feed supplement that improves the health of host animals by improving intestinal balance” (Fuller 1989). In the context of aquaculture, the intestinal microbiota are constantly influenced by the aquatic microflora. Thus, a modified definition of probiotics for aquaculture has been proposed (Verschuere et al. 2000). A probiotic is defined as a live microbial adjunct which has beneficial effect on the host by modifying the host associated or ambient microbial community, by ensuring improved use of feed or enhancing nutritional value, by ensuring improved use of feed or enhancing nutritional value, by enhancing response towards disease or by improving quality of ambient environment. Some of the common bacterial groups used as probiotics in aquaculture and their proposed mode of action is indicated in Table 1 above.

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