[5] Diseases Caused By Viral Pathogens

3.3.5.1 Disease Agent

ISAV is classified as the type species of the genus *Isavirus* within the viral family designated *Orthomyxoviridae* by the ICTV (King et al, 2012). ISAV is a pleomorphic enveloped virus, 100-130 nm in diameter, with 10-12 nm surface projections. The viral genome consists of eight single-stranded RNA segments of negative polarity. ISAV has been divided into two major clusters or genotypes, called the North American and the European, and the third genotype may exist.

3.3.5.2 Diagnostic Methods

ISA should be a consideration in Atlantic salmon with increased mortality and signs of anemia, or lesions consistent with this disease. It should always be investigated if the hematocrit is less than 10%. External clinical signs in infected fish are pale gills and muscle, petechial hemorrhage, dark liver and spleen, ascites are all present. The presence of renal interstitial hemorrhaging, and tubular epithelial degeneration, necrosis, and casting within the posterior kidney have all been noted. Liver sections show multifocal to confluent hemorrhagic hepatic necrosis, focal congestion, and dilatation of hepatic sinusoids.

3.3.5.3Control

Same as IHNV. Mixed vaccines are commercially available in Norway.

6) Other diseases

Erythrocytic inclusion body syndrome (EIBS), pancreas disease (PD), sleeping disease (SD), viral wiring disease (VWD) and viral erythrocytic necrosis (VEN) are reported. In case of EIBS, erythrocytic inclusions and typical virus particles have been detected in spawned adult salmon, but in fresh water the disease seems to be limited to juvenile and smolting fish. In salt water, EIBS has been diagnosed in fish as large as 500 g and has caused problems in netpen culture. Naturally occurring infections have been demonstrated in coho, Chinook and Atlantic salmon. The agent that causes EIBS has neither been isolated nor fully characterized. PD and SD are infectious diseases of farmed Atlantic salmon and rainbow trout, respectively. Salmonid alphaviruses are recognized as pathogen. The main pathological lesions are similar in PD and SD, with extensive loss of pancreatic acinar cells during the viraemic phase, concurrent cardiomyocytic degeneration and inflammation and subsequent skeletal muscle degeneration and fibrosis. VWD was first recognized in coho salmon in Japan (Oh et al, 1995). VEN is a condition characterized by the presence of viroplasmic inclusion bodies within the cytoplasm of affected erythrocytes. The causative agent is tentatively placed in the *Iridovirid*ae.

4. Shrimp Diseases

Toshiaki Itami and Raja Sudhakaran

4.1. Synopsis

Penaeid shrimp culture is an important industry in Asia and Americas. The total annual production in 2010 reached over 3.7 million tons in the world (FAO, 2012). However,

diseases, especially the viral diseases, are being a major negative factor to this industry apart from the bacterial, fungal diseases and parasites. Therefore, six important viral diseases are reviewed in this section.

4.2. Introduction

The shrimp culture industries have been spreading worldwide since the 1970s, originally starting from Japan. With their expansion, various viral and bacterial diseases have been occurring and causing serious damage to the shrimp culture industries worldwide. *Vibrio penaeicida* infection spread from the late 1980s, and then after 1990s, major diseases in shrimp aquaculture changed from bacterial diseases to viral diseases. White spot syndrome (WSS) which is viral disease and has high mortality rate has spread in shrimp culture in Asia and in central and south Americas (Lightner et al, 2012; Lightner2011; Flegel, 2012).

The World Animal Health Organization (the OIE: Office International des Epizooties) lists 6 virus diseases out of 8 diseases of crustacean including fungal and bacterial diseases (Table 4.1) (OIE, 2012). In this section, white spot syndrome, yellow head disease, infectious hypodermal and hematpoieitc necrosis, Taura syndrome, white tail disease and infectious myonecrosis are discussed which are damaging shrimp aquaculture industries.

4.3. Disease Agent (Characteristics, Genome Size, Serological Classification, Molecular Classification, Pathogenesis)

4.3.1 White Spot Syndrome

The causative agent of this disease is white spot syndrome virus (WSSV). WSSV is a large (80-120 x 250-380 nm), enveloped, double-stranded DNA virus and was assigned to a new genus *Whispovirus*, and family *Nimaviridae* (Mayo, 2002,2005) (Figure 4.1). Virions show tail-like appendages. The size of genome of this virus is ~300 kb: 305,107 bp (GenBankaccession No.AF332093), 292,967 bp (AF369029) and 307,287 bp (AF440570). Virulence is quite high and the mortality reaches up to 80-90 % in 3-10 days in juvenile shrimp. WSSV has a wide host range among over 70 species of crustaceans including shrimp, crabs and crayfish (Flegel, 2006).

4.3.2 Yellow Head Disease

The causative agent of this disease is yellow head virus (YHV, genotype 1), gill-associated virus (GAV, genotype 2) (Spann et al, 1997) and other related viruses known as genotype 3-6 (OIE, 2012 Mayo, 2002; Walker et al, 2005). YHV, the genus *Okavirus* in the family of *Roniviridae*, is enveloped, rod-shaped (40-60×150-200nm), single stranded RNA virus with a genome of ~27 kb (Figure 4.2). YHV (genotype 1) was observed mainly in Southeast Asian countries and Mexico, and GAV and other genotypes were found in Australia, Mozambique and Southeast Asia (Wijegoonawardane et al, 2008). Black tiger shrimp (*Penaeus monodon*) is the major susceptible host species and shows the high and rapid mortality. Natural infections were reported in other penaeid shrimp, *e.g. Marsupenaeus japonicus*, *Fenneropenaeus merguiensis*, *Litopenaeus*

stylirostris and *L. vannamei* (Soowannayan et al, 2003).

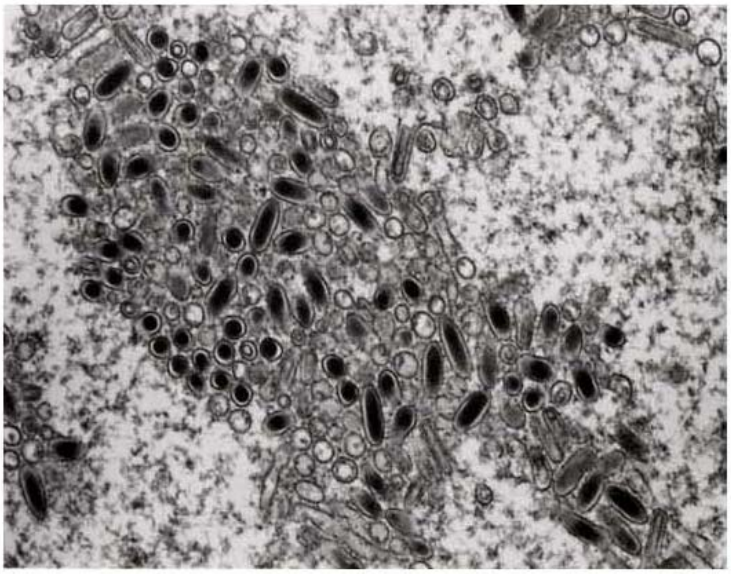

Figure 4.1. The electron micrograph of WSSV virions in the infected lymphoid organ. Photo courtesy of Dr. M. Maeda.

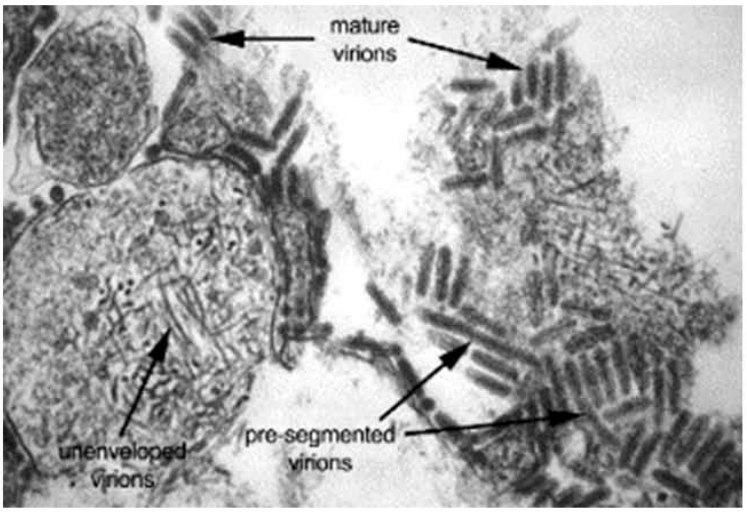

Figure 4.2. Transmission electron micrograph of a YHV-infected shrimp tissue section showing the unusual filamentous nucleocapsid precursors (on the left) and mature, rod-shaped, enveloped virions (on the right). (Flegel, 2006. *Aquaculture* 258: 1 - 33)

Disease name	**causative agents**		**susceptible species, stage and target organ**		
	virus name	**taxon**	**species**	**stage**	**target organ/tissue**
white spot syndrome, white spot disease	WSSV, WSDV	family *Nimaviridae* genus *Whispovirus*	penaeid shrimp	PL - adults	ectodermal and mesodermal origin
yellow head disease	YHV (gill-associated virus: GAV)	family *Roniviridae* genus *Okavirus*	penaeid shrimp	PL15-adults	ectodermal and mesodermal origin
infectious hypodermal and hematopoietic necrosis	IHHNV	genus Brevidensovirus family Parvoviridae	penaeid shrimp	egg - adults	ectodermal and mesodermal origin
Taura syndrome	TSV	family Dicistroviridae genus *Aparavirus*	penaeid shrimp	PL - adults	cuticular epithelium (the general exoskeleton, foregut, hindgut, gills and appendages), connective tissues, haematopoietic tissues, lymphoid organ, antennal gland
white tail disease	*Mr*NV XSV	MrNV: family Nodaviridae XSV: extra small virus	*Macrobrachium rosenbergii*	larvae - PL - juveniles	gill tissue, head muscle, heart, abdominal muscle, ovaries, pleopods tail muscle
infectious myonecrosis	IMNV	family *Totiviridae (closely related to Giardia lamblia virus)*	penaeid shrimp	juveniles - subadults	striated muscles, connective tissues, haemocytes, lymphoid organ

Table 4.1. OIE listed virus diseases in shrimp as of 2012 (OIE, 2012)

4.3.3 Infectious Hypodermal and Hematpoieitc Necrosis

The causative agent of this disease is infectious hypodermal and hematpoieitc necrosisvirus (IHHNV). This virus was assigned to the genus *Brevidensovirus*, family *Parvoviridae*. IHHNV is a 20-22 nm, non-enveloped icosahedron, containing single-stranded DNA with estimated size of 3.9 kb (Lightner2011; OIE, 2012; Lightner et al, 1983; Bonami and Lightner, 1991; Bonami et al, 1990 ; Rai et al, 2011). IHHNV has at least three genotypes: IHHNV-I from Americas/Philippines, IHHNV-II from Southeast Asia and IHHNV-III from East Africa and Australia (Lightner et al, 2012). The most penaeid shrimp show the susceptibility to IHHNV-I and IHHNV-II, and principal hosts are *L. stylirostris*, *L. vannamei* and *P. monodon*. IHHNV causes the acute epizootics and mass mortality in *L.stylirostris* (>90%), while this virus causes runt-deformity syndrome (RDS) and reduced, irregular growth, rather than heavy mortalities, in *L. vannamei* (Kalagayan et al, 1991). *P. monodon* usually show the subclinical infection, however IHHNV-infected shrimp have been reported to show RDS and low growth rate, causing economic damage (Chayaburakul et al, 2005; Primavera et al, 2000).

4.3.4 Taura Syndrome

The causative virus Taura syndrome virus (TSV) is 32 nm diameter and nonenveroped icosahedron (Figure 4.3). The genome consists of a linear single-stranded RNA of 10.2 kb. TSV has at least four genotypes: the Americas group, Southeast Asian group, the Belize group and the Venezuelan group (OIE, 2012; Wertheim et al, 2009). TSV has been assigned to the newly created genus *Aparavirus* in new family *Dicistroviridae* in the order of *Picornavirales* in the 9th report of the ICTV (Lightner et al, 2012; Chen et al, 2012). Major hosts of this virus are *L. vannamei*and*L.stylirostris*and other penaeid species can be infected with TSV by experimental challenge.

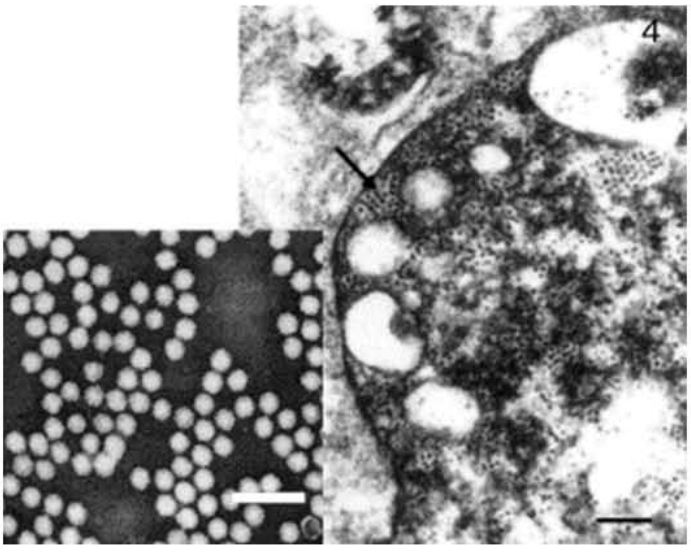

Figure 4.3. TSV by TEM. The electron micrograph above shows TSV in the cytoplasm by normal TEM using thin tissue sections from TSV infected *P. vannamei*. The upper inset micrograph shows purified TSV virions by negative staining. (Brock et al., 1997). (Flegel, 2006. *Aquaculture* 258: 1 - 33)

4.3.5 White Tail Disease

The causative agents of this disease are two viral pathogens: *Macrobrachium rosenbergii* nodavirus (*Mr*NV) and extra small virus (XSV) (Qian et al, 2003). *Mr*NV is necessary for the outbreak of WTD in *M. rosenbergii* but the role of XSV is not clear, known as satellite virus. *Mr*NV is a non-envelped, icosahydron (26-27 nm diameter), consisting of 2.9 kb and 1.26 kb single-stranded RNA (Figure 4.4) . XSV is a non-envelped, icosahydron (15nm diameter), consisting of 0.9 kb single-stranded RNA (Figure 4.4) (Bonami et al, 2005; Wang et al, 2008). The principal host species is *M.rosenbergii*, giant freshwater prawn, and are a few other host reported (Sudhakaran et al, 2006a; Sudhakaran et al, 2006b; Bonami and Widada, 2011). Severe mortality is observed in larvae and post larvae stages of *M.rosenbergii* and adults become resistant carriers (Sahul Hameed et al, 2004; Sudhakaran et al, 2006).

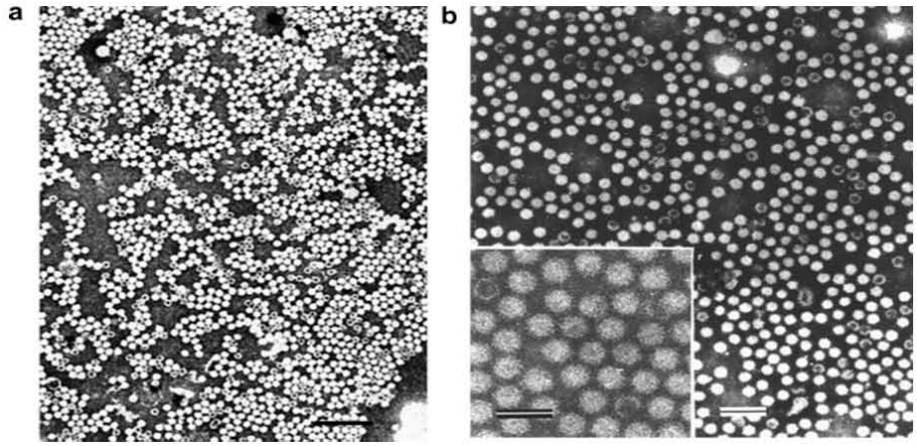

Figure 4.4. Purified XSV (a) and *M*rNV (b) particles after the CsCl gradient. TEM. PTA 2%. Bars =100 nm. Inset in b: higher magnification of *Mr*NV; bar= 50 nm. (Bonami and Widada, 2011. Journal of Invertebrate Pathology 106:131 - 142)

4.3.6 Infectious Myonecrosis

Infectious myonecrosis virus (IMNV) is icosahedron (40 nm in diameter) having double stranded RNA of 7.56 kb as genetic material. IMNV is closely related to genus *Giardiavirus*, family *Totiviridae* (Poulos et al, 2006). The principal host species is *L. vannamei, L.stylirostris* and *P. Monodon* is susceptible by experimental infection (Tang et al, 2005). Juveniles and sub-adults of *L. vannamei* are most severely infected by IMNV (Lightner, 2011).

4.4. Diagnostic Methods

4.4.1. White Spot Syndrome

The clinical sign of this disease is observation of typical white spots (0.5-2.0 mm in diameter) on the carapace (Figure 4.5). Moribund shrimp show a pinkish or reddish coloration of the exoskeleton. Typical histopathology of this disease is presence of eosinophilic to pale basophilic, Feulgen-positive intranuclear inclusion bodies in

hypertrophied nuclei of cuticular epithelial cells and connective tissue cells (Lightner, 2011). For the diagnosis, polymerase chain reaction (PCR) (OIE, 2012; Kiatpathomchai et al, 2001; Tsai et al, 2006; Vaseeharan et al, 2003) and quantitative real-time PCR using TaqMan probe (Durand et al, 2002, 2003; Powell et al, 2006) have been developed. Loop mediated isothermal amplification (LAMP) assay and quantitative LAMP assay using turbidimeter have also been developed (Mekata et al, 2009; Wongteerasupaya et al, 1997).

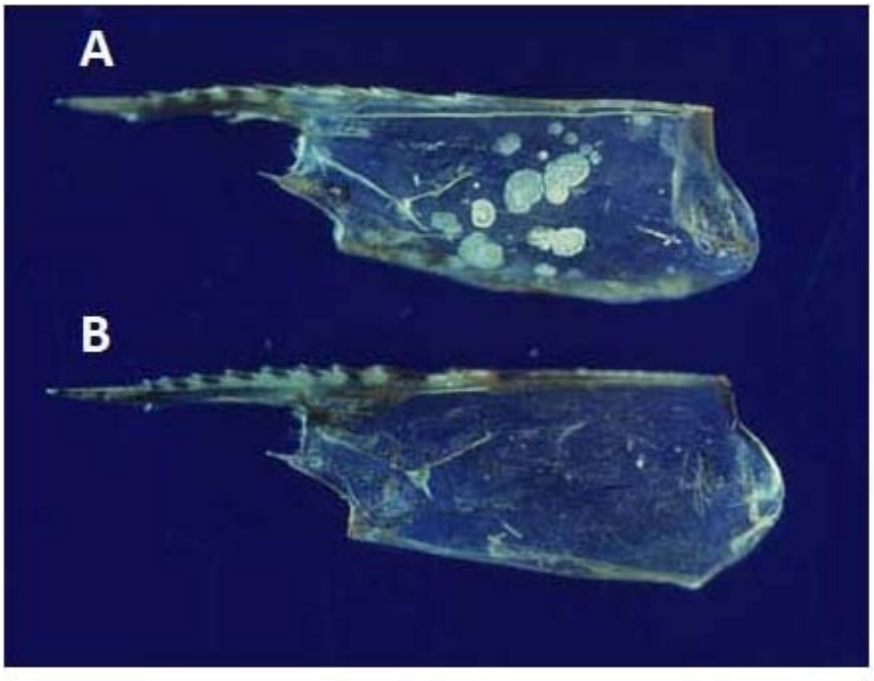

Figure 4.5. (A) Carapace from WSSV-infected kuruma shrimp (*Marsupenaeus japonicus*), presenting the typical white spots on its inner side. (B) Carapace from the normal shrimp.

4.4.2. Yellow Head Disease

The characteristic gross signs of this disease are yellow discoloration in the cephalothorax and very pale discoloration during moribund stage of infection (Figure 4.2). However, American penaeid shrimp, *e.g. L. setiferus, L. vannamei, Farfantepenaeus aztecus and F. duorarum*, did not show these signs in laboratory challenge test (Lightner et al, 2012). Densely basophilic inclusions can be found in gill sections by H&E stain (Flegel, 2006). Necrosis and inclusion bodies are found in lymphoid organ, hematopoietic tissue, epithelial tissues and hemocytes. Firstly, RT-PCR was developed by Wongteerasupaya *et al*. (Wongteerasupaya et al, 1997) to detect YHV (genotype 1) in Thailand and GAV (genotype 2) was detected by RT-nested PCR (Cowley et al, 2000). These two genotypes could be distinguished by multiplex RT-nested PCR designed by Cowley *et al*. (Cowley et al, 2004). Six genotype of YHV complex could be found using multiplex RT-nested PCR (Wijegoonawardane et al, 2010). Highly sensitive quantitative real time LAMP assay for YHV (genotype 1) has been reported (Mekata et al, 2009).

Figure 4.6. Gross signs of yellow head infection are seen here in the 3 shrimp on the right. They are generally bleached in color with a yellowish discoloration of the cephalothorax region when compared to shrimp of normal appearance on the left. (Flegel, 2006. *Aquaculture* 258:1 - 33)

4.4.3. Infectious Hypodermal and Hematpoieitc Necrosis

The gross signs of this disease in *L.stylirostris* are lethargic surface swimming, inverted sinking to the bottom and lack of mobility (Vega-Heredia et al, 2012). In *L. vannamei*, runt-deformity syndrome (RDS) and reduced, irregular growth can be found (Figure 4.7) (Kalagayan et al, 1991). However, high mortality and significant reduced or irregular growth have not been observed in *P. monodon* (Chayaburakul et al, 2005). Cowdry type A intranuclear inclusions are found in nerve cord, gills, cuticular hypodermis, haematopoietic tissues and connective tissues (Lightner, 1996). Aquatic Animal Health Manual (OIE, 2012) recommends the primer sets for PCR detection of IHHNV. Quantitative real-time PCR using TaqMan probe and SYBR have been developed (Tang and Lightner, 2001; Yue et al, 2006 ; Dhar et al, 2001). Qualitative (Sun et al, 2006) and quantitative LAMP assay (Sudhakaran et al, 2008), multiplex LAMP assay (He et al, 2011) and LAMP-LFD (chromatographic lateral flow dipstick) (Arunrut et al, 2011) assays have been reported.

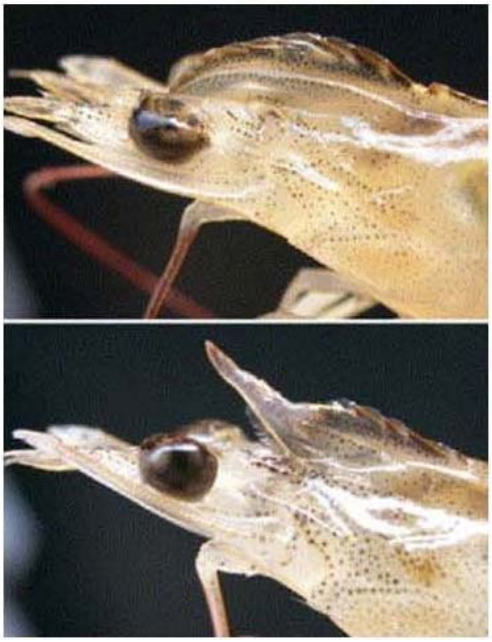

Figure 4.7. Runt deformity syndrome in *Litopenaeus vannamei*. Two shrimp specimens showing deformed rostra — one curved down and the other up and both shorter than normal. (Flegel, 2006. *Aquaculture* 258:1 - 33)

4.4.4. Taura Syndrome

The severely affected shrimp become hypoxic and move to the pond edges or pond surface. In an acute phase, gross signs of moribund shrimp are overall pale reddish coloration and red coloration in tail fan and pleopods (Figure 4.8). Acutely affected shrimp die during molting (Lightner et al, 2012). Surviving shrimp after the acute phase show irregularly shaped melanized cuticular lesions on the carapace (Figure 4.8). In the chronic phase after the recovery phase, shrimp do not show the obvious signs of disease though they are persistently infected with this virus. Therefore, these shrimp would be the prominent source for the next outbreak of this disease. In acute-phase lesions, necrosis in the cuticular epithelium of body surface, appendages, gills, hindgut and foregut by H&E staining (Lightner, 2011). OIE recommends the primer set for RT-PCR, 9992F and 9195R, amplifying a 231 bp sized PCR product. NASBA（Nucleic Acid Sequence-Based Amplification）, RT-LAMP and its modifications, RT-LAMP-DBH and RT-LAMP-LFD, have been reported (Teng et al, 2007, 2006;
Kiatpathomchai et al, 2007, 2008).

4.4.5 White Tail Disease

The affected prawn shows white, opaque muscle in the abdominal segment (Figure 4.9) and progressive reduction in feeding and swimming. In the histopathological observation, acute Zenker's necrosis of striated muscles and muscular lysis are observed. The large oval or irregular basophilic inclusion bodies are found in the cytoplasm of infected muscles and phagocytes of hepatopancreas (Arcier et al, 1999; Hsieh et al, 2006). OIE recommends the nested RT-PCR for detecting *Mr*NV and XSV. Multiplex RT-PCR have

been developed by Yoganandhan *et al*. (Yoganandhan et al, 2005) and Tripathy *et al*. (Haridas et al, 2010) to detect both viruses at the same time. RT-LAMP with loop-primer (Phillai et al, 2006; Puthawibool et al, 2010; Haridas et al, 2010) and real-time RT-PCR with TaqMan probe (Zhang et al, 2006) have been established.

Figure 4.8. Gross signs of Taura syndrome. On the left is a tail fan of *L. vannamei* with reddish necrotic areas (arrow). The right photo shows black lesions in the cuticle characteristic of the recovery stage of TSV infection (Lightner, 1996). (Flegel, 2006. Aquaculture 258:1 - 33)

Figure 4.9. Postlarvae of *M. rosenbergii* heavily infected with WTDV, showing whitish coloration of the muscle of head part.

4.4.6 Infectious Myonecrosis

The gross signs are extensive white necrotic areas in striated muscles in distal abdominal segments and tail fan (Figure 4.10). Lymphoid organs show hypertrophy that is 2-4 times

their normal size. In histopathological features, IMN shows the characteristic coagulative necrosis of striated muscle fibers, accompanied by infiltration and accumulation of hemocytes (Lightner, 2011). OIE recommends the nested RT-PCR (Poulos et al, 2006). Real-time RT-PCR using TaqMan probe and RT-LAMP have been developed (Andrade et al, 2007; Andrade et al, 2009).

Figure 4.10. Gross signs of infectious myonecrosis in naturally infected farmed *L. vannamei*, exhibiting various degrees of skeletal muscle necrosis, visible as an opaque, whitish discoloration of the abdomen. Source: DV Lightner (http://library.enaca.org/Health/FieldGuide/html/cv045im.htm#)

4.5. Control and Recent Topics (Prevention, Chemotherapy, Vaccine)

Routine virus monitoring by PCR or other detecting methods would help the shrimp farmers to reduce the mortality and to avoid the disease outbreak by partial harvest. This can be achieved standardizing the stocking density and by feeding the additional vitamins and immunostimulants to enhance the immune response.

In the laboratory experiment level, DNA vaccine and recombinant vaccine for WSSV have been demonstrated to be effective to control this disease. WSSV has five major proteins: VP28, VP26, VP24, VP19 and VP15. VP28 and VP19 are the envelope protein and others are nucleocapsid-associated proteins (Van Hulten et al, 2000; Van Hulten et al, 2000). DNA vaccines contracted by the plasmids encoding VP28 and/or VP281 showed the significant level of protection against WSSV artificial challenge test when the DNA vaccine was injected. Protection by DNA vaccination lasts till 7 weeks after the vaccination whereas the recombinant protein vaccination failed to protect after 3 weeks (Rout et al, 2007; Rajesh Kumar et al, 2008; Kono et al, 2009). The oral delivery of DNA construct containing VP28 gene of WSSV encapsulated in chitosan nanoparticles were effective to control this disease (Rajesh Kumar et al, 2009).

Oral administration of recombinant proteins, rVP28 or rVP26, were effective for prophylaxis of this disease. Although the duration of the vaccine is relatively short (within 55-75 days in this experiments), the booster administration of the homologous vaccine could extend its duration (Satoh et al, 2009, 2008; Ning et al, 2011).

An application of RNA interference technology against WSSV are promising and effective antiviral strategy. Sequence-specific viral inhibition in shrimp by long synthetic dsRNAs or siRNAs was highly efficient in inhibiting WSSV gene expression (Sarathi et al, 2008; Shekhar and Lu, 2009; Xu et al, 2007; Kim et al, 2007; Sudhakaran et al, 2010) and YHV gene expression (Tirasophon et al, 2007).

Since 2009, a newly emerging disease has been occurring in China, Vietnam (estimated loss: 75 million US dollar) and Thailand. This disease is characterized by massive degeneration of the hepatopancreas but the causative agent has not been identified yet. D.V. Lightner (University of Arizona) has named this condition acute hepatopancreatic necrosis syndrome (AHPNS). Close attention should be paid on the spread and impact of this disease on shrimp culture production (Flegel, 2012).

Glossary

WSSV **:** White spot syndrome virus

YHV **:** Yellow head virus

IHHNV **:** Infectious hypodermal and hematopoietic necrosis virus

TSV **:** Taura syndrome virus

WTD **:** White tail disease

***Mr*NV** **:** *Macrobrachium rosenbergii* nodavirus

XSV **:** Extra small virus

IMNV **:** Infectious myonecrosis virus

RT-PCR **:** Reverse transcription polymerase chain reaction

LAMP **:** Loop mediated isothermal amplification

RT-LAMP **:** Reverse transcription loop mediated isothermal amplification

H&E staining **:** Hemotoxylin and eosin staining

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