2. Viral diseases of shrimp in the Philippines

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Abstract. Shrimp is a high-value commodity and one of the major aquaculture species in the world, including the Philippines. The shrimp farming industry is dominated by the black tiger shrimp *Penaeus monodon* and the Pacific white shrimp, *Penaeus vannamei*. Intensification in shrimp aquaculture to meet the global demand resulted to several socio-economic and biophysical production bottlenecks. Consequently, the issues besetting the industry had raised several questions on its sustainability. In particular, viral diseases remain a constant threat and a significant concern in many shrimp producing countries especially in the developing world. In this chapter, current knowledge on major viral pathogens affecting shrimp aquaculture in the Philippines is presented and discussed. The discussion is focused on white spot syndrome virus (WSSV), monodon baculovirus (MBV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), hepatopancreatic parovirus (HPV), yellow head virus (YHV), and taura syndrome virus (TSV). Updates on their clinical signs, transmission and distribution are presented. Records of incidence in the Philippines are provided as well. The second half of the chapter discusses some of the methods.
how to control viral diseases in shrimp farming with a particular focus on vaccination, biosecurity and diagnostics.

Introduction

Aquaculture is major driver in the socio-economic development of poor and rural communities in many countries, particularly in Asia (Walker and Winton, 2010). It accounts for nearly 50% of the world’s food fish and is probably the fastest growing food-producing sector in the world, contributing significantly in the increasing demand of protein and essential micronutrients for human consumption (FAO, 2012). Particularly in the Philippines, aquaculture contributes approximately 39% to the total value of fish production, led by the top produced species including milkfish, grouper, tilapia, seaweed, mud crab and most importantly, shrimp (BAS, 2012).

Shrimp is a major aquaculture species in the Philippines mainly produced for export purposes in fresh/chilled/frozen form to Japan, USA, Korea and Hong Kong (BFAR, 2012; BAS, 2012). Two species dominate the Asian shrimp farming industry, the Black Tiger Shrimp *Penaeus monodon* and the Pacific White Shrimp, *Penaeus vannamei* (Flegel, 2009). In the early 2000, the lack of shrimp seed supply in farms shifted the native black tiger prawn culture to the exotic American whiteleg shrimp in major shrimp-producing countries in Asia including China, Thailand and Indonesia. At that time, *P. vannamei* was restricted in *P. monodon*-dominated shrimp aquaculture in the Philippines (Flegel, 2009).

In 2012, a total of 56,411.58 metric tons (MT) of shrimp from the total Philippine supply of 2,541,965.39 MT were produced for aquaculture purposes (BAS, 2012). The lifting of ban and the introduction of *P. vannamei* in the Philippines in 2007 led to an increased shrimp production and considered contributory catalyst in the recovery of the industry (Figure 1) (Guerrero, 2012).

![Figure 1. Total shrimp production in the Philippines from 1997 to 2013 (BAS, 1997-2013)](image-url)
**Challenges in shrimp aquaculture.** The intensification of aquaculture practices in hatchery and grow-out ponds may have positive effects in heightening the production, but it has concurrently contributed at a significant scale to the development of several farming bottlenecks resulting in growth retardation, physical deformity, reduced fecundity, physiological malfunction, and mortality in cultured shrimps (Moriarty, 1999; Lavilla-Pitogo et al. 2000; Walker and Winton, 2010). Besides anthropogenic and chemical-related factors, diseases caused by viruses, bacteria, fungi and/or parasites are constantly challenging the shrimp aquaculture sector. Massive devastations due to disease outbreaks have been documented worldwide especially in shrimp-producing countries in Asia including the Philippines (Menasveta, 2002; Lavilla-Pitogo et al. 2000).

Briefly, the Philippine shrimp industry started in 1970s, developed in the eighties and the decline in the nineties was mainly due to bacterial diseases including vibriosis and luminous bacterial infection (Platon, 1999; Rosario and Lopez, 2005). In addition, poor environmental conditions in ponds caused by the lack of technically qualified manpower, improper site selection, defective farm design, rapid intensification, overcrowding of farms in restricted locations and disproportionate development of the industry relative to supply of quality farm inputs were the key factors in the significant setback in shrimp farming sector experienced between 1995-1996 (Sangamaheswaran and Jeyaseelan, 2001). Most of the intensive shrimp farms in the Philippines especially in Western Visayas Region experienced a substantial decrease in production between the period of 1995-1999 due to disease outbreaks. Decline in production activities in a number of hatcheries and processors followed thereafter (Platon, 1999; Rosario and Lopez, 2005). Viral diseases have been a long standing concern in shrimp aquaculture. In fact, modern shrimp farming is, in a way, shaped by viral disease outbreaks in the nineties and early 2000’s. According to Lightner and Redman (1998), there are nearly 20 viral pathogens that can cause serious epizootics in penaeid shrimp. The major viruses infecting the shrimp industry not only in the Philippines but other countries as well include white spot syndrome virus (WSSV), monodon baculovirus (MBV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), hepatopancreatic parvovirus (HPV), yellow head virus (YHV), and taura syndrome virus (TSV).

This chapter will revisit the major viral pathogens affecting the Philippine shrimp aquaculture. Current updates on taxonomic classification, genomics, pathologies and geographical distribution are provided for each virus. Existing pro-active strategies to prevent viral outbreaks and promote healthy and robust shrimp stocks are discussed. These practices are
acknowledged in the global shrimp farming sector and the chapter dissects their applications and advances in the Philippines.

**Viral diseases in the Philippines**

**White Spot Syndrome Virus (WSSV).** White spot syndrome virus (WSSV), the largest animal DNA virus sequenced, is among the most lethal infectious agents that is rapidly replicating and has emerged globally as one of the most prevalent and widespread disease-causing agents in penaeid shrimps (van Hulten, 2001; Sanchez-Paz, 2010; Santos et al. 2013). The WSSV is a tailed, rod-shaped nucleocapsid, double-stranded circular DNA virus belonging to the family Nimaviridae (van Hulten, 2001; Sanchez-Paz, 2010; Santos et al. 2013). WSSV belongs to the genus Whispovirus under the family Nimaviridae; from the Latin word “nima” which means “thread”, because of its tail-like polar projection (Sanchez-Paz, 2010).

The viral envelope of WSSV is 6–7 nm thick and has a structure that appears to have a lipidic bilayer membrane, with an area between the envelope and the nucleocapsid varying between 2 and 7.5 nm. The nucleocapsid is cylindrical of about 200 x 65 nm with a 6 nm thick external wall consisting a core that is highly electron dense (Durand et al. 1997). The viral genome of 305,107 bp contains at least 181 open reading frames (ORF), most of which encode polypeptides with no homology to other known proteins (Yang et al. 2001).

**Clinical signs.** WSSV-infected shrimp may rapidly develop distinct white spots associated with calcium deposition (0.5–3.0 mm in diameter) that are most apparent on the exoskeleton, appendages and inside the epidermis circa 2 days after infection. Moribund shrimp display reddish to pink discoloration and have loose cuticle. Infected shrimp exhibit surface swimming and those with broken antennae usually gather at pond dikes. Other signs of WSSV include lethargy, sudden reduction in food consumption and empty gut (Durand et al. 1997; Lavilla-Pitogo, et al. 2000; Sanchez-Paz, 2010).

**Course of infection.** WSSV affects a wide host range and targets several tissues/organs including pleopods, gills, hemolymph, stomach, abdominal muscle, gonads, midgut, heart, peripods, lymphoid organ, integument, nervous tissue and the hepatopancreas resulting in a massive systemic infection. Shrimps weighing 4-15 grams are particularly susceptible but infection may occur from mysis to broodstock especially in shrimps at pre-molting stages. *P. indicus* suffers earlier and greater losses compared to *P. monodon*. Pandemic epizootic occur in extensive, semi-intensive and intensive culture systems regardless of water quality and salinities (Lavilla-Pitogo et al. 2000).
**Geographical distribution.** WSSV appeared in northeast Asia in 1992-1993 and spread very rapidly throughout the most of the shrimp growing regions of Asia and the Indo-Pacific region. It was in November 1995 that the first documented case of WSSV was documented in the Western Hemisphere. WSSV has been reported in different countries especially in major players in global shrimp aquaculture including Philippines, China, Japan, Korea, Thailand, Indonesia, Vietnam, Malaysia and India (Lightner, 1996; Magbanua et al. 2000).

**Transmission.** WSSV can occur in both vertical and horizontal pathways. The presence of viral inclusions in reproductive organs of *P. monodon* broodstock indicates the vertical transmission of the virus (Lo et al. 1997). Horizontal transmission is done by ingestion of infected organisms, direct exposure of body surfaces to virus particles in the water or through injection of cell-free extract of infected tissue (Sanchez-Paz, 2010; Santos et al. 2013).

**Host.** There are more than 93 species of arthropods that have been reported as WSSV carriers from culture facilities, the wild or through experimental infection (Sanchez-Paz, 2010). The virus infects mysis, postlarvae, juveniles and broodstock stages of shrimps. *Penaeus monodon, P. indicus, P. merguiensis, P. semisulcatus, P. setiferus, P. chinensis, Litopenaeus stylirostris, L. vannamei, Scylla serrata, Charybdis feriatus, Portunus pelagicus, Ascetes sp., P. sanguinolentus* are viral reservoirs of WSSV (Lavilla-Pitogo et al. 2000).

**Prevalence in the Philippines.** The group of Magbanua et al. (2000) conducted a nationwide screening of shrimps and results showed that the presence of WSSV in the Philippines was widespread both in hatcheries (i.e. 50% of the PL samples tested) and in grow-out ponds (i.e. 79% of the juvenile/adult shrimp samples tested). Another study by de la Pena, et al. (2007) determined the prevalence of WSSV in Palawan, Bohol, Quezon, Capiz, Negros Occidental, Misamis Occidental and Surigao del Sur using polymerase chain reaction (PCR) which revealed that WSSV infection was high during dry season (April-May) than the wet season (August-October). WSSV causes serious economic losses with high mortalities of up to 100% in 3-10 days (Lavilla-Pitogo et al. 2000).

**Monodon Baculovirus (MBV).** Among the viruses infecting shrimp, Monodon Baculovirus (MBV) is one of the broadly studied and well described virus. It was the causative agent of the first major crisis in the history of penaeid shrimp culture (Rajendran et al. 2012). Observation of the presence of occlusion bodies in the hepatopancreas of live larvae and postlarvae may be done to diagnose MBV using a light microscope. The occlusion bodies can be seen as small, raised, spherical clusters of
polyhedral particles that can be easily differentiated from the lipid droplets. The complete genome sequence of MBV is not yet available but its nucleic acid is rod-shaped, enveloped, double-stranded DNA virus recently discovered as type A baculovirus. The size of the virus range from 265-324 nm in length and 42-77 nm in diameter and genome size is predicted to range from 80-160 kbp. The virus codes for a 53 kDa major polyhedrin polypeptide and two minor 47 and 49 kDa polypeptides (Rajendran et al. 2012).

**Clinical signs.** Presence of multiple spherical inclusion bodies in the hepatopancreas and midgut epithelial cells is the major sign of MBV-infected shrimp. Infected shrimps exhibit pale-bluish gray to dark blue-black coloration, sluggish and inactive swimming movements, loss of appetite and retarded growth (Lavilla-Pitogo et al. 2000; Rajendran et al. 2012).

**Course of infection.** The virus infects the hepatopancreas and lining of the digestive tract. Spherical occlusion bodies occupy the enlarged nuclei of hepatopancreatic cells and release into the lumen after cells have been damaged after which, necrosis with secondary bacterial infection will follow (Lavilla-Pitogo et al. 2000).

**Host.** MBV affects a wide range of host such as several cultured and wild caught shrimp species including the freshwater prawn, *Macrobrachium rosenbergii*. MBV is believed to infect all life stages of *P. monodon* with late larval, postlarval and young juvenile as the most vulnerable stages while no reports documented on MBV in early larval stages. The presence of MBV has been documented in *P. penicillatus*, *P. indicus*, *P. semisulcatus*, *P. merguiensis*, *P. kerathurus*, *P. esculentus*, *Litopenaeus vannamei*, *Metapenaeus ensis* and *M. llysianassa* (Rajendran et al. 2012).

**Transmission.** The virus spreads horizontally through oral exposure to occlusion bodies, contaminated tissues or fomites. These are released in the shrimp feces and ingested by other larvae. While the virus can spread vertically from infected mother to the offspring through contamination, there has not been any report of transovarian transmission (Rajendran et al. 2012).

**Geographical distribution.** MBV has a wide geographic distribution and was reported to originate in Taiwan and believed to be enzootic in wild penaeids of the Indo-pacific coasts of Asia including China, Taiwan, Philippines, Malaysia, Singapore, Thailand, Sri Lanka and Indonesia. It is also present in Australia, South Africa, Israel, Kuwait and Italy (Lightner et al. 1989; Rajendran et al. 2012).

**Prevalence in the Philippines.** According to a study by Natividad and Lightner (1992), MBV was the most prevalent disease that occurred in *P. monodon* hatchery and grow-out ponds in the Philippines accounting for
249 cases or 66.9% prevalence in 12 major provinces. A study conducted by de la Pena, et al. (2008) determined the prevalence of MBV in *P. monodon* in the Philippines through PCR method in all 7 primary sources of broodstock and spawners. Results showed that MBV was detected in all sites except Palawan during the dry season and Negros Occidental and Bohol during the wet season. Reports showed 20-100% incidence rate of MBV and accumulated mortality of 70% among *P. monodon* juveniles reared in raceways and tanks (Lavilla-Pitogo *et al.* 2000)

**Infectious Hypodermal and Hepatopoietic Necrosis Virus (IHHNV).** Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) is the smallest known virus to affect several commercially farmed penaeid shrimp. IHHNV appears to be harmless in some species such as *P. monodon* but can cause severe mortality and growth retardation in other shrimp species (Turkmen and Toksen, 2010). IHHNV is a member of the *Paroviridae* family with a single stranded DNA that is non-enveloped icosahedrons with an average diameter of 22 nm, a density of 1.40 g∙ml in cesium chloride, an estimated size of 4.1 kb, a capsid that has polypeptides with molecular weights of 74, 47, 39 and 37.5 kDa (Bonami *et al.* 1990).

Occurrence of IHHNV in the Americas in the early 80s was due to the introduction of imported live *P. monodon* from Asia and had subsequently infected *P. stylirostris* and *P. vannamei*. This caused 90% mortality in juvenile and sub-adult *P. stylirostris* reared in a super intensive raceway system in Hawaii (Lightner, 2011). Cultured *P. vannamei* can also be chronically infected with IHHNV despite their relative resistance to the disease and can undergo runt deformity syndrome (RDS) which was linked to IHHNV infection (Lightner, 1999).

**Clinical signs.** Gross signs of IHHNV are not pathognomonic but penaeid shrimps infected with IHHNV exhibit an erratic swimming behavior, rising slowly to the water surface within 4-12 hours, hanging and rolling over until the ventral side is up. Infected shrimp becomes weak and lose their appetite. *L. stylirostris* infected with IHHNV usually have white opaque abdominal muscles, bluish to a clear blue cuticular color often with mottle buff to tan pigment patches in the hypodermis and very soft cuticles are observed. (Lavilla-Pitogo *et al.* 2000; Lightner, 2011). In addition, abnormal deformities in tail fin and rostrum, wrinkled antennal flagella, bubble-heads together with variation in sizes and growth reduction are some of the signs in IHHNV-infected *P. stylirostris* (Rai *et al.* 2012). RDS occurs in *P. vannamei* as a result of IHHNV infection where infected juvenile shrimp show a 45-90° bent or deformed rostrum, a deformed 6th abdominal segment, wrinkled antennal flagella, cuticular roughness, “bubble head” and smaller than expected shrimp (Lightner, 2011).
**Course of infection.** IHHNV is a systemic virus and it does not infect organ systems of endodermal origin such as hepatopancreas, mid-gut epithelium, anterior mid-gut caecum or posterior midgut caecum (Rai *et al.* 2012). No adverse effect has been recorded in larval or postlarval stages but produced a serious epizootic in *P. stylirostris* and *P. monodon* shrimp of size range from 0.05-2 grams (Lightner, 1985).

**Host.** Natural infections by IHHNV have been observed in *P. stylirostris*, *P. vannamei*, *P. occidentalis* (western white shrimp), *P. californiensis* (yellow-leg brown shrimp), *P. monodon* (giant tiger prawn), *P. semisulcatus* (green tiger prawn), and *P. japonicus* (Kuruma or Japanese tiger prawn). Other penaeid shrimps have been experimentally infected including *P. setiferus* (northern white shrimp), *P. duorarum*, and *P. aztecus* (northern brown shrimp). All stages of susceptible host species may be infected with the virus but the juvenile stages are the most severely affected (Lightner, 1999).

**Transmission.** Transmission of IHHNV in the environment is both horizontal and vertical in nature where *P. stylirostris* and *P. vannamei* survivors of IHHNV infection may bear the virus for life and carry it on to their offsprings and other shrimp populations and shrimp (Lightner, 2011).

**Geographical distribution.** IHHNV is considered to be a cosmopolitan disease because it has been reported in several regions around the world including North, South, Central America, the Caribbean and the Indo-Pacific region (Rai *et al.* 2012). IHHNV in captive *P. japonicus* and *P. monodon* broodstock was documented to occur in East and South East Asia countries including Japan, Singapore, Malaysia, Indonesia, Thailand and the Philippines (Lightner, 1999).

**Prevalence in the Philippines.** The presence of IHHNV in naturally-infected *P. monodon* and *L. vannamei* in the Philippines was molecularly documented using its capsid protein gene (Caipang *et al.*, 2011). It was further revealed that there is a possibility of at least two strains of IHHNV is present in the Philippines.

**Hepatopancreatic parvovirus (HPV).** HPV, also known as *Penaeus monodon* densovirus (*PmoDNV*) is an emerging shrimp virus reported to cause significant loss in shrimp aquaculture and infects several penaeid shrimp species (Flegel, 2006). Based on virion characteristics, HPV is considered a member of the family *Parvoviridae*. However, their distinctively different genome structure, unusual capsid proteins and size suggest that HPV may be considered as a new member of *Densovirinae*, a subfamily capable of infecting both vertebrates and invertebrates. HPV particles are in average 22-24 nm in diameter, icosahedral, and have a buoyant density of 1.412-1.425 g/ml in cesium chloride. The genome
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consists of a negative single stranded DNA molecule of approximately 5 to 6 Kbp. Complete sequencing of an isolate infecting *P. monodon* from Thailand revealed the presence of 3 large open reading frames (ORFs) (OIE, 2007; Safeena *et al.* 2012; Bonami *et al.* 1995). Presently, four complete genome sequences of HPV are available and these have been isolated from Thailand, Australia, India and South Korea. The South Korean isolate had the largest genome (6, 366 bp) followed by Thai (6, 321 bp), Indian (6, 310 bp) and Australian (6, 299 bp) strains (Safeena *et al.* 2012).

**Clinical signs.** HPV infections have been reported during the early larval stages and post larval stages of shrimp and up to 100% mortality was recorded during outbreaks (Safeena *et al.* 2012; Spann *et al.* 1997). The effect of HPV infection on adult shrimp is unknown, however, it may compromise their survival if the infection is severe and the shrimp is in a highly demanding metabolic state (i.e., during gonadal maturation). Mortalities due to HPV infection are difficult to document as it is seldom observed alone in epizootics and usually occurs in association with other pathogens (OIE, 2007; Safeena *et al.* 2012). Nonspecific clinical signs of the disease include poor growth rate, atrophy of the hepatopancreas, anorexia, decreased preening activity, increased surface and gill fouling by epicommensal organisms and sporadic opacity of tail musculature (Safeena *et al.* 2012).

**Course of infection.** HPV is reported to cause mortalities in early larval and postlarval stages of shrimp and stunted growth in juveniles. Additionally, the HPV-associated cumulative mortality was reported to be 50-100% after 4-8 weeks in juvenile in *P. merguiensis* (Safeena *et al.* 2012).

**Geographical distribution.** HPV was first discovered in Asia, later documented in the Indo-Pacific region and eventually in the Americas via importation of live shrimp (Lightner, 1996). HPV occurs in wild, cultured and hatchery reared shrimps throughout the world especially in China, Korea, Taiwan, Thailand Singapore, Malaysia Indonesia, Philippines, Australia, Kenya, Madagascar, Israel Kuwait, Mexico Honduras, El Salvador, Colombia, Ecuador, Peru and Brazil (OIE, 2007; Safeena *et al.* 2012).

**Transmission.** The first report on successful horizontal transmission by HPV by oral challenge in postlarvae of *P. monodon* was documented in 2003 (Catap *et al.* 2003). HPV is believed to be transmitted vertically (i.e. parental broodstock to progeny) and horizontally (i.e. contaminated water and cannibalism) (OIE, 2007).

**Host.** Natural host range includes an increasing number of cultured and captured shrimp species such as post larvae, juveniles and adults of *P. monodon, P. merguiensis, P. vannamei, P. esculentus, P. semisulcatus,*
P. monodon, P. indicus, P. japonicus, P. stylirostris, P. penicillatus and P. chinensis from all around the world (Safeena et al. 2012).

**Prevalence in the Philippines.** Postlarvae (PL1-PL19) from three hatcheries in Iloilo, Philippines showed prevalence rates of 7.8 to 26.4 % (Lio-Po et al. 2001). Catap et al. (2003) also revealed that HPV was detected in samples of P. monodon postlarvae (PL-13, PL-18, PL-19, PL-26) from 2 hatcheries in 2 provinces (i.e. Samar and Iloilo) in the Philippines. The percentage of infection was 20 to 100 % in postlarvae obtained from the hatchery in Samar in August 2001. Postlarvae from the hatchery in Iloilo, sampled in October and November 2001, had 70 to 99 % HPV infection.

**Yellow Head Virus (YHV).** YHV, one of six known genotypes in the yellow head complex of viruses, was the cause of the second most serious P. monodon viral epizootic in Thailand during 1990. YHV is classified in the Roniviridae family, genus Okavirus within Nidovirales order. YHV is an enveloped rod-shaped virion (approximately 40-50 x 150-170 nm) with prominent spike-like projections of 11 nm on the surface and an inner helical nucleocapsid (Wongteerasupaya et al. 1995). The virion contains three major structural proteins with molecular masses of 116, 64 and 20 kDa, respectively. YHV genome is approximately 26, 662 nucleotides with a 3’-polyadenylated tail and four functional ORFs (ORF-1a, -1b, -2 and -3) (Jitrapakdee et al. 2003; Sittidilokratna et al. 2008).

**Clinical signs.** Infection is usually characterized by a pale-yellowish cephalothorax due to hepatopancreas and gill discoloration. Histologically, YHV infection is characterized by necrosis containing vacuolated cells with hypertrophied nuclei and basophilic viral inclusions in the cytoplasm of infected cells. YHV targets tissues of ectodermal and mesodermal origin which includes the lymphoid organ, haemocytes, haematopoietic tissue, gill lamellae and spongy connective tissue of the subcutis, gut, antennal gland, gonads, nerve tracts and ganglia (Chantanachookin et al., 1993; Lightner, 1996).

**Course of infection.** Infection may occur in culture ponds about 50-70 days after stocking usually when shrimps are 5-15 g in size. Animals initially cease feeding and mortalities soon begin. Deaths reach massive proportions (up to 50 % mortality per day) and cumulative mortalities run to nearly 100 % 3-5 days after onset (Chantankachookin et al. 1993). Either age or size appears to influence yellow head disease with younger shrimp are less susceptible than older individuals (Lightner, 1996).

**Geographical Distribution.** YHV was first documented in Thailand in 1990. From then, subsequent YHV-related outbreaks have been reported from cultivated shrimp in many countries in Asia including China, India,
Indonesia, Malaysia Philippines, Sri Lanka, Vietnam and Taiwan as well as in Mexico (OIE, 2009; Seibert and Pinto, 2012; Walker and Winton, 2010).

**Transmission.** YHV is transmitted by feeding infected carcasses and by inoculation with cell-free extract from infected shrimp as observed in the laboratory (Lotz, 1997). In addition, the virus can remain infectious in water for more than 72 h (Flegel et al. 1995).

**Host.** YHV can have multiple hosts including many penaeid shrimp species (Chantanachookin et al. 1993; Seibert and Pinto, 2012). It has been shown that the shrimp *Palaemonstyliferus* and *Acetes* sp. served as reservoirs of YHV (Chantankachookin et al. 1993; Flegel et al. 1995) and *P. merguensis* and *Metapenaeusenssis* shown to act as asymptomatic carriers (Chantankachookin et al. 1993).

**Prevalence in the Philippines.** Natividad (1999) reported the first incidence of YHV in the country. Among the ten provinces chosen as sampling sites, selected shrimp farms from Misamis Occidental had the highest rate of infection for YHV followed by South Cotabato, Negros Occidental and Capiz. Agusan del Norte and Bohol selected shrimp farms were also positive for YHV but at lower percentages. Fifty-three (24.2 %) out of 219 samples of *P. monodon* from these sampling sites were found positive for YHV by Western blot assay.

**Taura Syndrome Virus (TSV).** TSV is the causative agent of Taura syndrome, a disease that was first reported in 1992 in *P. vannamei* collected from farms in Taura River, Ecuador. The disease outbreaks caused catastrophic losses with cumulative mortality rates of 60 to > 90 % in pond-cultured shrimp (Lightner et al. 1995; Lightner, 1999). TSV is a member of the *Dicistroviridae* family, order *Picornavirales* consisting of a small, non-enveloped icosahedral virion with 31-32 nm in diameter. Its genome contains 10,250 nucleotides in a single-stranded linear RNA of positive polarity, with two large non-overlapping ORFs separated by a 207-nucleotide intergenic region (Mari et al. 2002).

**Clinical Signs.** TSV-infected shrimps are characterized by lethargy, anorexia, opaque musculature and reddish discoloration in the tail fan and pleopods during the acute phase (Lightner et al. 1995). In the recovery phase, animals exhibit melanized cuticular lesions on the cephalothorax, tail and appendages, although clinical signs become undetectable in the chronic phase. Histopathological evaluation revealed five main anatomical regions infected by TSV which include the cuticle, gills, appendages, foregut and hindgut; however some individuals can exhibit signs in abdominal muscle tissue. Moreover, the disease is characterized by multifocal to diffuse necrosis and the presence of eosinophilic and basophilic viral inclusions in the cytoplasm of infected cells (Seibert and Pinto, 2012).
Course of infection. TSV is a rapidly progressing disease marked by extensive mortalities that become evident 25-35 days after a shrimp pond is stocked with post larvae (Brock et al. 1995). Elevated death rates last only a few days but commonly reach 25% per day and leave a mere 5-25% alive shrimp. Survivors carry latent infections for at least a year and probably for life. TSV is a particularly virulent pathogen of *P. vannamei* and can infect several other shrimp species including *P. monodon, P. azteicus, P. duorarum, L. setiferus, L. stylirostris, Marsupenaeus japonicas, M. rosenbergii, Metapenaeusensis, F. chinensis* and *L. schmitti*.

Geographical distribution. TSV is widely distributed in shrimp-farming regions of the Americas, Southeast Asia and Middle East. Moreover, there is an evidence that TSV is present in natural populations of *P. vannamei* in Central America such as Mexico and Ecuador and probably elsewhere (Lightner 1996; OIE, 2012). The rapid spread of TSV in the Americas and Asia has been attributed to the international trade of live shrimp (Walker and Winton, 2010).

Transmission. TSV can be transmitted by horizontal or vertical routes. Horizontal transmission occurs by cannibalism and by contact with water containing infected animals (Brock et al. 1995). It can also be transmitted experimentally by injection of cell-free extract of infected shrimp. Unlike IHHNV, TSV appears not to be transmitted vertically through oocytes of the female to the nauplii (Lotz, 1997). Vertical transmission from infected adult broodstock to their offspring is strongly suspected but has not been confirmed experimentally. Additionally, neither age nor size influences the susceptibility to TSV infection (Overstreet et al. 1997, OIE, 2012).

Other significant factors contributed to the spread of TSV are aquatic insects like the water-boatman (*Trichocorixa reticulata*) and by gulls that carry the virus in the gut and is eventually released through their feces. The survivors of TSV infection remain persistently infected by the virus, probably for life, gives the virus the opportunity for both horizontal and vertical transmission (Lightner, 1999).

Host. The principal host species for TSV are the Pacific white shrimp *Penaeus vannamei* and the Pacific blue shrimp *P. stylirostris*. According to Lightner (1996) TSV has been documented in all life stages (i.e. post larvae, juvenile, adults) of *P. vannamei* except in eggs, zygote and larvae.

Prevalence in the Philippines. To our knowledge, there is no documented report regarding TSV presence in the Philippines but it might be in the country in the future if strict biosecurity measures in the international trade of shrimp are not cautiously followed.
Methods of control

Diseases have emerged as the most serious limiting factor in the sustainable growth of shrimp culture in the Asia-Pacific region particularly in the Philippines. Various strategies are being employed to mitigate the risk of diseases in shrimp culture. Available methods of controlling viral shrimp diseases include the stocking of high-health seed, reduced water exchange rates and screening of influent water (Pruder, 2004). Moreover, short term interventions, such as the use of immunostimulants, vaccines and RNA interference (RNAi) have been studied as attempts to boost the immune response of shrimp against viral infections. Biosecurity measures comprising prevention, control and eradication of viruses in shrimp farms are important in preventing shrimp viral diseases (Seibert and Pinto, 2012).

Immunostimulants (see Chapters III and IV), probiotics (see Chapter V) and vaccines are considered promising approaches in preventing shrimp diseases. Immunostimulants are chemical compounds that activate the non-specific immune responses (Raa, 1996), probiotics are beneficial microorganisms (Lazado et al., 2015), whereas, vaccines include live, attenuated or killed bacteria or inactivated virus that rely on acquired or specific immune responses of the animal (Melamed et al., 2002).

Vaccination. One of the central dogmas of comparative (evolutionary) immunology for the last 30-40 years indicates that invertebrates do not possess acquired, specific, or adaptive immune system similar to that of vertebrates (Lee and Söderhäll, 2002; Rowley and Pope, 2012). Adaptive immunity is believed to be absent in invertebrates, including shrimp, because of the lack of immunoglobulin (Ig), T cell receptor (TCR) and Major histocompatibility complex (Mhc) high diversity molecules. Hence, non-specific immune system is a crucial defense mechanism in invertebrates (Söderhäll and Cerenius, 1998; Ara-Chavez and Sequeira, 2000).

Vaccination depends upon the specific or adaptive immune mechanisms realized through recognition of antigens by immune receptors on lymphocytes and memory cells. A second encounter between the host and the pathogen in the vaccine results in heightened immunity that is specific for that particular pathogen. Knowing this, the logical conclusion is that invertebrates, apparently lacking a specific immune system, thus cannot be vaccinated. However, several recent reports have suggested that the invertebrate innate immune system may be capable of some form of immune memory, described as “immune priming”, “specific immune priming” or “line specific memory” (Rowley and Pope, 2012).
Types of vaccines. Vaccines could be in the form of the following: 1) attenuated vaccines contain bacteria or viruses that have been altered so they can not cause disease; 2) killed vaccines contained killed bacteria or inactivated viruses; 3) toxoid vaccines contain inactive toxin bacterial (only applied in vertebrates); 4) recombinant vector vaccines are experimental vaccines similar to DNA vaccines, but they use an attenuated virus or bacterium; 5) DNA vaccines contain genes into a vector to produce a microbe’s antigens introduced into the body; 6) RNAi vaccines (see Chapter VII) contain dsRNA from viral specific gene (sometimes endogen gene) and applied for intramuscular injection induces an antiviral activity response for blocking of respective gene (Badhul Haq et al. 2012).

WSSV is extremely virulent and possesses a wide range of host specificity and targets various tissues. Presently, two kinds of "vaccines", protein and DNA, have been used to protect against crustacean WSSV. VP19, VP26, VP28 and VP292 of WSSV were used as protein vaccines and VP28, VP281, VP15 and VP35 as DNA vaccines. Some of these subunit vaccines, such as VP28, have been shown to efficiently protect shrimp or crayfish against WSSV (Johnson et al. 2008). In the Philippines, a study by Amar and Faisan (2011) evaluated the use of formalin-inactivated virus (FIV) by administering in different levels either by injection, bath-immersion, or oral delivery. The study revealed that vaccination could protect shrimp against WSSV and survival was further enhanced when FIV was provided together with diets supplemented with wheat grass and methyl sulfonyl methane (MSM).

Characteristics of vaccine. Ideally, a vaccine must be 1) safe for both the fish, the administrator and the consumer; 2) have a broad strain or pathogen coverage; 3) provide 100 % protection; 4) give a long-lasting protection, preferable as long as the production cycle; 5) be easy to apply; 6) be applicable in various species; 7) be cost effective; and 8) be readily licensed or registered (Grisez and Tan, 2005). Moreover, different methods or routes are being used in order to administer vaccines, namely: a) oral vaccination, vaccine is either mixed with the feed, coated on top of the feeds or bio-encapsulated; b) immersion vaccination, with two application methods, dip vaccination (i.e. animals immersed for a short duration) or bath vaccination (i.e. animals are exposed for a longer time); and c) injection vaccination - initially perceived by farmers as unfavorable because of the stress resulting from manipulation and injection, impractical when dealing with large number of samples and the process is time consuming. However, there are a number of advantages that make them the preferred method, for instance by providing long duration of protection (Grisez and Tan, 2005).
**Biosecurity.** Biosecurity is defined as the sum of all procedures in place to protect living organisms from contracting, carrying and spreading disease and other non-desirable health conditions, it is the practice of exclusion of specific pathogens from cultured aquatic stocks in broodstock facilities, hatcheries and farms or from entire regions or countries for the purpose of disease prevention; it is also the concept of protecting cultured shrimps from contamination by diseases and of preventing the spread of diseases (Moss *et al.*, 1998; Lightner 2002; Menasveta 2002; Lightner 2005). Biosecurity is a vital activity in aquaculture that is interactive and interdependent. It is a shared responsibility that each individual in the process of animal production plays a different but critical role in the implementation of the overall program (Pruder, 2004).

Biosecurity would include the physical, chemical and biological precautionary measures against potential disease outbreaks (Horowitz and Horowitz, 2003). Physical measures aim at preventing the intrusion of disease-carrying vectors to the farm site. This includes defined structure and barriers, such as the fences and gates in place. The facility should be constricted with materials that can be disinfected easily should a disease outbreak occur and is free from unauthorized access such as vehicles or people. It should have structures that prevent the escape of target animals and the entry of other organisms. It should be located away from hazards that are potential sources of infection or contamination. Untreated surface water should not be used as the source water because it may contain pathogens. The ideal system should have appropriate back-up water, life support systems and operational procedures that allow one-way flow, so that nothing can be returned to the facility without disease screening (Pruder, 2004). Fill and exchange water should be disinfected. Controlled water source is accomplished through better farm sitting, farm design and water management. This is achieved by several strategies such as inland shrimp farming, “zero” water exchange and the use of water treatment devices that remove potential vectors from the water source (Pruder 2004; Browdy *et al.*, 2001).

Chemical measures in biosecurity would include those being used to treat materials before they enter the facility. Chlorination and ozonization are commonly used to treat incoming water and iodine and chlorine are used to treat other potential vectors such as tools, footwear and clothing. On the other hand, biological measures include the use of specific pathogen free (SPF) shrimp which is readily available commercially (Horowitz and Horowitz, 2003).

The application of biosecurity concept in shrimp farming is not something that can be accomplished easily or in short term. According to Lightner (2002), the following are the principles and tools that are key
efforts at excluding pathogens: 1) Knowledge of disease of concern; 2) Availability of diagnostic and detection methods and services; 3) A list of excludable diseases/ pathogens; 4) Control of shrimp stocks that are farmed; 5) Adequate environmental control to prevent the introduction of pathogens; 6) The use of effective management practices that ensure continuous implementation of pathogen exclusion methods and that policies are in place and practiced; 7) Disinfection and pathogen eradication plans in place to contain and eradicate disease outbreaks.

**Biosecurity in the Philippines.** The Philippine Shrimp Congress is an annual event organized by the Bureau of Fisheries and Aquatic Resources (BFAR) together with the Philippine Shrimp Industry Association/ Negros Prawn Producers Marketing Cooperative Inc. (PHILSHRIMP/NPPC), Southeast Asian Development Center (SEAFDEC), Department of Science and Technology and other private sectors that brings together renowned local and international scientists and shrimp experts to discuss the status of the shrimp industry, overview of the latest updates in shrimp farming and government policies (PhilStar, 2002). This is where different sectors including the government, research institutions, academe and industry players exchange ideas aiming at improving the country’s shrimp production through intensified research and development. In the 2008 Philippine Shrimp Congress, local and foreign experts have agreed on the following significant points in order to ensure success in farming shrimp specifically *P. vannamei: 1) the use of specific-pathogen free (SPF) or specific-pathogen resistant (SPR) broodstock and "high-health" fry. Hatcheries producing "high-health" fry from imported broodstock should apply for accreditation from the Bureau of Fisheries and Aquatic Resources (BFAR). Accreditation is contingent upon water treatment (incoming and effluent water), physical isolation, aeration, sanitation and disinfection facilities and practices; 2) the use of best management practices (BMPs) in grow-out farms which includes provision of settling and treatment ponds, filtration system and reservoirs, the use of probiotics which are commercially available; strict compliance to biosecurity measures, including tire bath at farm entrance, footbath & hand disinfection at the pond entrance, nets & high-density polyethylene liners as crab fence, bird scaring devices, individual paraphernalia for each pond, and hygiene facilities for farm personnel as well as continuous monitoring of shrimp stock for disease symptoms. BFAR and other institutions like Southeast Asian Fisheries Development Center / Aquaculture Department (SEAFDEC/AQD) have fish health diagnostic laboratories where shrimp farmers can send shrimp samples for screening of possible pathogens. Moreover, grow-out farms also need to get certification from BFAR before operation; 3) the marketing of the right size of shrimp demanded by consumers, and in compliance with regulations on food safety
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(e.g. HACCP), traceability, environmental and social components (e.g. fair trade) (SEAFDEC-AQD News, 2008). Additionally, the Philippine Biosafety and Biosecurity Association (PhBBA) comprising of the National Laboratory Biosafety and Biosecurity Action Plan Task Force aims to assist the Department of Health and Agriculture in their efforts to create a National Policy (Standards and Guidelines) and an Implementation Plan for laboratory biosafety and biosecurity throughout the country. It plays a major role in harmonizing the needs and requirement of the concerned stakeholders (https://phbba.wordpress.com/about/).

**Control of shrimp stocks: The use SPF and SPR shrimp.** The single most important principle of biosecurity is stock control. The latter defined as the use of captive or domesticated stocks, cultured under controlled conditions and which have been the subject of an active disease surveillance and control program (Lightner 2003). To help mitigate the risk of having diseased or disease carrying shrimp, the use of domesticated shrimp stocks that have a known history of being free of pathogens of concern or the concept of having a specific pathogen-free stocks is of utmost importance (Pruder 2004 and Lightner 2002). The application of the specific pathogen-free (SPF) concept is dependent on the absence of the pathogen(s) of concern in the stocks being reared (or that are present), the availability of sensitive and accurate detection and diagnostic methods for the pathogen(s) and the presence of an effective barrier (i.e. facility design and geographic location, government mandated import restrictions, etc.) to prevent the introduction of the pathogen(s) intended to be excluded. In cases where specific pathogens may not be excludable, the development and use of specific pathogen-resistant (SPR) stocks may provide a valuable alternative to using exclusively SPF stocks (Lightner 2002). The development and use of SPF stocks is emerging as the best management strategy for stock control in farms, regions or countries with biosecurity programs. The process of establishing SPF stocks is shown in Figure 2 (modified from Pruder 2004).

**Adequate diagnostic and detection methods.** At present, there is no universally effective treatment for viral infections hence preventive measures and practices are required to keep pathogens from spreading. Rapid diagnosis is one of the most effective strategies among promising proactive measures (Seibert and Pinto 2012). The application of biosecurity in shrimp culture is dependent upon the availability of sensitive, accurate, cost effective disease diagnosis and pathogen detection methods. Diagnostic methods may be applied to determine the cause of disease(s) that are adversely affecting the culture performance or survival of farmed shrimp stocks or they may be used for surveillance purposes to screen for the presence of specific pathogens or perhaps in healthy shrimp for the purpose of disease control (Lightner 2002).
Figure 2. Development of SPF shrimp. In the development of an SPF shrimp, each “candidate SPF population” of wild or cultured shrimp stocks of interest is identified. Samples of the stock are taken and tested using appropriate diagnostic protocols. If negative result is found, a founder population (F₀) of the “candidate SPF” stock is acquired and reared in primary quarantine. During the primary quarantine, the F₀ stock is monitored for signs of disease, sampled and tested periodically for specific pathogens. If a pathogen is detected, the stock is then discarded. Stocks that tested negative for pathogens through primary quarantine (running from 30 days to as much as 1 year for some stocks) are moved to a separate secondary quarantine facility for maturation, selection, mating and production of second (F₁) generation. The F₁ stocks are maintained in quarantine for further testing for specific pathogens. Those that tested negative are designated as SPF and can be used to produce lines of SPF and “high health” shrimp (Wyban et al., 1992; Pruder et al. 1995).

Rapid detection methods of shrimp viruses in the Philippines. Infectious diseases are commonly encountered in hatchery and grow-out operations. Moreover, dependence of the shrimp industry on wild *P. monodon* broodstock or the introduction of *P. vannamei* into the endemic stock is also a possible source of asymptomatic carriers of these pathogens (Geduspan et al. 2013).

Molecular methods (i.e. gene probes and DNA amplification using PCR) are increasingly becoming the standard for the detection and diagnosis of shrimp viruses. The use of these techniques over traditional methods of
diagnosing disease has been widely accepted because of its high degree of specificity and sensitivity, rapidity and its ability to detect the presence of pathogens even in extremely low amounts. Early and rapid detection can reduce the risk brought about by disease(s) and in the long run lead to increased shrimp production (Lightner 2008; Geduspan et al. 2013). Kindly refer to Chapter IX for an in depth discussion on molecular detection assays for shrimp farming.

The prevalence and geographic distribution of WSSV infection among cultured penaeid shrimp in the Philippines was determined using PCR and Western blot assays and reported for the first time, the presence of WSSV in the Philippines (Magbanua et al. 2000). PCR assays described by de la Peña et al. (2007, 2008) confirmed the presence of WSSV and MBV in several wild stocks of *P. monodon* in the Philippines. The current source of broodstock is from the wild thus the protocols assisted broodstock collectors in selecting organisms free of WSSV and MBV infection. Geduspan et al. (2013) optimized published PCR protocols mainly by improving sensitivity of the assay for the detection of commercially important viral pathogens, including WSSV, MBV and IHHNV in shrimp. The optimized PCR assays are suited to Philippine conditions and can be used as a management tool for the prevention and control of viral diseases in shrimp aquaculture in the country through early detection of the pathogen. SDS-western-blot-enzyme immunoassays and *in situ* hybridization assays have been used as well in screening for shrimp viruses in the Philippines (Albaladejo et al., 1998).

**Concluding remarks**

It is a fact that the burgeoning world population requires additional food supply. This immense challenge of providing food for mankind defines the very driving nature of modern aquaculture – the fastest food-producing sector in the world. Aquaculture is considered a major economic activity in many countries. Along with its developments, there are several impediments that persistently challenge the sustainability of the industry. Diseases, particularly those of viral origin, have caused massive economic losses and even set the shrimp aquaculture industry to a significant setback a decade ago. It is important to understand the causative agents of these viral diseases because it will be essential in the development of effective strategies to prevent them. In the last 20 years, several shrimp viruses have been identified and characterized highlighting their complexity and threats. However, the research arm of shrimp aquaculture is still facing with several questions yet to be explored and answered. In the advent of more sophisticated and technologically advanced tools and techniques, it is not a
farfetched possibility that a greater and holistic understanding of each of the major viruses affecting the shrimp industry will come to fruition soon.

Given the nature of viral diseases, a proactive approach is by far the most effective disease control strategy in shrimp aquaculture. Several strategies are now widely available and their novelty and effectiveness are documented. It is important that laboratory data be translated into practical knowledge that should be adopted and practiced in shrimp farms.

The Philippine shrimp aquaculture has slowly recovered from its challenging past. It is still under the constant threat of viral diseases and these challenges must be faced head-on. A daunting task does not only lie with the researches to understand these viral diseases and develop preventive measures, but also with the aquaculturists to apply science-based approaches in their farming practices. An improved cooperative research and development between the government, the industry and the academe will be a decisive action for the advancement of a healthy, eco-friendly and sustainable Philippine shrimp aquaculture.

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