The Penaeid Shrimp Viral Pandemics due to IHHNV, WSSV, TSV and YHV: History in the Americas and Current Status

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Abstract

At least four virus caused pandemics have adversely affected the global penaeid shrimp farming industry since 1980. These viruses in the approximate order of their discovery are Infectious hypodermal and hematopoietic necrosis virus (IHHNV), Yellow Head Virus (YHV), Taura Syndrome Virus (TSV), and White Spot Syndrome Virus (WSSV). The socioeconomic impact of the diseases caused by these viruses have been so severe in some shrimp producing countries of Asia and the western hemisphere (Americas) that they were listed by the World Animal Health Organization (or 'Office International des Epizootics,' OIE) as posing a significant disease threat to cultured and wild crustaceans as a consequence of international trade or movement of infected crustaceans. IHHNV, TSV, and WSSV have had major impacts on cultured and wild shrimp in the Americas. Despite some unconfirmed reports, YHV has not been found in wild or cultured penaeid shrimp in the Americas. Nonetheless, YHV is considered to pose a significant threat to wild and cultured shrimp in the Americas because of the experimental demonstration of high susceptibility of American penaeids to the virus. Additional virus-caused diseases found in shrimp have been recognized recently. For example, Infectious Myonecrosis (IMN) has severely affected shrimp farms in northeast Brazil in 2003. This emerging disease was shown to have a viral etiology and the virus was named IMNV for the disease it causes. This paper reviews aspects of the biology and epizootiology of WSSV, TSV, IHHNV, YHV, and the emerging disease IMN.

Introduction

The shrimp farming industry of the Western Hemisphere (the Americas) produces approximately 25% of the world's farmed shrimp. With 91,000 metric tons (MT) of production for 2003, Brazil has become the region's leading producer. Brazil has thus replaced Ecuador which has not fully recovered from the WSSV pandemic of 1999, but still produced 81,400 MT in 2003 (I. Rocha, unpublished data, 'Presidente Assoccação Brasileria de Criadores de Camarão,' Recife, Brazil; Rosenberry 2003). In terms of significant production, only two farmed penaeid shrimp species have been significant in the Americas. Of these, *Litopenaeus vannamei* (the Pacific white shrimp; Perez Farafante and Kensley 1997) currently accounts for more than 90% of the total production. Other penaeid shrimp species have been cultured, and these make up the remaining percent of the total production. The second most important species is *L. stylirostris*, the Pacific blue shrimp, once accounted for nearly 20% of the hemisphere's production. However, the high susceptibility of the species to WSSV and to new strains of TSV resulted in the abandonment of the species in 1999-2000 by most of the industry (Zarin-Herzberg and Ascencio-Valle 2001; Erickson et al. 2002; OIE 2003). Also cultured in some locations are

L. setiferus and *L. schmitti* (the Gulf of Mexico and Caribbean white shrimp, respectively) and *Farfantepenaeus aztecus*, *Fa. subtilis*, and *Fa. californiensis* (the Gulf of Mexico, Caribbean, and Pacific brown shrimp, respectively) (Perez Farafante and Kensley 1997; Rosenberry 2001, 2003).

The most important disease of cultured penaeid shrimp, in terms of economic impact, in Asia, the Indo-Pacific, and the Americas have infectious agents as their cause (Flegel and Alday-Sanz 1998; Lightner 1999; OIE 2003). Among the infectious diseases of cultured shrimp, certain virus-caused diseases stand out as the most significant **(Tables 1 & 2).** Some of the most important diseases (and their etiological agents) were once limited in distribution to either the Western or Eastern Hemisphere (Lightner, 1996a; Flegel and Alday-Sanz 1998; OIE 2003). However, the international movement of live (for aquaculture) and dead (commodity shrimp for reprocessing and commerce) has led to the transfer and establishment of certain pathogens from one hemisphere to the other (Lightner 1996b; Durand et al., 2000; AQUIS, 2000). Frozen commodity shrimp have been implicated as the route by which WSSV was moved from Asia to the Americas, while TSV was moved in the opposite direction with infected live broodstock from Central America (Nunan et al. 1998; Tu et al. 1999; Yu and Song 2000; Durand et al. 2000).

Table 1. Viruses of concern to penaeid shrimp aquaculture in the Americas (as of January 2004; modified from Lightner 1996a and Lightner 1999).

Virus Type / Family	**Pathogen/Pathogen Group**[1]	
DNA VIRUSES		
dsDNA - *Nimaviridae*	* WSSV	- genus *Whispovirus*
dsDNA - *Baculoviridae*	** BP[2]	- an occluded enteric baculovirus
	** MBV[2]	- an occluded enteric baculovirus
	** BMN[2,3]	- a nonoccluded enteric baculovirus
ssDNA - *Parvoviridae*	** IHHNV	- a systemic parvovirus
	HPV	- enteric parvoviruses

* Listed by OIE (OIE 2003a, 2000b).

** Listed by OIE with "Other Significant Diseases" as of May 1999 (OIE 2000a, 2000b, 2001).

[1] For more information on these pathogens and the most appropriate diagnostic methods see: Lightner 1996 and OIE, 2003, Manual of Diagnostic Tests for Aquatic Animals.

[2] The most recent report on virus taxonomy from the International Committee on Taxonomy of Viruses (van Regenmortel et al. 2000) lists only MBV as a member of the baculovirus family. The omission of BP was almost certainly an oversight. Because BMN is nonoccluded, it was removed from the baculovirus family by the ICTV in 1995 (Murphy et al. 1995). Nonetheless, for the purpose of this list BMN, MBV, and BP will be listed a baculoviruses.

[3] BMN was de-listed in 2002 by OIE from its list of Crustacean Diseases. Nonetheless, it has been kept on the USMSFC list.

RNA VIRUSES	
ssRNA - *Dicistroviridae*	* TSV - genus *Cripavirus*
ssRNA - *Roniviridae*	* YHV/GAV/LOV[34] - genus *Okavirus*
dsRNA - *Totiviridae*	IMNV - *Myonecvirus* (proposed new genus)

* Listed by OIE (OIE 2003a, 2000b).
** Listed by OIE with "Other Significant Diseases" as of May 1999 (OIE 2000a, 2000b, 2001).

Table 2. Estimated economic losses since the emergence of certain diseases in penaeid shrimp aquaculture.

Virus	Year of emergence to 2001	Product Loss (dollars)
WSSV - Asia	1992	$4-6 billion
WSSV-Americas	1999	>$ 1 billion
TSV	1991-92	$1-2 billion
YHV	1991	$ 0.1-0.5 billion
IHHNV*	1981	0.5-1.0 billion

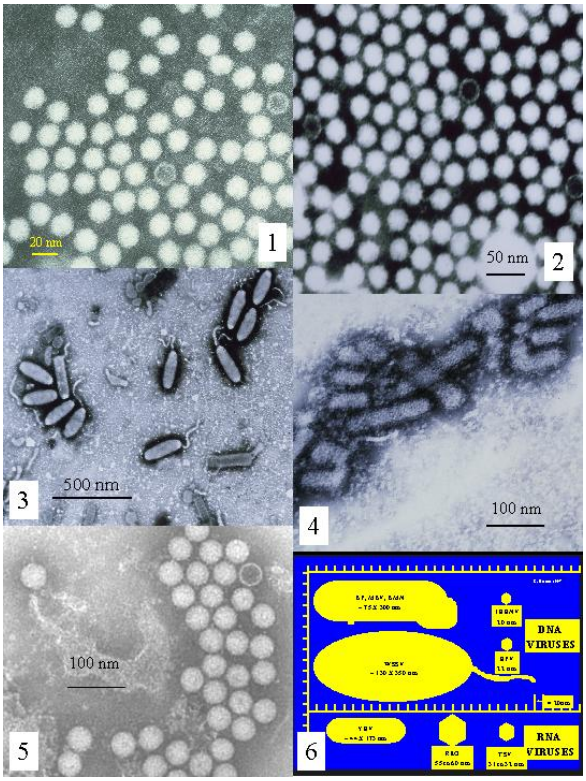
* Includes Gulf of California fishery losses for 1989-1994.

As a consequence of the rapid growth and development of the penaeid aquaculture industry, many of the most significant shrimp pathogens were moved from the regions where they initially appeared to new regions even before the "new" pathogen had been recognized, named, proven to cause the disease, and before reliable diagnostic methods were developed. The diseases caused bye to the shrimp viruses IHHNV, TSV and WSSV were all transferred with live shrimp stocks from country to country and from one continent to another well before their etiology was understood and diagnostic methods were available. The pandemics due to the penaeid viruses WSSV and TSV, and to a lesser extent to IHHNV and YHV, have cost the penaeid shrimp industry billions of dollars in lost crops, jobs, and export revenue **(Table 2)**. The social and economic impacts of the pandemics caused by these pathogens have been profound in countries in which shrimp farming constitutes a significant industry. This paper reviews the current status of the virus diseases due to WSSV, TSV, YHV, IHHNV and IMNV in the Americas in terms of their biology, history, distribution, production impacts, methods for their management in shrimp aquaculture in the Americas. Details on diagnostic methods for these diseases (except IMN) and detection methods for their causative agents are available from several sources, with the most recent being Manual of Diagnostic Tests for Aquatic Animals (OIE 2003).

[4] The OIE (2003a, 2003b) lists YHV and includes GAV/LOV as a distinct strain of YHV.

The Major Viruses In The Americas

Although nearly 10 of the known shrimp viruses have been found in cultured penaeid shrimp in the Western Hemisphere **(Table 1)**, only four of these have caused panzootic disease with serious economic consequences in the Americas (Lightner 1996a, 1996b, 2003) during the past decade. These viruses, or perhaps more correctly groups of different strains of the same virus species, are IHHNV, TSV, WSSV, and IMNV **(Table 1; Figures 1-4 and 6)**. IMN (infectious myonecrosis; **Figure 5)** recently emerged in 2002-2003 in shrimp farms in the northeastern states of Brazil where it has caused serious mortality and production losses at affected farms (Lightner et al. 2004).



Figures 1-5. Transmission electron micrographs of purified (by ultracentrifugation in sucrose or cesium chloride gradients) preparations of penaeid shrimp viruses stained with 2% PTA. Scale bars are in nanometers as indicated on the figure plate. Fig. 1: IHHNV; Fig. 2: TSV; Fig. 3: WSSV; Fig. 4: YHV; and Fig. 5: IMNV.

Figure 6. Schematic of the principal viruses of penaeid shrimp grouped by nucleic acid type of the genome, and showing their relative sizes and their general profile. The spacing on the scale lines is 20 nm.

Infectious hypodermal and hematopoietic necrosis virus (IHHNV)

IHHNV is the smallest of the known penaeid shrimp viruses. The IHHN virion is a 22 nm diameter, nonenveloped icosahedron **(Table 1; Figures 1 and 6)**, with a density of 1.40 g/ml in CsCl. It genome is linear single-stranded DNA of 4.1 kb in length, and its capsid consists of four polypeptides with molecular weights of 74, 47, 39, and 37.5 kD. Because of these characteristics, IHHNV has been classified as a member of the *Parvoviridae* and a probable member genus *Brevidensovirus* (Bonami et al. 1990; Bonami and Lightner 1991; Mari et al. 1993; Nunan et al. 2000; Shike et al. 2000).

The disease IHHN, and later its causative agent, IHHNV, was first described as the cause of acute epizootics and mass mortalities (> 90%) in juvenile and subadult *L. stylirostris* farmed in super-intensive raceway systems in Hawaii (Brock et al. 1983; Lightner 1983, 1988; Lightner et al. 1983a, 1983b; Brock and Lightner 1990). Shortly after its discovery in *L. stylirostris*, the virus was found in *L. vannamei* being cultured at the same facility in Hawaii and these *L. vannamei* were shown to be asymptomatic carriers of the virus (Lightner et al, 1983b; Bell and Lightner 1984). Some members of populations of *L. stylirostris* and *L. vannamei* that survive IHHNV infections and/or epizootics, may carry the virus for life and pass the virus on to their progeny and other populations by vertical and horizontal transmission (Bell and Lightner 1984; Lightner 1996a; Morales-Covarrubias et al. 1999, Morales-Covarrubias and Chavez-Sanchez 1999; Motte et al. 2003). A few years after it was reported that *L. vannamei* could be infected with IHHNV and not present significant mortalities (Lightner et al, 1983b; Bell and Lightner 1984), IHHNV was shown to be the cause of 'runt deformity syndrome' (RDS) in *L. vannamei*. With RDS, irregular, reduced growth and cuticular deformities, rather than mortalities, were found to be the principal effect of infection (Kalagayan et al. 1991; Browdy et al. 1993; Bray et al. 1994; Brock and Main 1994; Lightner 1996a). Hence, the economic and production impacts of IHHNV infection in *L. vannamei* are due to reduced and irregular growth and small sized shrimp at harvest and not to elevated mortality. To mitigate this effect, several strategies have been used. With one strategy, selected lines of *L. stylirostris*, which were not only resistant to IHHN disease, but are also refractory to infection, were developed (Tang et al. 2000; Dhar et al. 2001). IHHNV-free lines of *L. vannamei* were also developed as SPF (specific pathogen-free) lines and these stocks were the first developed in the SPF stock development program (Pruder et al. 1995).

After its initial discovery in cultured shrimp in Hawaii in 1981, IHHNV was subsequently found to be widely distributed in cultured shrimp in the Americas and in wild shrimp collected along the Pacific coast. As of 2003, the only country in the Americas, which can claim to have IHHNV-free zones, is the United States. This was achieved with the development and use of SPF shrimp stocks (Pruder et al. 1995). The introduction of IHHNV into shrimp farms in northwestern Mexico and wild shrimp stocks in Mexico's Gulf of California during the late 1980's and early 1990's resulted not only in significant losses in farmed *L. stylirostris,* but also in a collapse in 1990 of the wild fishery for *L. stylirostris* in the northern Gulf of California (Lightner et al. 1992; Martinez-Cordova 1992; Lightner 1996b; Pantoja et al 1999; Morales-Covarrubias and Chavez-Sanchez 1999; Morales-Covarrubias et al. 1999). A decade later, the *L. stylirostris* fishery had recovered sufficiently to support commercial fishing, but the prevalence of IHHNV infection in adult *L. stylirostris* collected from the northern Gulf of

California fishery remained high (80% to 100% females and 60% in males) (Morales-Covarrubias et al. 1999; Morales-Covarrubias and Chavez-Sanchez 1999).

IHHNV has been found to be widely distributed in wild and cultured *Penaeus monodon* in east and SE Asia where it does not seem to cause production losses (Flegel 1997; Primavera and Quinito 2000; Tang et al. 2003). Molecular studies show considerable variation among Asian isolates of the virus (Tang et al. 2003), while little variation was found in Americas isolates (Tang and Lightner 2002). All isolates of IHHNV from the Americas are nearly identical with IHHNV from the Philippines, suggesting, along with other aspects of its history and epidemiology of IHHN in the Americas, its introduction from the Philippines, perhaps with live *P. monodon* that were imported as a candidate aquaculture species during the very early development of shrimp farming in the Americas (Lightner 1996b; Tang et al. 2003).

Taura Syndrome Virus (TSV)

TSV is a small, simple RNA virus. The TSV virion is a 32 nm diameter, nonenveloped icosahedron with a buoyant density of 1.338 g/ml **(Table 1; Figures 2 and 6).** The genome of TSV consists of a linear, positive-sense single-stranded RNA of 10,205 nucleotides, excluding the 3' poly-A tail, and it contains two large open reading frames (ORFs). ORF 1 contains the sequence motifs for nonstructural proteins, such as helicase, protease and RNA-dependent RNA polymerase. ORF 2 contains the sequences for TSV structural proteins, including the three major capsid proteins VP1, VP2 and VP3 (55, 40, and 24 kDa, respectively) (Bonami et al. 1997; Mari et al. 1998; Mari et al. 2002; Robles-Sikisaka et al. 2001). The virus replicates in the cytoplasm of host cells. Based on its characteristics, TSV has been assigned by the International Committee on Taxonomy of Viruses (ICTV) to the newly created genus *Cripavirus* in new family *Dicistroviridae* (in the superfamily of picoranviruses) (Mayo 2002a, 2000b).

The principal host for TSV is the Pacific white shrimp, *L. vannamei*, although other species can be infected and present disease (Aguirre and Valle 2000; Hasson et al. 1995, 1999; Lightner 1999; Overstreet et al. 1997; Robles-Sikisaka at al. 2001). Cumulative mortalities due to TSV epizootics have ranged from 40 to >90% in cultured populations of postlarval (PL), juvenile, and subadult *L. vannamei*. Survivors of TSV infections may carry the virus for life (Brock et al. 1995, 1997; Hasson et al. 1999; Lightner 1996a, 1996b; Lotz 1997). TSV has been demonstrated to remain infectious in the feces of sea gulls that have ingested infected shrimp carcasses, which may implicate birds as being an important route of transmission of the virus within affected farms or farming regions (Garza et al. 1997; Lightner 1999). TSV can also infect other Western Hemisphere penaeid species (i.e. *L. stylirostris*, *L. setiferus*, and *L. schmitti*), sometimes resulting in disease and mortalities in PL or yearly juvenile stages, but also in asymptomatic persistent infections (Brock et al. 1997; Overstreet et al. 1997). Other Western Hemisphere penaeids (*Farfantepenaeus aztecus* and *Fa. duorarum*) and Eastern Hemisphere penaeids (*Fenneropenaeus chinensis*, *P. monodon*, and *Marsupenaeus japonicus*) have been experimentally infected with TSV (Brock et al. 1997; Overstreet et al. 1997).

TSV emerged from an unknown source in Ecuador in 1991. The disease was recognized as a major new disease of farmed *L. vannamei* by early 1992 and it was named Taura Syndrome (Jimenez 1992; Lightner et al. 1995). The viral etiology of TS was confirmed in 1994 and the virus was named Taura syndrome virus (TSV) (Hasson et al. 1995). In the interest of supporting

litigation brought by a group of Ecuadorian shrimp farmers against several companies that had been implicated as the cause of a toxicity syndrome they called 'Taura Syndrome' (Intriago et al. 1997), Jimenez et al. (2000) reported on the epizootiology of the disease in Ecuador assigned to TSV the synonym infectious cuticular epithelial necrosis virus (ICENV).

By 1994, when the viral etiology of TS had been established, the virus had been moved with live shrimp transfers to many of the shrimp growing countries of the Americas (Brock et al. 1995; Hasson et al. 1995, 1999; Bonami et al. 1997; Lightner 1996a, 1996b). While wild postlarvae with TSV infections were reported as being found near shrimp farms with ongoing TSV epizootics (Lightner et al. 1995), TSV infections in wild shrimp have not been further documented, suggesting that TSV does not have a discernable impact on wild populations of shrimp (Brock 1997). By 1998, TSV reached Asia with infected stocks of *L. vannamei*, introduced for aquaculture purposes (Tu et al. 1999; Yu and Song 2000). Physicochemical and more recent molecular studies of TSV suggest that a single strain of the virus was present in the initial TSV pandemic, but that new strains are emerging which differ in host range and virulence (Yu and Song 2000; Zarin-Herzberg and Ascencio-Valle 2001; Erickson et al. 2002).

White Spot Syndrome

WSSV has a wide host range among decapod crustaceans (Lo et al. 1996; Flegel 1997; Flegel and Alday-Sanz 1998), and is potentially lethal to most of the commercially cultivated penaeid shrimp species (OIE 2003). The causative agent of WSD is white spot syndrome virus (WSSV) or white spot virus (WSV), is a very large double-stranded DNA (dsDNA) virus recently assigned by the ICTV to its own new genus, *Whispovirus*, and family, *Nimaviridae* (Table 1) (Mayo 2002a, 2002b). Virions are large (80-120 x 250-380 nm), rod-shaped to elliptical, and with a trilaminar envelope (Wang, et al. 1995; Durand et al. 1997; Inouye et al. 1994, 1996; Kanchanaphum et al. 1998; van Hulten, et al. 2001). Negatively stained virions purified from shrimp hemolymph show unique, tail-like appendages (Figures 3 and 6) (Wang et al. 1995). The virions are generated in hypertrophied nuclei of infected cells without the production of occlusion bodies. In initial reports, WSSV was described as a non-occluded baculovirus, but WSSV DNA sequence analysis has shown that it is not related to the baculoviruses (van Hulten et al. 2001; Yang et al. 2001). The size of the WSSV genome has been differently reported for different isolates: 305,107 bp (GenBank Accession No. AF332093), 292,967 bp (GenBank Accession No. AF369029) and 307,287 bp (GenBank Accession No. AF440570) for viruses isolated from the People's Republic of China, Thailand and Taipei China, respectively. The sequences of these three isolates are almost identical, with the size differences being due mostly to several small insertions and one large (~12 kBp) deletion. In accordance with a genome size of ~300 kb, a total of 531 putative open reading frames (ORFs) were identified by sequence analysis, among which 181 ORFs are likely to encode functional proteins. Thirty-six of these 181 ORFs have been identified by screening and sequencing a WSSV cDNA library or else have already been reported to encode functional proteins many of which show little homology to proteins from other viruses (OIE 2003).

White spot disease (WSD caused by WSSV) emerged in east Asia in 1992-93 and it was quickly dispersed with infected seed and broodstock across the Asian continent to SE Asia and India where it caused a major pandemic, and continues to cause significant losses in some

regions. WSD outbreaks were first reported from farmed Ma. *japonicus* in Japan in 1993 (Inouye et al. 1994, 1996; Nakano et al. 1994) and the causative agent was named penaeid rod-shaped DNA virus (PRDV) or rod-shaped nuclear virus of *Ma. japonicus* (RV-PJ). Later, outbreaks of viral disease with similar gross signs caused by similar rod-shaped viruses were reported from elsewhere in Asia and other names were applied: hypodermal and hematopoietic necrosis baculovirus (HHNBV) in the People's Republic of China (Huang et al. 1995a, 1995b); white spot baculovirus (WSBV) and PmNOBIII in Taipei China (Chou et al. 1995, Lo et al. 1996); and systemic ectodermal and mesodermal baculovirus (SEMBV) or PmNOBII in Thailand (Wongteerasupaya et al. 1995). The virus from the People's Republic of China has also been called Chinese baculovirus (CBV) (Lu et al. 1997). Shrimp exhibiting the gross signs and histopathology of WSD have also been reported from Korea (Kim et al. 1998), India (Karunasagar et al. 1998), the Philippines, and the USA (Lightner 1996; Durand et al. 2000). WSSV has even reached shrimp farms in southeastern Europe (1997) and the Middle East (1999) via live shrimp movements, and Australia and Spain with introductions of frozen infected shrimp that were used as fresh food for broodstock (OIE 2003; Lightner unpublished data).

During 1999, WSD also had a severe impact on the shrimp industries of both Central and South America (Durand et al. 2000; Vidal et al. 2001; Lightner 2003). Despite the absence of evidence of live shrimp introductions from Asia to the Americas, WSSV was diagnosed at several sites in 1995-1997 in captive wild shrimp or crayfish and in cultured domesticated shrimp stocks in the eastern and southeastern U.S. (Nunan et al. 1998; Durand et al. 2000; Lightner et al. 2001). Early in 1999, WSSV was diagnosed as the cause of serious epizootics in Central American shrimp farms. By mid to late 1999, WSSV was causing major losses in Ecuador (then among the world's top producers of farmed shrimp), and by 2000-01, export of shrimp from Ecuador was down nearly 70% from pre-WSSV levels (Rosenberry 2001; Lightner 2003). Although the documentation is sketchy, WSSV has been found in wild shrimp stocks in the Americas (Nunan et al. 2001). In the US, the virus was successfully eradicated from shrimp farms and it has not been reported from farmed shrimp stocks since 1997. However, its sporadic detection in wild shrimp stocks (Gulf of Mexico and SE Atlantic states) (Thomas McIlwain, U.S. DOC, NOAA, National Marine Fisheries Service, St. Petersburg, FL, unpublished data) suggests that it has become established in wild penaeid shrimp stocks in SE US coastal waters or that it continues to be introduced perhaps, with wastes (peeled shells, etc.) from value-added reprocessing of imported shrimp in coastal packing plants. It has been proposed that the introductions of WSSV to the Americas were the result of importation of frozen shrimp products from WSSV-affected areas of Asia and the value-added reprocessing of those frozen shrimp for the US market in coastal processing plants (Nunan et al. 1998; Durand et al. 2000; Lightner et al. 2001; Lightner 2003). WSSV also reached Spain and Australia in 2000-2001. In both cases successful containment and eradication was reported, and for both events the importation and use of infected frozen shrimp as a fresh feed for broodstock was implicated as the route of introduction. Regardless of where they were obtained, isolates of WSSV have shown little genetic or biological variation, suggesting that the virus emerged and was spread from a single source (OIE 2003).

Yellow Head Disease

Yellow head disease (YHD) was first described in 1991 as an epizootic in Thai shrimp farms (Limuswan 1991), and subsequent outbreaks have been reported from other shrimp farming countries in Asia. A closely related strain of YHV, named Gill-Associated virus (GAV), has been reported from Australian shrimp farms (Walker et al. 2001). Laboratory trials have shown that YHV can cause high mortality in representative cultured and wild penaeid species from the Americas (Lu et al. 1994, 1997; Lightner 1999; Pantoja & Lightner 2003). When it occurs in farms rearing *P. monodon*, YHD is characterized by high and rapid mortality that is sometimes accompanied by the gross signs of yellowing of the cephalothorax and general bleaching of body color from which the disease got its name. In laboratory studies, American penaeids challenged with YHV did not develop yellow heads or signs of marked discoloration (Lightner and Redman 1998). YHV is potentially lethal to most of the commercially cultivated penaeid shrimp species (OIE 2003).

The causative agent of YHD is YHV, GAV and other closely related strains of the same virus (Table 1) (OIE 2003). Transmission electron microscopy (TEM) of YHV-infected tissues shows enveloped bacilliform virions. They range from approximately 150 nm to 200 nm in length and from 40 nm to 50 nm in diameter and are located within vesicles in the cytoplasm of infected cells and in intercellular spaces. The virions arise from longer, filamentous nucleocapsids (approximately 15 nm x 130-800 nm), which accumulate in the cytoplasm and obtain an envelope by budding at the endoplasmic reticulum into intracellular vesicles. Negatively stained YHV virions show regular arrays of short spikes on the viral envelope (Figures 4 and 6) (Boonyaratpalin et al. 1993; Chantanachookin et al. 1993; Lightner 1996a).

YHV was originally described mistakenly as a granulosis-like virus (Boonyaratpalin et al. 1993; Chantanachookin et al. 1993), but it was later found to be a single-stranded, positive sense RNA (ssRNA) virus (Tang and Lightner 1999) related to nidoviruses in the Coronaviridae and Arteriviridae (Sittidilokratna et al. 2002). GAV, the Australian strain of YHV has been recognized as the type species for the new virus genus *Okavirus* in the new family *Roniviridae* (Mayo 2002a, 2002b; OIE 2003).

Although YHD was first described as an epizootic from Thai shrimp farms (Limsuwan 1991), subsequent outbreaks of YHD have been reported from cultivated shrimp in many locations in Asia (OIE 2003). YHV has also been reported in frozen imported commodity shrimp in the United States (Nunan et al. 1998; Durand et al. 2000), and it has been incorrectly reported in farmed shrimp from the Americas based on the presentation of severe necrosis of the lymphoid organ, a lesion once thought to be pathognomonic for YHD (Lightner 1996a; Lightner et al. 1998; Lightner and Redman 1998) . However, the diagnosis of YHV infection in these cases was not confirmed with a second diagnostic method until after the errant reports were published. More recent work has shown that the presumptive histological diagnoses were due to severe infections with white spot virus, which can cause histopathology in the lymphoid organ which mimics that occurring in severe acute YHD (Pantoja and Lightner 2003). Because of the risk of introduction of YHV into the Western Hemisphere with frozen commodity shrimp still remains (Nunan et al. 1998, Durand et al. 2000), and because the possibility that concurrent WSSV/YHV infections may occur, in those severe WSSV cases in which YHV infection may also be suspected, the samples should be further analyzed by another method (i.e. RT-PCR or

ISH with a YHV specific probe) to confirm or rule out the presence of YHV. Hence, despite some early reports of YHV in the Americas, it has not been found infecting farmed shrimp in the Americas.

Infectious Myonecrosis (IMN)

Infectious myonecrosis (IMN) is a recently identified disease in cultured *L. vannamei* in northeast Brazil (Table 1). IMN causes significant disease and mortalities in juvenile and subadult pond-reared stocks of *L. vannamei*. In 2003, IMN is reported to have been responsible for millions of dollars in losses in northeast Brazil (unpublished data, ABCC, Brazil). Outbreaks of the disease seemed to be associated with certain types of environment and physical stresses (i.e. extremes in salinity and temperature, collection by cast net, etc.), and possibly with the use of low quality feeds. IMN presents as a disease in *L. vannamei* with an acute onset of gross signs and elevated mortalities, but it progresses with a more chronic course accompanied by persistent low level mortalities. To date, IMN appears to be limited to northeast Brazil, but shrimp with similar gross signs have been also reported from other countries where *L. vannamei* are cultured. Affected shrimp present extensive white necrotic areas in the striated muscle, especially of the distal abdominal segments and tail fan. These may become necrotic and reddened in some individual shrimp. By histopathology, shrimp with acute phase disease present lesions with coagulative muscle necrosis, often with edema. In shrimp recovering from acute disease or those in the more chronic phase of the disease, the myonecrosis appears to progress from coagulative to liquefactive necrosis. This progression of myonecrosis is accompanied with hemocytic infiltration and fibrosis. Significant lymphoid organ spheroid formation is typically present, and ectopic lymphoid organ spheroids are often found in the hemocoel and loose connective tissues, especially in the heart lumen and adjacent to antennal gland tubules. In some histological preparations, perinuclear pale basophilic to darkly basophilic inclusion bodies are evident in muscle cells, connective tissue cells, hemocytes, and in cells that comprise lymphoid organ spheroids (Lightner, unpublished data).

The infectious nature of the disease has been demonstrated by transmission of the disease into SPF indicator shrimp by injection and per os challenge studies using cell-free filtrates prepared from diseased shrimp or chopped diseased shrimp carcasses, respectively. A 40 nm diameter spherical virus has been isolated from naturally infected shrimp with the disease (Figure 5). The virus has been partially characterized and portions of its nucleic acid (RNA) genome have been cloned and sequenced. Molecular probes and RT-PCR methods for diagnosis of the disease and detection of IMNV have been developed and will be reported elsewhere (Lightner et al. 2004).

Disease Management

Until the WSSV pandemic, the penaeid shrimp farming industry in Asia and the Americas remained largely dependent on wild shrimp for stocking its farms. This was accomplished by the practice of collection and use of wild seed (postlarvae) for stocking of its farms directly or by the use of captive wild broodstock for the production of seed stock in hatcheries. This dependence has fostered the intensification and spread of the viral diseases in

shrimp aquaculture and in wild populations. The shrimp farming industry as a whole has recognized this fact and it has begun to change its farming practices in order to continue to develop, if not survive. While many of the shrimp stocks currently used to stock farms are produced from captive wild broodstock, only those that test negative for WSSV in Asia and WSSV and TSV in the Americas are used to stock biosecure farms. Biosecure production systems (that are designed to exclude potentially infected wild shrimp seed) stocked with shrimp stocks known to be free of the major shrimp pathogens have become a common practice in many shrimp growing regions. A further sign of a maturing industry is its movement towards the expanded development and use of specific pathogen-free (SPF) domesticated shrimp stocks of the most important shrimp species (Pruder et al. 1995; Moss et al. 2002; Lightner 2003; Lee and O'Bryen 2003). The techniques have advanced shrimp farming and made the industry far more sustainable than it was before the emergence of the virus caused diseases discussed in the present paper.

Acknowledgments

Grant support for much of the work cited in this review was provided by the United States Marine Shrimp Farming Consortium CSREES, under Grant No. 99-38808-7431, and by a series of special grants from the National Fisheries Institute.

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