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The Penaeid Shrimp Viruses TSV, IHHNV, WSSV, and YHV: Current Status in the Americas, Available Diagnostic Methods, and Management Strategies

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ABSTRACT. Viral diseases have emerged during this decade as serious economic impediments to successful shrimp farming. While nearly 20 distinct viruses or groups of viruses are known to infect penaeid shrimp, only four, WSSV, YHV, IHHNV, and TSV, pose a threat to the future of penaeid shrimp culture in the Western Hemisphere. TSV and IHHNV have caused serious disease epizootics throughout the Americas and Hawaii. IHHNV was described nearly 20 years ago when it was found to be responsible for cumulative losses of cultured *Penaeus stylirostris* that often exceed 90%. The threat of high mortalities posed by IHHNV has historically curtailed interest in the culture of this species in favor of the more IHHNV-resistant *P. vannamei*. While relatively resistant, IHHNV nonetheless infects *P. vannamei* and causes runt deformity syndrome (RDS) in which affected shrimp display reduced growth, highly variable sizes, lower production, and sometimes reduced survival. Taura Syndrome in *P. vannamei* is the virtual "mirror image" of IHHN disease in *P. stylirostris*. Following its recognition in 1992 as a distinct disease of *P. vannamei* in Ecuador, Taura Syndrome and its viral agent TSV spread rapidly throughout many of the shrimp farming regions of the Americas. Cumulative mortalities due to TSV in affected juvenile *P. vannamei* populations have ranged from 40 to 95%. Because *P. stylirostris* was found to be innately TSV-resistant, genetically selected IHHNV-resistant lines of this species disease are being developed and marketed in the Americas. Some shrimp farmers are

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using domesticated, selected stocks of *P. vannamei* that show improved resistance to TSV, while others have continued to use wild stocks that are also showing increased resistance, perhaps through intense natural selection. The viruses of the Yellow Head Disease (YHV) and the White Spot Syndrome (WSSV) were first recognized in 1991-92 in Asia, and by 1996 the two diseases had spread and had caused major pandemics throughout much of the shrimp farming regions of East Asia, Southeast Asia, Indonesia, and India. In late 1995, WSSV was found in North America for the first time in *P. setiferus* at a shrimp farm in south Texas. WSSV has since been detected in cultured and wild shrimp, crabs, and freshwater crayfish at multiple sites in the eastern and southeastern U.S., as well as being commonly found (along with YHV) in imported frozen commodity shrimp. Because Western Hemisphere penaeids are highly susceptible to WSSV and YHV, the introduction and establishment of either of the viruses poses a significant threat to the shrimp farming industry. The available detection methods for IHHNV, TSV, WSSV, and YHV include traditional methods that employ gross signs, clinical history, histopathology, and bioassay with susceptible shrimp hosts. Molecular and serological methods have also been developed, and specific gene probes, monoclonal antibodies, or PCR methods are readily available for each of the four viruses. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: getinfo@haworthpressinc.com <Website: http://www.haworthpressinc.com>]*

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INTRODUCTION

Twenty-five years have elapsed since John Couch described *Baculovirus penaei,* the first recognized virus of penaeid shrimp, in *Penaeus duorarum* (northern pink shrimp) from the Gulf of Mexico in Florida (Couch 1974). Since then the list of viruses infecting this group of marine invertebrate animals has grown to include nearly 20 viruses (Table 1), and viruses have emerged as important pathogens of penaeid shrimp virtually everywhere in the world where penaeid shrimp are cultured (Lightner 1996a). Currently, nine viruses (or groups of closely related viruses) are known to be enzootic in Western Hemisphere penaeids, and five of these pathogens have emerged as serious pathogens in one or more species of cultured shrimp (Table 2). Virus diseases have also severely impacted the shrimp farming industries of the Eastern Hemisphere. In the shrimp growing regions of the

TABLE 1. Viruses of penaeid shrimp (as of January 1999; modified from Lightner 1996a and Lightner et al. 1998b).

DNA VIRUSES		
PARVOVIRUSES:		
IHHNV	=	infectious hypodermal and hematopoietic necrosis virus
HPV	=	hepatopancreatic parvovirus
SMV	=	spawner-isolated mortality virus
LPV	=	lymphoidal parvo-like virus
BACULOVIRUSES and BACULO-LIKE VIRUSES:		
BP-type	=	*Baculovirus penaei* type viruses (PvSNPV type sp.): BP strains from the Gulf of Mexico, Hawaii & Eastern Pacific
MBV-type	=	*Penaeus monodon*-type baculoviruses (PmSNPV type sp.): MBV strains from East & SE Asia, Australia, the Indo-Pacific, and India
BMN-type	=	baculoviral midgut gland necrosis type viruses:
BMN	=	from *P. japonicus* in Japan
TCBV	=	type C baculovirus of *P. monodon*
PHRV	=	hemocyte-infecting nonoccluded bacule-like virus
WHITE SPOT SYNDROME BACULO-LIKE VIRUSES (PmNOBII-type):		
SEMBV	=	systemic ectodermal & mesodermal baculo-like virus
RV-PJ	=	rod shaped virus of *P. japonicus*
PAV	=	penaeid acute viremia virus
HHNBV	=	hypodermal & hematopoietic necrosis baculo-like virus; agent of "SEEDS" (shrimp explosive epidemic disease)
WSBV	=	white spot bacule-like virus
WSSV/WSV	=	white spot syndrome virus
IRIDOVIRUS:		
IRDO	=	shrimp iridovirus
RNA VIRUSES		
PICORNAVIRUS:		
TSV	=	Taura syndrome virus
REOVIRUSES:		
REO-III & IV	=	reo-like virus type II and IV
TOGA-LIKE VIRUS:		
LOVV	=	lymphoid organ vacuolization virus
RHABDOVIRUS:		
RPS	=	rhabdovirus of penaeid shrimp
YELLOW HEAD VIRUS GROUP:		
YHV/"YBV"	=	yellowhead ("yellowhead baculovirus") virus of *P. monodon*
GAV	=	gill associated virus of *P. monodon*
LOV	=	lymphoid organ virus of *P. monodon*

Indo-Pacific and East Asia at least 12 viruses (or groups of closely related viruses) are recognized. Of the 12 virus groups, five have been documented to be responsible for serious disease epizootics regionally, with two of these viruses having caused panzootics throughout much of the industry in the Indo-Pacific and East Asia. Although there

TABLE 2. Viruses reported from Eastern (Asian, Australian, European, and African) and Western Hemisphere (the Americas and Hawaii) of penaeid shrimp (modified from Lightner 1996a; Lightner et al. 1998b).

Virus or Virus Group	Eastern Hemisphere	Western Hemisphere
Baculo and baculo-like viruses	MBV-group BMN-group WSSV PHRV	BP WSSV
Parvo and parvo-like viruses	IHHNV HPV SMV LPV	IHHNV HPV
Picornavirus	none	TSV
Rod-shaped ssRNA viruses	YHV LOV/GAV	none
Reo-like viruses	REO-III REO-IV	REO-III
Toga-like viruses	none	LOVV
Rhabdovirus	none	RPS
Iridovirus	none	IRDO

are nearly 20 different viruses recognized in penacid shrimp, only four seem to stand out as being especially important from their historical, current and potential future adverse effects on the international shrimp farming industries. These four viruses are white spot syndrome virus (WSSV), yellow head virus (YHV), taura syndrome virus (TSV), and infectious hypodermal and hematopoietic necrosis virus (IHHNV). TSV and IHHNV have caused serious disease problems in many of the shrimp growing countries in the Americas. Wide-spread epizootics due to YHV and WSSV emerged early in this decade in the Asian shrimp farming industry, and together they have caused catastrophic disease losses (with a total negative economic impact averaging from U.S.$1 to 3 billion per year since 1994) collectively to major shrimp growing countries like China, Thailand, India, Indonesia, Bangladesh, Malaysia, Taiwan, Vietnam and Japan (ADB/NACA in press). This paper will discuss the biology, hosts, the available detection and diagnostic methods, and what is perceived as the best management practices for the shrimp culture industry of the Western Hemisphere to most effectively deal with these viruses. The taxonomic (Latin binomial and common) names used here are according to Holthuis (1980).

THE VIRUSES OF CONCERN: BIOLOGY, RANGE AND HOSTS

Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV)

The virion of IHHN is among the smallest animal viruses and it is the smallest of the known penaeid shrimp viruses. The IHHN virion is a non-enveloped icosahedron of 22 nm in diameter with a 4.1 Kb ssDNA genome and nuclear replication. Its characteristics place IHHNV within the family Parvoviridae (Bonami et al. 1990). IHHNV was first recognized in 1981 when it was shown to be the cause of acute, catastrophic epizootics with cumulative mortality rates of ~60 to 90% in semi-intensively or intensively cultured juvenile *P. stylirostris* (blue shrimp) stocks that originated from Mexico, Ecuador, or Panama (Lightner et al. 1983a, b; Bell and Lightner 1987; Lightner 1996a, b). In *P. vannamei* (white leg shrimp) IHHNV was recognized soon after its discovery to infect and cause disease, but not significant mortalities (Bell and Lightner 1984). Despite their relative resistance to IHHN disease, cultured *P. vannamei* can be chronically infected with IHHNV and suffer runt deformity syndrome (RDS) as a consequence. RDS was linked by epizootiological data to infection by IHHNV. Shrimp with RDS often show greatly reduced growth rates and a variety of cuticular deformities affecting the rostrum (= "bent rostrum"), antennae, and other cephalothoracic and abdominal areas of the exoskeleton (Kalagayan et al. 1991; Browdy et al. 1993). RDS is an economically significant disease of cultured *P. vannamei*, which has been observed in virtually every country in the Americas where the species is cultured (Brock and Lightner 1990; Brock and Main 1994; Lightner 1996a). Cultured populations affected by RDS may contain up to 30% runts and consequently a wide distribution of size ("count" or the number of shrimp per pound) classes. Because runted shrimp have a lower market value than unaffected shrimp, RDS significantly reduces the market value of affected *P. vannamei* crops, resulting in revenue losses that can range from 10 to 50% of the value of similar IHHNV-free (and RDS-free) crops (Wyban et al. 1992).

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). Because other penaeid species including *P. setiferus* (northern white shrimp), *P. duorarum,* and *P. aztecus* (northern brown shrimp) have been experimentally infected, natural infections by the virus probably occur in a number of other penaeid species. However, species like *P. indicus* (Indian white prawn) and *P. merguiensis* (banana prawn) seem to be refractory to IHHN (Lightner 1996a). While all life stages of susceptible host species may be infected by IHHNV, the juvenile stages are the most severely affected (Table 3).

IHHNV has been documented to occur in East and Southeast Asia (Japan, Singapore, Malaysia, Indonesia, Thailand, and the Philippines) in shrimp culture facilities using only captive-wild *P. japonicus* and *P. monodon* broodstock, and where American penaeids had not been introduced. Except for a single report from the Philippines that implicated IHHNV as the cause of a serious epizootic in *P. monodon* (Rosenberry 1992), the virus is increasingly viewed as a generally insignificant pathogen in Asia (Baticados et al. 1990; Lightner et al. 1992; Flegel et al. 1995a; Lightner 1996a, b; Flegel 1997). The occurrence of IHHNV in captive-wild broodstocks and in their cultured progeny suggests that East and Southeast Asia is within the virus' natural geographic range and that *P. monodon* and *P. japonicus* may be

TABLE 3. Susceptibility and severity of disease of important American penaeids to the viruses IHHNV, TSV, BP, WSSV and YHV as determined from natural and experimental infections (modified from Lightner et al. 1997; Lightner and Redman 1998).[1]

Species	IHHNV				TSV				WSSV				YHV			
	L	PL	J	A	L	PL	J	A	L	PL	J	A	L	PL	J	A
P. vannamei	–	+	+	+	–	+/+	++	++	?	+/+	+/+	?	–	–	++	?
P. stylirostris	–	+	++	+	–	–	+	–	?	?	+/+	?	?	?	++	?
P. schmitti	–	?	+	?	–	–	+	–	?	?	?	?	?	?	?	?
P. setiferus	–	–	+	?	–	+/+	+	?	?	+/+	+/+	?	?	–	++	?
P. aztecus	–	–	+	?	–	+	+	?	?	+/+	+/–	?	?	_	++	?
P. duorarum	–	–	+	?	–	–	–	?	?	+/+	–	?	?	–	++	?
P. californiensis	–	–	+	+	–	–	–	?	?	?	+/–	?	?	?	?	?

[1] Key to symbols for each pathogen and life stages:

L = larvae; PL = postlarvae; J = juvenile; A = adult.
– = infection occurs but without disease expression in this life stage.
? = no data available.
+ = infection accompanied by expression of moderate disease.
++ = infection accompanied by expression of significant disease.

among its natural host species. Based on the apparently stable host-pathogen relationship of IHHNV to its shrimp hosts in Asia versus the more serious history of IHHNV in the Americas, it has been suggested that IHHNV was introduced into the Americas from Asia during the early 1970's (Lightner 1996b). The hypothesis that IHHNV was introduced into the Americas with Asian *P. monodon* in the mid-1970s goes a long way towards explaining why the developing shrimp culture industry in Latin America and in the U.S. shifted from culturing *P. stylirostris* to *P. vannamei* during that period.

Taura Syndrome Virus (TSV)

TSV has been classified as a picornavirus based on its virion structure (i.e., 30 nm diameter, non-enveloped, icosahedron with cytoplasmic replication, and a ssRNA genome) (Bonami et al. 1997; Mari et al. 1998). Taura Syndrome (TS) was first recognized in shrimp farms located near the mouth of the Taura River in the Gulf of Guayaquil, Ecuador, in mid-1992 (Jimenez 1992; Lightner et al. 1995) where the disease caused catastrophic disease losses with cumulative mortality rates of 60 to > 90% of affected pond-cultured juvenile *P. vannamei*. Following its recognition as a distinct disease of cultured *P. vannamei* in Ecuador in 1992, TSV spread rapidly to virtually all of the shrimp growing regions of Latin America and to parts of the U.S. (Brock et al. 1995, 1997; Lightner 1996a, 1996b, 1998; Hasson et al. 1995, 1997a, b, 1999). The epidemiological and laboratory studies that followed its discovery and spread from Ecuador showed that TSV had a viral etiology and that of the American (*P. vannamei*, *P. stylirostris*, *P. schmitti* [the southern white shrimp], *P. setiferus* and *P. aztecus*) and Asian (*P. monodon*, *P. japonicus*, *P. chinensis* [fleshy prawn or Chinese white shrimp]) penaeid species naturally or experimentally infected by the virus, *P. vannamei* was by far the most severely affected (Brock et al. 1995, 1997; Hasson et al. 1995, 1997a, 1999; Lightner 1996a, 1997; Overstreet et al. 1997).

Because shrimp culture in the Americas was, and still is, a virtual monoculture based on *P. vannamei* (Rosenberry 1993, 1998), TS has seriously impacted the shrimp culture industry. In Ecuador, the disease resulted in production losses that reached between 15 and 30% of the country's production in 1993 and 1994 (Rosenberry 1994a, b; Wigglesworth 1994). At shrimp wholesale prices in 1992-1994 (~$13.00/kg for 31/35 count tails), a 30% reduction in production relative to

Ecuador's 1991 production of 100,000 t translates to nearly a $400 million loss in revenue per year. TS has had a similarly devastating impact on the farms of other countries as it spread from Ecuador (Lightner 1996b; Brock 1997).

Since shortly after the discovery of TSV, a controversy over the etiology of TS began and it has persisted to the present. Although both toxic and infectious etiologies have been proposed for TS, the disease is caused by a virus (Bonami et al. 1997; Hasson et al. 1995) and this has been demonstrated and confirmed by several independent laboratories (Brock et al. 1995, 1997; Hasson et al. 1999a). The hypothesis that TS has a toxic etiology has not held up to scientific scrutiny, and the hypothesis has endured only in the interest of ongoing litigation. Unfortunately, the controversy on the etiology of TS also contributed to the geographic spread of the disease (Lightner 1996b; Hasson et al. 1999a). The transfer of TSV from Ecuador into other countries of the region with TSV-infected PL *P. vannamei* may not have happened so rapidly, if at all, had there not been so much reluctance by the Ecuadorian shrimp farming industry to accept the notion that TS had a viral etiology. Other important factors in the biology of TSV that have contributed to its spread include its being carried in the gut contents and feces (at least within and among adjacent farms) by aquatic insects like the water boatman and by gulls (Lightner 1996b; Garza et al. 1997). That survivors of TSV infections (in either susceptible *P. vannamei* or in resistant *P. stylirostris*) remain persistently infected by the virus, perhaps for life, provides the virus with the opportunity for both horizontal and vertical transmission (Hasson et al. 1999b).

White Spot Syndrome Baculo-Like Virus (WSSV) Complex

At least five viruses in the white spot syndrome (WSS) complex have been named in the literature (for review see Lightner 1996a; Lightner et al. 1998). They appear to be very similar viruses. The names of the viruses and the diseases they cause are summarized in Table 1. All are very similar in morphology and replicate in the nuclei of infected cells, which are typically in tissues of ectodermal and mesodermal origin. Infected nuclei in enteric tissues (i.e., midgut mucosa and hepatopancreatic tubule epithelium) are rarely, if ever, present (Lightner 1996a).

Isolated virions from this WSS complex, when contrasted by negative staining and viewed by TEM, are enveloped, elliptical rods, aver-

aging approximately 130 nm in diameter by 350 nm in length with size variations ranging from 100 to 140 nm and 270 to 420 nm, respectively. Some virions possess a tail-like appendage at one extremity that is an extension of the envelope. Nucleocapsids are rod shaped with blunted ends, measure 90 nm by 360 nm (range of 70 to 95 nm by 300 to 420 nm, respectively), and display a superficially segmented appearance with an angle of 90° to the long axis of the particle. The nucleic acid of WSS viruses is a large single molecule of circular dsDNA that is between 100 and 200 kbp in length (Wongteerasupaya et al. 1995; Durand et al. 1996; Lo et al. 1996). The characteristics of the WSSV complex are most like members of the family Baculoviridae (Francki et al. 1991; Murphy et al. 1995). However, the non-occluded viruses in this family were removed from the family and placed temporarily in a group of viruses of uncertain taxonomic position (Murphy et al. 1995).

The WSSV-complex infects and causes serious disease in many species of penaeid shrimp and in a variety of other decapod crustaceans. Among the Asian penaeids reported to be infected by WSSV-complex viruses are *P. monodon*, *P. semisulcatus*, *P. japonicus*, *P. chinensis*, *P. penicillatus* (redtail prawn), *P. indicus*, *P. merguiensis*, *Trachypenaeus curvirostris* (southern rough shrimp), and *Metapenaeus ensis* (greasyback shrimp) (Chang et al. 1998; Lightner 1996a; Wang et al. 1998). Western Hemisphere penaeids are also susceptible for WSSV infection and disease (Lightner 1996a; Tapay et al., 1997; Lightner et al. 1998a). WSSV infects and can cause serious disease in *Macrobrachium rosenbergii* (giant river prawn) and in the North American crayfish, *Procambarus clarkii* (red swamp crayfish). WSSV also can infect but does not seem to cause significant disease in a variety of marine crabs and spiny lobsters (Chang et al. 1998; Wang et al. 1998).

Following its appearance in 1992-1993 in northeast Asia, the WSSV has spread very rapidly throughout most of the shrimp growing regions of Asia and the Indo-Pacific. Documented reports of the WSSV epizootics in Asia now include Taiwan, China, Korea, Thailand, Indonesia, Vietnam, Malaysia, India, Sri Lanka, and Bangladesh (Inouye et al. 1994, 1996; Nakano et al. 1994; Takahashi et al. 1994; Chen 1995; Chou et al. 1995; Huang et al. 1995; Wang et al. 1995; Wongteerasupaya et al. 1995; Lo et al. 1996; Chang et al. 1998; Kasornchandra et al. 1998; Wang et al. 1998).

In the American penaeids, natural infections by WSSV have been

documented every year since an initial outbreak in late 1995 involving *P. setiferus* cultured in Texas (Lightner 1996a; Lightner et al. 1998; Lightner and Redman 1998). Since that initial outbreak, WSSV was detected on two separate occasions in 1996 and 1997 in captive-wild crayfish being held at the National Zoo in Washington, DC, where the virus was associated with severe disease and mortalities in the crayfish, *Orochnectes punctimanus* and *Procambarus* sp. (Richman et al. 1997). In penaeids, WSSV has been found in two separate regions in the U.S. since 1995. The virus was detected in wild *P. setiferus* and *P. duorarum* collected off Texas in 1997 and 1998. In South Carolina, WSSV was found in 1997 and 1998 in wild *P. setiferus* and in a variety of other wild decapod crustaceans, in cultured stocks of *P. setiferus* in 1997, and in cultured stocks of P. *stylirostris* and *P. vannamei* in 1998. In the latter two cases, WSSV infections occurred at shrimp farms and were associated with > 95% cumulative losses to the affected farms within days to weeks after the onset of disease.

Laboratory studies have provided some information on the susceptibility of American penaeids to WSSV. Postlarval (PL) and juvenile stages of *P. vannamei*, *P. stylirostris*, *P. setiferus*, *P. aztecus*, and *P. duorarum* were experimentally infected by a WSSV isolate that was originally derived from cultured *P. monodon* from Thailand. Challenge of PL with the virus resulted in severe infections in *P. setiferus* and *P. vannamei* and in less severe infections in PL *P. aztecus* and PL *P. duorarum*. The results from WSSV challenge studies with juveniles followed a similar trend to that observed with PL. Challenge resulted in positive infections in juveniles of all four species, with severe infections and 100% cumulative mortalities resulting in juvenile *P. setiferus* and *P. vannamei*, while only moderate infection, disease and mortalities occurred in challenged juvenile *P. aztecus*, and no signs of disease nor mortality resulted in challenged juvenile *P. duorarum* (Lightner et al. 1998).

Yellow Head Virus Group (YHV)

Yellow head virus (YHV) from southeast Asia (Booryaratpalin et al. 1993; Chantanachookin et al. 1993; Wongteerasupaya et al. 1995) and the morphologically similar lymphoid organ virus (LOV) (Spann et al. 1995) and gill associated virus (GAV) from Australian *P. monodon* (Spann et al. 1997) are rod shaped, enveloped viruses that replicate in the cytoplasm of infected cells. For the purpose of this paper

these viruses will be called YHV. The YHV virion is enveloped and measures 44 nm by 173 nm in length (with a range of 38 to 50 nm by 160 to 186 nm, respectively), contains a cylindrical nucleocapsid of ~15 nm in diameter and a single piece of ssRNA as its genome. While not yet adequately characterized, YHV has been suggested to be a member of the families Rhabdoviridae, Paramyxoviridae (Boonyaratpalin et al. 1993; Chantanachookin et al. 1993; Flegel et al. 1995b; Kasornchandra et al. 1995; Spann et al. 1995; Lightner 1996a), and most recently, the Coronaviridae (P.J. Walker, personal communication, CSIRO, Brisbane, Australia).

Yellow head virus causes a serious disease in *P. monodon* in intensive culture systems in southeast Asia and India. YHV is widespread in cultured stocks of *P. monodon* in the southeast Asian and Indo-Pacific countries of Thailand, China, Malaysia, Indonesia, India (Flegel et al. 1995b, 1997; Lightner 1996a). The virus was found in Taiwan in *P. japonicus* that were co-infected with WSSV (Wang et al. 1998). Likewise, the closely related virus, GAV, causes serious disease in *P. monodon* cultured in Australia (Spann et al. 1997). Disease due to YHV and GAV infection typically occurs in juveniles and sub-adults (Boonyaratpalin et al. 1993; Flegel et al. 1995b; Lightner 1996a). The brackish water shrimps, *Palaemon styliferus* (grass shrimp) and *Eubasia superba* and *Acetes* sp. (planktonic shrimps), which are often resident in shrimp ponds in Thailand, were found in bioassays with healthy *P. monodon* to carry YHV (Flegel et al. 1995b; Alda De Grainorge and Flegel 1999). Although resistant to YHV in ponds, *P. merguiensis* and *M. ensis* were found to be experimentally infected by YHV in laboratory challenge studies (Flegel et al. 1995b).

American penaeids were found to be highly susceptible to experimental infection by YHV. While the PL stages were refractory to infection, juveniles of *P. vannamei, P. stylirostris, P. setiferus, P. aztecus,* and *P. duorarum* were found to be susceptible to challenge by the virus and to suffer significant disease (Lu et al. 1995; Lightner 1996a; Lightner et al. 1998).

DIAGNOSTIC METHODS

Methods for the detection of penaeid shrimp viruses and diagnosis of the diseases that they cause can be as simple as the observation of specific gross signs that accompany infection by particular viruses

(Table 4). Of the four viruses of being reviewed in the present paper, all four can cause unique signs of infection that may aid in diagnosis. Specifically, chronic infections by IHHNV typically result in RDS in *P. vannamei* and sometimes in *P. stylirostris* with its unique cuticular deformities and high numbers of under-sized shrimp in affected populations. TSV produces unique cuticular melanized spots in the transitional phase of its disease cycle in *P. vannamei.* As the name implies, some shrimp in the recovery phase of infection by WSSV may display prominent subcuticular white spots. Likewise, in populations of *P. monodon* with epizootic disease due to YHV, some affected shrimp display yellowish discoloration of the gills and hepatopancreatic re-

TABLE 4. Summary of diagnostic and detection methods for the major viruses of concern to the shrimp culture industries of the Americas (modified from Lightner 1996a; Lightner and Redman 1998).[1]

METHOD	IHHNV	TSV	YHV	WSSV
Direct BF/LM	–	++	++	++
Phase contrast	–	+	–	+
Dark-field LM	–	–	–	++
Histopathology	++	+++	+++	++
Enhancement/histology	++	+	+	+
Bioassay/histology	+	+++	+++	++
Transmission EM	+	+	+	+
Fluorescent antibody	r&d	r&d	–	r&d
ELISA with PABs/MABs	r&d	r&d	r&d	–
DNA Probes	+++/K	+++/K	+++/K	+++/K
PCR/RT-PCR	+++	+++	+++	+++

[1] Definitions for virus acronyms and symbols:

– = no known or published application of technique.
\+ = application of technique is known or published.
++ = application of technique is considered to provide sufficient diagnostic accuracy or pathogen detection sensitivity for most applications.
+++ = technique provides a high degree of sensitivity in pathogen detection.
K = diagnostic kit available from DiagXotics, Inc. (Wilton, Connecticut).
r&d = techniques in research and development phase.

Methods:
BF = bright field LM of tissue impression smears, wet-mounts, stained whole mounts.
LM = light microscopy.
EM = electron microscopy of sections or of purified or semi-purified virus.
ELISA = enzyme-linked immunosorbent assay.
PABs = polyclonal antibodies.
MABs = monoclonal antibodies.
PCR = DNA amplification by polymerase chain reaction.
RT-PCR = PCR after reverse transcription of viral RNA genome.

gion of the gnathothorax (Lightner 1996a). However, in American penaeids, infection by WSSV or YHV may result in acute disease and near 100% cumulative moralities, and hence, these diagnostic gross signs may seldom, if ever, be present.

Histology is among the most important and commonly used diagnostic method for penaeid diseases in general (Lightner 1996a; Bell and Lightner 1988), and each of the four viruses reviewed in the present paper (IHHNV, TSV, WSSV, and YHV) produce unique lesions at some phase of their infection cycles to provide a definitive diagnosis (Table 4). These specific lesions and methods used for histological diagnosis of infections by these viruses are readily available from a number of sources (Baticados et al. 1990; Brock and Lightner 1990; Lightner 1993, 1996a; Brock and Main 1994, Chanratchakool et al. 1994; Johnson 1995; OIE 1997).

Several serodiagnostic methods have been developed for use in shrimp disease diagnosis (Table 4). However, despite the considerable research and development efforts (by research groups in Asia, Europe, and the North America) that have gone into attempts to develop monoclonal antibodies (MAbs) to the penaeid viruses IHHNV, YHV, TSV, and WSSV, only for TSV has a serodiagnositc method been developed and made available commercially to the industry (Poulos et al. in press). While serodiagnostic methods for IHHNV were developed (Poulos et al. 1994), the MAbs developed were of the IGM class. These antibodies reacted specifically with purified IHHNV or its capsid proteins in Western blots. However, they also reacted nonspecifically with components in normal shrimp tissue, resulting in false positive reactions with uninfected shrimp tissue samples in ELISA-based assays (Poulos et al. 1994; Lightner and Redman 1998b). Although the development of serological tests for the more important shrimp pathogens has lagged behind the development of molecular detection and classical diagnostic methods, it is very likely that the use of tests based on polyclonal and monoclonal antibodies will become much more common in shrimp diagnostic laboratories in the next few years. Because of their speed, versatility, relatively low cost, simplicity, and reasonably good sensitivity, monoclonal antibody based tests are potentially very useful as routine diagnostic tests, even in the most modestly equipped diagnostic laboratories (Reddington and Lightner 1994).

Molecular methods (gene probes and DNA amplification using the

polymerase chain reaction [PCR]) are increasingly becoming the standard for the detection and diagnosis of shrimp viruses. Nearly a decade has passed since the first non-radioactively labeled gene probe was developed and applied to the diagnosis of the IHHNV (Table 4) (Mari et al. 1993). Since then, molecular probe methods for all of the important penaeid shrimp viruses have been developed and made commercially available as diagnostic kits and as labeled probes marketed under the product name 'ShrimProbes™' (DiagXotics, Wilton, Connecticut[1]).

As genome sequence information became available for each of the viruses of concern, PCR and Reverse Transcriptase PCR (RT-PCR) methodologies were developed that have added even more potential sensitivity to the detection capabilities for shrimp viruses. In PCR, small, often undetectable amounts of DNA can be amplified to produce detectable quantities of the target DNA. This is accomplished by using unique and specific oligonucleotide primers designed to bind to the target sequences of the positive and negative sense strands of the DNA molecule. The primers, along with a buffered reaction mixture containing nucleotides and DNA polymerase, fill in the specific nucleotide sequence of the positive and negative DNA strands that lie between the primers. Using a programmable thermal cycler, the process of PCR is repeated from 20 to 40 times. In the case of the RNA viruses (YHV and TSV), a step is added to the assay in which the ssRNA strand is converted to cDNA by reverse transcription. After that step, the resulting cDNA is amplified by PCR as would be the specific gene sequence from a DNA virus. The resultant PCR or RT-PCR product may then be compared to a known standard for the virus being assayed, using gel electrophoresis. Alternatively, the PCR product may be blotted onto a membrane (or transferred using the Southern transfer method) and tested with a specific genomic probe by reaction with a specific DNA probe. In some applications PCR products themselves may be labeled with DIG and used as specific DNA probes (Innis et al. 1990; Perkin Elmer 1992). Details of these methods are available from a number of recent publications: IHHNV (Lightner 1996a); TSV (Nunan et al. 1998b); YHV (Wongterrasupaya et al. 1997; Tang et al. 1999); and WSSV (Kimura et al. 1996; Lo et al. 1996; Takahashi et al. 1996; Nunan and Lightner 1997).

1. Use of trade or manufacturer name does not imply endorsement.

THE THREAT OF WSSV AND YHV TO THE AMERICAS

As discussed earlier in this review, WSSV and YHV were thought to be limited to Asia, until WSSV appeared November 1995 in cultured *P. setiferus* in Texas (Lightner et al. 1997, 1998; Lotz 1997; Nunan et al. 1998a). Since that initial recognition of the virus in Texas, it has been found every year in wild or cultured penaeids in Texas or South Carolina, as well as in wild crabs and in captive-wild freshwater crayfish (see WSSV section). Following the WSSV outbreak in Texas, various sources for introduction of the pathogen were considered. The stocks being cultured at the affected farm had been produced from captive-wild broodstock of *P. setiferus*, and it was at the end of the culture cycle (the affected shrimp were late juvenile to sub-adults). The occurrence of a WSSV-caused epizootic late in the culture cycle of a highly susceptible species indicated that the virus was introduced to the farm during culture, rather than being introduced with the PL when the farm was stocked months earlier. A similar scenario applies to the WSSV epizootics of 1997 and 1998 at the affected farms in South Carolina. The affected farms were culturing domesticated, SPF lines (Wyban et al. 1992; Lotz et al. 1995) of *P. vannamei* or *P. stylirostris* with no prior history of WSSV, and since only SPF (i.e., free of TSV, IHHNV, WSSV, YHV, and other important pathogens) *P. vannamei* had been cultured in Texas and South Carolina for 2-3 years preceding the initial appearance in these states, it is extremely unlikely that these viruses were not introduced with the SPF shrimp stocks. Their source had to have been from some other activity.

That source could be imported commodity shrimp. Despite the presence of large monocultures of highly susceptible *P. vannamei* and *P. stylirostris* being grown in a number of major shrimp growing countries in Latin America, nowhere but in the U.S. has WSSV been detected in wild or cultured shrimp stocks. The difference between these WSSV-negative countries and the U.S. is imports. The U.S. is a major market for shrimp, and it imports each year thousand of tons of cultured penaeid shrimp from Asian countries (Filose 1995) where WSSV and YHV have been enzootic and causing serious epizootics since 1992. In marked contrast, the other countries of the Americas where penaeid shrimp are farmed or fished do not import significant quantities of Asian shrimp. According to U.S.D.C. data, imports of penaeid shrimp for the U.S. market have increased significantly in

recent years, and since 1995 over half of the imported shrimp marketed in the United States now comes from farm-cultured stocks (Filose 1995; New 1997; U.S.D.C. 1997). More than half of those imports come from countries (Thailand, India, and China) that, since 1992, have been severely impacted by WSSV and YHV. Imported commodity shrimp are distributed throughout the U.S., and some of the imported shrimp are re-processed at shrimp packing plants situated on coastal bays and estuaries where native penaeid nursery grounds also occur. Imported small sized, heads-on shrimp are also packed specifically for sale as bait to U.S. sport fishermen (JSA 1997; U.S.D.C. 1997; Lightner et al. 1997).

Emergency harvests are commonly employed in Asia to salvage marketable shrimp crops with developing epizootics due to these viruses (Flegel et al. 1995a, b; Jory 1996; Lightner et al. 1997). Because of this practice small count size (40 to 90 count) *P. monodon* displaying gross signs of WSSV infection (i.e., cuticular white spots and reddish pigmentation) began to appear in U.S. retails outlets about 1994 and are still frequently found in U.S. retail outlets (Nunan et al. 1998a; Lightner et al. in press). PCR assays of samples of these shrimp confirmed the presence of WSSV and YHV. Live shrimp bioassays with SPF *P. vannamei* or *P. stylirostris* as the indicator for infectious virus have given positive results for both WSSV and YHV (Table 5). These studies have confirmed that frozen imported commodity shrimp do in fact contain non-indigenous viruses that are infectious to Western Hemisphere penaeids and other decapods.

Genetic and virulence comparisons of WSSV isolates from Asia, Texas, South Carolina, the National Zoo, and from imported commodity shrimp have shown little or no difference among the isolates (Lo et al. 1999; Wang et al. 1999). These results suggest that WSSV is pandemic and that the same or a few very closely related strains of the virus have spread from the initial epicenter of the disease in East Asia.

STRATEGIES FOR CONTROL OF VIRUS DISEASE

Numerous strategies have been attempted for the control of viral diseases in penaeid shrimp aquaculture. These strategies have ranged from the use of improved husbandry practices to stocking specific pathogen-free (SPF) or specific pathogen resistant (SPR) species or stocks (Lotz 1997). In the Americas many strategies have been employed

TABLE 5. Results of PCR assays for WSSV and YHV using samples of frozen imported *Penaeus monodon* tails from Thailand and other SE Asian sources.[1]

UAZ ID#	Source/ Origin	Species/ Size	Gross Signs	PCR Primers WSSV	PCR Primers YHV	PCR Results	Bioassay UAZ ID#	Bioassay Indicator Species	Bioassay Results
95-204	Tucson Retail Thailand	*P. monodon* 51/60 count	reddish w/spots	F-6581 R-7632	N/A	++++ WSSV	95-204	*P. stylirostris*	+++ YHV + WSSV
95-319	California Retail/Asia	*P. monodon* ~40 count	reddish w/spots	F-6581 R-7632	N/A	+++ WSSV	N/A	N/A	N/A
95-320	Washington Retail/Asia	*P. monodon* 26/30 count	none	F-6581 R-7632	N/A	Negative WSSV	N/A	N/A	N/A
96-87	Tucson Retail Thailand	*P. monodon* ~40 count	reddish w/spots	F-6581 R-7632	N/A	++++ WSSV	N/A	N/A	N/A
96-101	Tucson Retail Thailand	*P. monodon* ~40 count	none	F-6581 R-7632	N/A	Negative WSSV	N/A	N/A	N/A
96-104	Texas Retail Asia	*P. monodon* ~40 count	none	F-6581 R-7632	N/A	Negative WSSV	N/A	N/A	N/A
96-109	Tucson Retail Thailand	*Macro-brachium rosenbergii*	w/spots	F-6581 R-7632	N/A	+ WSSV	N/A	N/A	N/A
96-115	Tucson Retail Thailand	*P. monodon* ~40 count	reddish w/spots	F-6581 R-7632	N/A	++ WSSV	N/A	N/A	N/A
98-78	Tucson Retail Asia	*P. monodon* 90 count	reddish w/spots	E-12 E-14	Thai	++++ WSSV + YHV	B98-210/2	*P. vannamei*	++++ WSSV (100% mortality) Negative YHV
98-111/A	Blue Sky/ Thailand	*P. monodon*	N/D	E-12 E-14	Thai	+++ WSSV Negative YHV	B98-146	*P. vannamei*	Negative WSSV Negative YHV
98-111/B	Suratthani Thailand	"White Tiger"[2]	N/D	E-12 E-14	Thai	+++ WSSV Negative YHV	B98-146	*P. vannamei*	Negative WSSV Negative YHV
98-111/C	Transamut Thailand	*P. monodon*	N/D	E-12 E-14	Thai	+ WSSV Negative YHV	N/A	N/A	N/A
98-111/D	Transamut Thailand	*P. monodon* & "White Tiger"	N/D	E-12 E-14	Thai	+ WSSV Negative YHV	N/A	N/A	N/A
98-111/E	Blue Sky Thailand	*P. monodon*	N/D	E-12 E-14	Thai	+ WSSV + YHV	B98-146	*P. vannamei*	Negative WSSV + YHV (PCR +; no mortality)
98-111/F	Suratthani Thailand	"White Tiger"	N/D	E-12 E-14	Thai	Negative WSSV Negative YHV	N/A	N/A	N/A
98-131	Tucson Retail Thailand	*P. monodon* 40 count	reddish w/ spots	E-12 E-14	Thai	++++ WSSV Negative YHV	B98-210/3	*P. vannamei*	+++ WSSV (100% mortality) Negative YHV

[1] 98-111 samples were provided by the National Fisheries Institute; the remaining samples were purchased from retails outlets in Tucson, AZ, Washington State, California, and Texas.
N/D = not described.
N/A = not analyzed.
+ = a weakly positive test result; ++ = strong positive test result; ++++ = very strong positive test result.
F-6581 and R-7632 from Nunan and Lightner (1997).
E-12 and E-14 = WSSV primers developed by S. Durand (unpublished, University of Arizona).
Thai = YHV primers developed by Wongteerasupaya et al. (1997).

[2] "White tiger" shrimp may be a marketing name for a pale variety of *P. monodon* or *P. semisulcatus*.

in efforts to reduce production losses due to the enzootic viruses *Baculovirus penaei* (BP) (Couch 1974; Lightner 1996a), IHHNV, and TSV. Improved husbandry practices have been successfully used to control BP, and for nearly a decade, this virus has seldom been reported as an economic constraint to successful shrimp culture (OIE 1997; Lightner and Redman 1998).

Until recently, the popularity and use of the relatively IHHNV resistant species *P. vannamei,* in preference to the culture of the more IHHNV susceptible *P. stylirostris,* was characteristic of the shrimp farming industries of the Americas. The popularity of *P. vannamei* began to decline when TSV emerged as a very serious pathogen of this species in 1992 and then spread to virtually all of the shrimp-growing regions of the Americas during the ensuing four years. Because *P. stylirostris* was found to be innately TSV resistant, at least two domesticated, genetically selected SPR strains of this species, which are resistant to IHHN disease, are currently being developed and marketed in the Americas. In some regions, these SPR stocks of TSV and IHHNV resistant *P. stylirostris* are replacing *P. vannamei* stocks in culture. Other shrimp farming interests are using wild or domesticated stocks of *P. vannamei* that show improved resistance to TSV. While resistance to TSV was used as a selection criteria for the domesticated stocks of *P. vannamei,* natural selection for TSV resistance appears to be occurring in wild stocks where TSV has been enzootic for several years. The same selective process for IHHNV resistance seems to be occurring in some wild stocks of *P. stylirostris.* Through selective breeding programs by broodstock producers, we will very likely see continued improvements in TSV and IHHNV resistance in domesticated lines of *P. vannamei* and *P. stylirostris.* However, because of natural selection where these viruses have become enzootic in wild populations, improved resistance in wild stocks of these species is very likely developing now and will improve with time (Lightner and Redman 1998; Lightner et al. in press).

Control strategies for WSSV and YHV in the Western Hemisphere should be based on prevention by exclusion. Success with this depends in large part upon the availability and the use of sensitive diagnostic tools and on the avoidance of introduction of these viruses through exclusion of live and frozen shrimp products from regions or facilities that have a history of infections by these viruses.

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