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REVIEW ARTICLE

Shrimp Infectious Diseases and Diagnostics in the Philippines

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ABSTRACT

Shrimp farming accounts for a significant percentage of commercial aquaculture and is an integral part of the continuous growth of the economy, particularly in the Philippines. Shrimp hatcheries and farms contribute to food security and export revenues of the country. Hence, it is essential to review and make an update on the various diseases that may affect shrimp production and the available technologies for diagnosis. This paper reports notable diseases of viral, bacterial, fungal, and parasitic origins that have been known to be present in the Philippines, with an emphasis on diagnostic methods for each disease.

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1. INTRODUCTION

Shrimp aquaculture is considered an economically important industry, providing a significant source of income, especially in countries with large coastal areas such as the Philippines (Dhar et al. 2014). However, the industry has been constantly plagued with several aquatic animal diseases caused by viruses, bacteria, fungi, and parasites, with emerging pathogens being identified yearly (NACA-FAO 2021). Diseases are major challenges to the sustainable growth of the aquaculture industry, costing approximately 6 billion USD in yield loss each year (Stentiford et al. 2017). In the shrimp sector, 40% of global production capacity was lost due to disease outbreaks (Stentiford et al. 2017).

Rapid and early diagnosis of pathogens and effective control measures are crucial in decreasing the occurrence and spread of outbreaks in aquaculture (Ninawe et al. 2017). Various disease detection methods and technologies were developed worldwide to contribute to the recovery and enhancement of aquaculture production. Such detection platforms were categorized into levels I, II & III to provide additional information to standardize diagnostic methods for OIE-listed pathogens (FAO 2001). Level I involves gross morphological and behavioral observations in farmed samples, providing a basis for conducting higher diagnostics (FAO 2001). Level II diagnostics, the traditional detection method, uses basic equipment and protocols to perform microscopic evaluation and histopathological examinations (Adams and Thompson 2011). These methods are simple and inexpensive, however, are also labor-intensive and time-consuming (Guo et al. 2016). With these constraints, the growth of new diagnostic methods (Level III) utilizing the principle of molecular biology and immunology is being poised as an alternative for accurate and cost-effective detection of shrimp diseases. The most commonly used molecular tool, polymerase chain reaction (PCR), amplifies a few copies of DNA sequences so that amplicons can be detected (Joshi and Deshpande 2010). PCR comes in various forms, such as multiplex, duplex, reverse transcriptase, nested, and real-time. Loop-Mediated Isothermal Amplification (LAMP) is also an alternative method for amplifying target DNA sequence under isothermal conditions using a

specialized polymerase with high strand displacement activity (Notomi et al. 2000). Immunological methods such as lateral flow assay and enzyme-linked immunosorbent assay (ELISA) are also utilized for the rapid screening of pathogens (Ninawe et al. 2017).

The Philippines is no exception to these diseases that plague farmed shrimp species, and yet knowledge of the pathogens and accessibility to diagnostic methods are not within the reach of farm operators. This review highlights the different diagnostic methods for detecting specific shrimp pathogens along with their profiles and pathogenesis. This is followed by a summary of susceptible shrimp species, their occurrence in the Philippines, and available diagnostics with their corresponding OIE levels. Compiling this pathogen information would contribute to a more diverse approach to diagnosing and managing diseases in aquaculture.

2. Viral diseases

2.1 *Monodon Baculovirus (MBV) Disease*

MBV is a double-stranded DNA virus that is rod-shaped with a single envelope, is occluded in paracrystalline intranuclear occlusion bodies, and can replicate within the host nucleus with a genome size ranging from 80 to 160 kbp (Rajendran et al. 2012). The International Committee on Taxonomy of Viruses (ICTV) lists MBV in the genus Nucleopolyhedrovirus under the tentative species name PemoNPV (Theilmann et al. 2005).

Lightner et al. (1983) reported three stages in the pathogenesis of MBV disease as seen in the hepatopancreas as follows: (1) starting with a few completed virions, hepatopancreatocytes have slightly hypertrophied nuclei but with no occlusion bodies; (2) presence of developing occlusion bodies in the hypertrophied cell nuclei with a completed virion and (3) abundant numbers of completed free and occluded virions in the cell nuclei. This will be followed by cell necrosis and cytolysis or sloughing. MBV-infected shrimps exhibit lethargy, anorexia, and notably reduced growth rate, feeding, and preening activities (Rajendran et al. 2012).

The presence of occlusion bodies (OB) in the hepatopancreas, which can be seen as small, raised spherical clusters of polyhedral particles, can be observed through direct microscopic examination of the transparent carapace (Rajendran et al. 2012). Lightner (1985) has observed OB in a squash mount preparation of freshly collected hepatopancreas samples stained with 0.05% malachite green. Stained sections of the hepatopancreas and anterior midgut show the OB in the hypertrophied nucleus and the tubules of the hepatopancreas with fragmented and marginated chromatin (Rajendran et al. 2012). Level III diagnostic methods include ELISA (Enzyme-linked immunosorbent assay), which uses polyclonal antibodies against the OB and MBV polyhedrin in tissue samples at nanogram levels (Hsu et al. 2000; Satidkanitkul et al. 2005). Polymerase chain reaction (PCR) has also been used extensively for its sensitivity in detecting MBV. Belcher et al. (1998) developed a colorimetric PCR assay for the MBV polyhedrin gene. However, the primers were later reported to amplify the polyhedrin of other insect baculoviruses. In 2000, Hsu et al. designed PCR primers specific for MBV and no other baculoviruses. Loop-mediated isothermal amplification (LAMP) and LAMP in combination with a lateral flow dipstick have also been developed, with a detection limit of as low as 0.7 fg. (Chaivisuthangkura et al. 2009; Nimitphak et al. 2010).

The first record of MBV in the country was documented from post-larval shrimp collections from 13 Philippine regions in 1981, with significant prevalence (Natividad 1991). In 2004, the slow growth rate of intensively farmed shrimps in the country was attributed to MBV (Flegel et al.). MBV has also been found in wild populations of *P. monodon* across the country, including Negros Occidental and Bohol, which serve as sources of broodstock and spawners for hatcheries (de la Peña et al. 2008). MBV screening of Orosco and Lluisma (2017) also revealed ~30% cumulative MBV prevalence among sampled *P. monodon* in the country in 2017.

2.2 Mid-Crop Mortality Syndrome

Spawner-isolated mortality virus (SMV), a small ~20 nm icosahedron ssDNA virus, is the causative agent of mid-crop mortality syndrome (Fraser and Owens 1996). This parvovirus is among the less characterized pathogens reported to infect shrimp.

Infected hosts exhibit non-specific lesions consisting of granular deposits under the cuticle and digestive gland (Munday and Owens 1998). Haemocytic enteritis and hypertrophied nuclei with marginated chromatin also appear in muscle tissues of experimentally infected prawns (Munday and Owens 1998). Moribund shrimps become dark red and produce red feces before the onset of mortality (Fraser and Owens 1996). Histopathological analysis is not always advised as lesions and other tissue changes are not specific and might be confused with

bacterial infections (Fraser and Owens 1996). Electron microscopy and in-situ DNA hybridization in target endodermal tissues can be employed for Level II and III detection of the virions (Owens et al. 1998)

SMV was detected in *P. monodon* samples from selected farms in 1998 through in situ hybridization (ISH) performed in Australia (NACA-FAO 1998). There were no further reports of MCMS until it was delisted by OIE in 2006.

2.3 Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) Disease

IHHNV is a single-stranded DNA virus, virions of which are non-enveloped icosahedrons. It belongs to the family Parvoviridae (Bonami et al. 1990; Shike et al. 2000). The genome size is 4.1 kb with three open reading frames (ORFs) (Shike et al. 2000).

The target of IHHNV infection are tissues of ectodermal and mesodermal origin such as cuticular epidermis, hypodermal epithelium of the fore and hindgut, nerve cord and nerve ganglia, hematopoietic organs, connective tissue, and striated muscles. This is due to the viruses' dependence on the host cells for DNA replication and multiplication (Rai et al. 2012). Observable manifestations for Level I detection in shrimps include growth retardation, deformed rostrums, wrinkled antennal flagella, cuticular roughness and cuticular abnormalities of the abdominal segments and tail fan (Melena et al. 2015; Pazir et al. 2012). Mortalities vary among shrimp species, catastrophic for *P. stylirostris* and subtler with low mortalities for *P. vannamei* and *P. monodon* populations (OIE 2017).

IHHNV can be detected by examination of histological sections showing Cowdry type A, which are eosinophilic, intranuclear inclusion bodies surrounded by marginated chromatin in hypertrophied nuclei of cells (Wongteerasupaya et al. 1995). Level III diagnostic methods include dot blot hybridization (Yang et al. 2007), LAMP (Sun et al. 2006), and PCR (Nunan et al. 2000 ; Flegel 2006; Tang et al. 2007), which are reliable and highly sensitive tools for diagnosis. However, PCR-based IHHNV diagnostics were complicated by the reports of integration of non-replicating IHHNV genome fragments in the genome of *P. monodon* (Tang and Lightner 2006 ; Rai et al. 2009), yielding false positive results in the previously developed diagnosis and commercial kits. Tang et al. (2007) designed a primer set IHHNV309F/R to distinguish only the IHHNV infectious form and was specific to isolates from the western hemisphere, including the Philippines.

IHHNV has been found in both *P. monodon* and *P. vannamei*; at least two strains of the virus are suspected to be present in the Philippines, as confirmed by samples of post-larvae from Iloilo (Caipang et al. 2011). Recently, IHHNV was detected among market-collected *P. vannamei* in Zambales, Bulacan, and Pampanga (Maralit and Genavia 2022) and still found prevalent in all BFAR-screened *P. monodon* stages from Luzon and Visayas (NACA-FAO 2022).

2.4 Hepatopancreatic Parvo-like Virus (HPV) Disease

HPV contains a single-stranded DNA of icosahedral shape 22 nm in diameter and constitutes a novel group in the Parvoviridae family. The genome size is approximately 5 kb encoding for a single polypeptide of 54 kDa (Bonami et al. 1995). HPV is also known as *Penaeus monodon* densovirus (PmoDNV) (Flegel 2006).

There are three stages to HPV infection, starting with the virion attaching to the host microvilli and entering the cell through pinocytosis (1); HPV matures in the nucleoplasm and is later accumulated in the main occlusion bodies (2); the nuclear membrane is disrupted because of the accumulation of mature parvovirus particles and new virions are released into the cytoplasm (3) (Fig. 1) (Safeena et al. 2012). There is no specific observable macroscopic sign of HPV for Level I diagnosis; infected shrimps exhibit anorexia and poor growth, which can easily be linked to other pathogens (Catap et al. 2003). However, chronic mortalities resulting from stunted growth have been observed in early or postlarval stages of affected shrimp (OIE 2007).

Conventional diagnostic methods, specifically histopathology, are widely accepted for detecting HPV. Infection is characterized by the presence of single, large basophilic intranuclear inclusion bodies in hypertrophied nuclei of the hepatopancreas and midgut cells (Catap et al. 2003). Nucleic acid-based techniques are also available for Level III diagnosis of HPV including conventional and real-time PCR, duplex PCR, LAMP, PCR-ELISA, non-radioactive labeling, ISH, and monoclonal antibody-based techniques (Safeena et al. 2012;), whereas PCR has been developed for early diagnosis with careful consideration on primer design due to the presence of insertions and deletions in the HPV genome (Dhar et al. 2019).

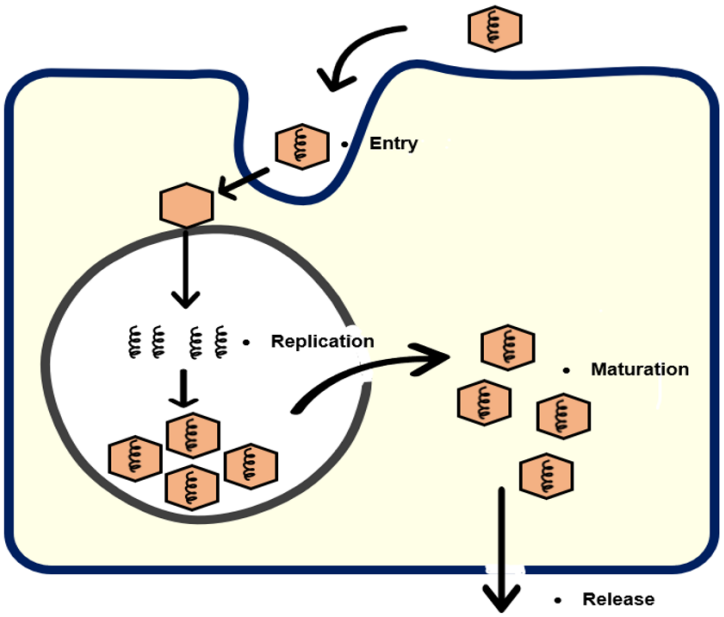

Figure 1. Stages of HPV infection (Safeena et al. 2012).

Following Lightner and Redman's (1985) initial detection of the disease in 1985, Catap et al. (2003) detected HPV-positive samples in PLs from Samar and Iloilo. HPV and MBV have now been delisted from OIE report due to economic insignificance (Thitamadee et al. 2016).

2.5 White Spot Disease (WSD)

White spot syndrome virus (WSSV) is an enveloped, non-occluded, bacilliform, circular double-stranded DNA virus. It has been recognized as the type species of the genus Whispovirus, family Nimaviridae by the International Committee on Taxonomy of Viruses. The WSSV genome is circular and 305 kb in size, different from 293 kb determined for a Thailand isolate and 307 kb for a Taiwan isolate. (Lan et al. 2006).

A proposed model of the morphogenesis of WSSV (Fig. 2) has been characterized by several authors and compiled by Escobedo-Bonilla et al. (2008) as follows: (1) an infectious WSSV virion attaches to a susceptible cell using envelope proteins with a cell attachment motif; (2) WSSV enters the cell; (3) the envelope of the WSSV virion probably fuses with the endosome and the naked nucleocapsid is transported to the nucleus, in a similar way as in baculoviruses; (4) the naked WSSV nucleocapsid attaches to the nuclear membrane and the WSSV genome is released into the nucleus; (5) the WSSV replication starts. In the cytoplasm, the mitochondria start degenerating; (6) in the nucleus, the early virogenic stroma appears composed of loose granular material, cellular chromatin accumulates near the nuclear membrane, and the rough endoplasmic reticulum becomes enlarged and active; (7) the marginated chromatin is transformed in a dense ring zone, here the virogenic stroma is less dense and starts forming vesicles that will form the viral envelope. The vesicles are probably formed with the membranous material found in the ring zone, as in baculoviruses. A viral nucleosome is also observed as a filamentous structure in the virogenic stroma. This structure contains proteins that will form the nucleocapsid; (8) new WSSV particles are assembled in the nucleus within an electron-dense inclusion. The empty envelopes are filled with a nucleocapsid. In the cytoplasm, organelles become disintegrated and the cellular and nuclear membranes are disrupted; (9) WSSV virions are completely formed and are ready to be released from the disrupted cell to begin the cycle in other susceptible cells. WSSV infections of penaeid shrimp are characterized by rapid mortality accompanied by gross signs in moribund shrimp. This is a lethal disease where 90–100% mortality occurs 3–10 days after the first signs of disease appear. The signs observed are white deposits of 0.5–2.0 mm diameter calcium on the shrimp cephalothorax or carapace cuticle. Diseased shrimp exhibit reduced feeding and increased lethargy (Sanchez-Martinez et al. 2007).

It is not advised to rely on the presence of white spots for diagnosis of WSD since the presence of a different rod-shaped bacteria may produce white spots that closely resemble that of WSSV. Histology is the gold standard for detecting the disease (Level II); however, more sensitive detection methods, such as PCR, are also available. Commercial diagnostic kits involving in situ hybridization, PCR and immunodetection can also be used. PCR is the more popular option due to its high sensitivity and rapid results (Pradeep et al. 2012). Recently, developing a simple, rapid, and cost-effective diagnostic kit for WSSV is promising for use in the field (Arabit et al. 2015). In 2014, Nicolasora et al. optimized a LAMP technology protocol for specific detection of WSSV isolates in the country.

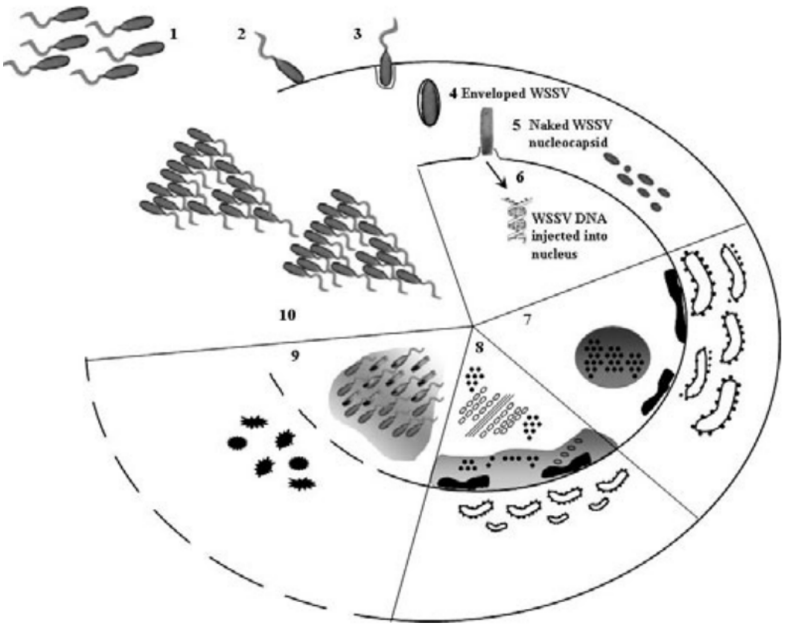

Figure 2. Stages of WSSV infection (Escobedo-Bonilla et al. 2008).

Since its emergence in 1999, WSD has remained prevalent in several BFAR-tested shrimp farms yearly (NACA-FAO 2022), making it the most destructive viral pathogen in the industry. In the study of WSD occurrence in wild P. monodon, the pathogen was found to be already established and endemic in the local marine environment, posing a threat due to the vertical transmission of the disease (dela Peña et al. 2007). It should also be noted that WSSV is more prevalent during wet than dry seasons (dela Peña et al. 2007).

2.6 Yellow Head Disease (YHD)

Yellow Head Virus Genotype 1 (YHV-1) is the aetiological agent of YHD and is classified by the International Committee on Taxonomy of Viruses as a single species in the genus Okavirus of the family Roniviridae. The virions are enveloped (studded with prominent peplomers), rod-shaped particles with helical nucleocapsids, and a positive, single-stranded RNA genome (OIE 2009). Among the eight identified YHV genotypes, YHV-1 is the most virulent in *P. monodon* host (Dhar et al. 2004).

Duangsuwan et al. (2011) proposed a putative pathway for YHV infection that begins with viral particles entering through endocytosis, uncoating, and passing into the cytoplasm, where replication occurs and nucleocapsid proteins are synthesized. The particles are then encapsulated and transported into the RER-Golgi compartment for release through exocytosis in secretory vesicles. The yellowing of the cephalothorax and general bleaching of body color characterizes YHD. However, these signs are not always developed and devastating losses due to high mortality caused by YHD have been reported in cultured *P. monodon* and, therefore considered potentially lethal to commercially cultivated penaeid shrimps (Lightner 1999).

Microscopic examinations of the lymphoid organ, stomach, and gills are used for Level II diagnosis of the disease, and a transmission electron microscope is the best tool for this purpose. However, since YHD histopathology could not be observed earlier than 42 hours post-infection or near the onset of morbidity, Level III diagnostics reverse-transcription PCR (Wongteerasupaya et al. 1997) and LAMP combined with lateral flow dipstick (Khunthong et al. 2013) were developed for a rapid, sensitive, and specific detection of the pathogen. Monoclonal antibodies and in-situ hybridization diagnostics were also developed to detect the virus in shrimp tissue (OIE 2009).

The presence of YHV was detected in five of the ten provinces selected for sampling namely, South Cotabato, Negros Occidental, Capiz, Agusan del Norte, and Bohol, and its prevalence in the country involving cultured P. monodon was established (Natividad et al. 2002). According to OIE reports, the last occurrence of YHD in the country was in 1999.

2.7 Gill-Associated Virus (GAV) Disease

Another member of the yellow head complex, the less virulent rod-shaped and enveloped Gill-Associated Virus or Yellow Head Virus Genotype 2 (YHV-2), causes GAV disease in *P. monodon*. YHV-1 and YHV-2 are the only YHV genotypes known to cause infections in shrimps (OIE 2019). All eight genotypes are classified as a single species in the genus Okavirus due to evidence of genetic recombination, however, they represent distinct genetic lineages due to variation in geographical origin (Cowley et al. 1999).

The morphology and pathology in GAVD closely resemble that in YHD, hence, diagnostics by gross sign monitoring (Level I) and lymphoid organ tissue examination (Level II) in GAVD-suspected shrimps should be accompanied with more specific molecular and immunological (Level III) analyses (Chantanachookin et al. 1993). Sensitive and specific multiplex RT-PCR detection that can differentiate GAV from YHV-1 was established by Cowley et al. (2004). Munro and Owens (2007) developed monoclonal- and polyclonal-based ELISA for GAV with the same sensitivity limit as PCR to further reduce the cost of equipment.

GAVD was reported in the country in late 1998 from *P. monodon* samples showing histopathological lesions associated with the disease (NACA-FAO 1998) and was delisted from the OIE list in the year 2006.

2.8 Monodon Slow-Growth Syndrome (MSGS)

Laem-Singh virus (LSNV) is a positive-sense single-stranded RNA virus closely related to the family Leuteoviridae and is considered the cause of MSGS (Chayaburakul et al. 2004). The disease was first detected in Thailand and is said to be why shrimp farmers shifted from the cultivation of *P. monodon* to *P. vannamei* (Thitamadee et al. 2016).

Viral-like particles have been observed in the lymphoid organ, which is suggested to be the preference for it to complete its processes. The progression of the infection into the optic nerve is also suspected to be caused by the shrimp's slow growth (Poornima et al. 2012).

Histopathology to reveal pathognomonic lesions in the eyestalk is the traditional means of diagnosis for LSNV-infected samples (Level II). Other diagnostic methods involve RT-PCR, nested PCR, RT-LAMP-LFD qRT-PCR, and ISH, which have also been used in several studies for Level III diagnostics. The following are the gross signs of MSGS, of which compliance to three of the signs increases the positive diagnosis for the disease: unusually dark color, a decline in weight gain, averaging to less than 0.1 g/day or about 1 g in four months, bright yellow markings that are highly unusual, "bamboo-shaped" abdominal segments, and brittle antennae (Poornima et al. 2012).

MSGS was not detected until 2015, when LSNV is reportedly present in *P. monodon* samples from Bulacan, supported with 100% identity in sequence alignment and analysis with the LSNV isolate of India (Cruz et al. 2015).

2.9 Taura Syndrome Virus (TSV)

The pathogenic agent of Taura syndrome is from the Genus Aparavirus of Family Dicistroviridae and Order Picornavirales, known as Taura syndrome virus (TSV) (OIE 2019). There is variability in the attack rate in shrimp populations. Cumulative mortality ranges from 5 to more than 95%, and the course of infection may be classified as acute if lasting for 5–20 days or chronic if lasting throughout grow-out. Histological changes are also evident in the acute and chronic forms, the presence of multi-focal areas of necrosis in both the cuticular epithelium and sub-cuticular connective tissue are in the former, while multiple, irregular-shaped and sized melanized lesions of the cuticle and an apparent enlargement of the lymphoid organ can be observed in the latter (Brock 1997).

Histopathology remains a standard protocol in diagnosing TSV, which can be accompanied by routine RT-PCR for Level III testing (Erickson et al. 2002).

TSV was not known to occur in the country since BFAR monitoring in the early 2000s. However, in 2019, Vergel et al. reported the disease occurrence in Bulacan, Batangas (Luzon), Bohol, and Cebu (Visayas).

An overview of documented bacterial diseases affecting shrimp in the Philippines, including the list of susceptible species and the diagnostic methods utilized was presented in Table 1.

3. Bacterial diseases

3.1 *Vibriosis/Penaied Bacterial Septicemia/ Luminescent vibriosis/Red-leg Disease*

There are several causative agents of Vibriosis and these are *Vibrio harveyi, V. parahaemolyticus, V. alginolyticus, V. anguillarum, V. vulnificus, V. splendidus* (Jayasree et al. 2006), *V. cambellii-like, V. penaeicida* (Saulnier et al. 2000), *V. damsela* (Song et al. 1993).

Varying virulence among various species of Vibrio is observed, with *V. harveyi* affected by loose shell syndrome being the most virulent and isolates from *V. anguillarum* to be least pathogenic. Any of the five diseases are external symptoms of Vibriosis: (1) Tail necrosis: necrosis of uropods and pleopods; swimming ability is lost and diseased shrimp exhibit erratic gliding movements and will tend to stay at the edge of the pond, (2) shell disease: the presence

Table 1. A summary of shrimp viral diseases in the Philippines and diagnostic methods used

Disease	Susceptible host species	Diagnostics	Remarks
MBV	-*P. vannamei, P. monodon, P. esculentus, P. semisulcatus, P. merguiensis, P. penicillatus, P. plebejus, P. kerathurus* (Lightner et al. 1989) -*Metapenaeus ensis* (Chen et al. 1989) -*M. lysianassa* (Hao 1999) -*M. monoceroros, M. elegans* (Manivannan et al. 2004) -*Macrobrachium rosenbergii* (Gangnonngiw et al. 2010)	-Direct microscopic examination (Level II) -ELISA, PCR (Level III) (Hsu et al. 2000) -LAMP (Chaivisuthangkura et al. 2009) -LAMP + lateral flow dipstick (Level III) (Nimitphak et al. 2010)	-Prevalent in 13 Philippine regions in 1981 (Natividad 1991) -Detected in wild populations of *P. monodon* from Negros Occidental and Bohol (de la Peña et al. 2008).
MCMS	-*P. monodon* (Fraser and Owens 1996)	-Direct microscopic examination (Level II) (Munday and Owens 1998) -ISH (Level III) (Owens et al. 1998)	-Detected in *P. monodon* samples from selected farms (NACA-FAO 1998)
IHHNV	-*Macrobrachium rosenbergii, Penaeus californiensis, P. monodon, P. setiferus, P. stylirostris, P. vannamei, P. duorarum, P. occidentalis, P. japonicus, P. semisulcatus, Artemesia longinaruis* (OIE 2017)	-Direct microscopic examination (Level II) (Wongteerasupaya et al. 1995) -ELISA, ISH, PCR (Level III) (Rai et al. 2012)	-Found present in both *P. monodon* and *P. vannamei* from Iloilo (Caipang et al. 2011) -Detected among market-collected *P. vannamei* in Zambales, Bulacan, and Pampanga (Maralit and Genavia 2022)
HPV	-*Penaeus monodon, P. esculentus, P. merguiensis, P. japonicus, P. chinensis, P. semisulcatus, P. indicus, P. penicillatus, P. schmitti, P. vannamei, P. stylirostris* (OIE 2007)	-Histopathology (Level II) (Catap et al. 2003) -PCR, LAMP, PCR-ELISA, non-radioactive labeling, ISH, Monoclonal Antibody based techniques (Level III) (Safeena et al. 2012; Catap et al. 2003)	-Detected in postlarval samples from Samar and Iloilo (Catap et al. 2003)
WSD	-All cultured, wild marine shrimps (*P. indicus, P. japonicus, P. chinensis, P. penicillatus, P. azteus, P. merguiensis, F. duorarum, P. stylirostris, P. monodon, P. vannamei*), *Alpheus brevicristatus, Alpheus lobidens, Aristeus* sp., *Callianassa* sp., *Exopalaemon orientalis, Farfantepenaeus aztecus, Fenneropenaeus penicillatus, F. chinensis, Heterocarpus* sp. and freshwater cultures of *Macrobrachium rosenbergii* (Pradeep et al. 2012).	-Histology (Level II) (Pradeep et al. 2012) -ISH, PCR, Immunodetection (Level III) (Pradeep et al. 2012; Arabit et al. 2015)	-Recently detected in 17 farms across the country (NACA-FAO 2022) and found more prevalent in the wet season as compared to the dry season (dela Peña et al. 2007).
YHD	-The two most economically important species of shrimp, *P. monodon* and *P. vannamei* have reported outbreaks of the disease. However other species can be infected as well, *P. japonicus, P. merguiensis, P. stylirostris, P. setiferus, Metapenaeus ensis, Palaemon styliferus, Euphasia superba, P. esculentus, P. aztecus, P. duorarum, Metapenaeus bennettae, Macrobrachium sintangense, Palaemon serrifer, Ascetes* sp (OIE 2009).	-Microscopic examinations (Level II) -Immunoblot detection, PCR, ISH, LAMP (Level III) (Wongteerasupaya et al. 2007; Khunthong et al. 2013; OIE 2009)	-Found present in *P. monodon* cultures from selected sampling sites including South Cotabato, Negros Occidental, Capiz, Agusan del Norte and Bohol (Natividad et al. 2002)

Continuation of Table 1. A summary of shrimp viral diseases in the Philippines and diagnostic methods used

Disease	Susceptible host species	Diagnostics	Remarks
GAV	-*Penaeus monodon* (Cowley et al. 1999)	-RT-PCR, LAMP (Level III) (Cowley et al. 2004; Munro & Owens 2007)	-Reported from P. monodon samples showing histopathological lesions (NACA-FAO 1998)
MSGS	-*Penaeus monodon, Fenneropenaeus merguiensis, Metapenaeus dobsoni, Litopenaeus vannamei* (Kumar et al. 2011).	-Histopathology (Level II), PCR, LAMP, ISH (Level III) (Poornima et al. 2012)	-Detected in P. monodon samples from Bulacan, supported with 100% identity with the LSNV (Cruz et al. 2015)
TSV	-*Metapenaeus ensis, P. aztecus, P. monodon, P. setiferus, P. stylirostris* and *P. vannamei* (OIE 2019)	-PCR, Southern blot, Western blot, immunohistochemistry, PAb, MAb (Level III) (Erickson et al. 2002) -Histopathology (Level II), ISH (Level III) (Vergel et al. 2019)	-Reported in 2019 by Vergel et al. in the islands of Luzon and Visayas.

of black spots and lesions on the exoskeleton and appendages with low mortality (symptoms 1 and 2 are accompanied with low mortality), (3) red disease: red coloration all over the shrimp body, (4) loose shell syndrome (LSS): soft and loose muscle with condensed and melanized hepatopancreas with shrimps appearing sluggish and (5) white gut disease (WGD): stunted growth and opaque white gut visible through the transparent cuticle as a white streak, diseased shrimp consumed large quantities of white fecal matter (3, 4 and 5 are accompanied with mass mortality) (Jayasree et al. 2006).

As gram-negative bacteria, isolation from shrimp samples involves stock cultures in Thiosulphate-Citrate-Bile-Salt Sucrose (TCBS) agar, where bacterial isolates are to be observed with scanning electron microscopy and biochemical reaction tests incorporated into API 20 E miniaturized identification test strip (Chatterjee and Haldar 2012; Hettiarachchi et al. 2005). Other Level III diagnostic methods include ribotyping, amplified fragment length polymorphism (AFLP), fluorescence in situ hybridization (FISH), random amplified polymorphic DNA (RAPD), repetitive extragenic palindrome-PCR (rep-PCR), and restriction fragment length polymorphism (RFLP) (Chatterjee and Haldar 2012).

Epizootics due to Vibriosis have been reported in pond-cultured *P. monodon* in a pond environment and was directly linked to luminescent Vibrios (Lavilla-Pitogo and Paner 1998). Dela Pena et al. (2001) isolated various luminescent Vibrio species from hepatopancreatic tissues of shrimps from 11 provinces of the country.

3.2 Bacterial Gill Disease

The disease may be caused by *Thiothrix sp., Flexibacter sp., Pseudomonas spp., Flavobacteria sp.*, and *Aeromonas formicans*. The filamentous bacteria attach to the body surfaces of the shrimp, preferably in the gills and accessory gill structures of juvenile and adult penaeids (Lightner 1985), and is manifested by the fouling of the affected structures as mentioned earlier. Some biological processes, such as molting, are also impaired, and death can result from hypoxia (Chandarakala and Priya 2017).

Gram-negative filamentous bacteria can be cultured using Remel 2A (R2A) agar while gram-positive filamentous bacteria can be grown in blood or serum agar. The procedure should be followed with an API 20 E miniaturized identification test strip and observed under a microscope (Level II) (Lightner 1978). Conventional PCR can also be used for Level III confirmatory tests.

The disease is cited as Filamentous Bacterial Disease by Baticados et al. (1990) in a book on Diseases of Penaeid Shrimps in the Philippines. Also, according to the authors, mortalities brought by these bacteria have been documented, however, the occurrence locations were not specified.

3.3 Acute Hepatopancreatic Necrosis Disease (AHPND)

Virulent strains of *Vibrio parahaemolyticus* (VpAHPND) containing the PirA and PirB toxins (OIE 2017) are identified as aetiological agents of

AHPND. The effects of AHPND appear to be limited to the hepatopancreas (HP), beginning with a marked reduction of fat storage and decreased activity of secretory cells. As it spreads, the characteristic sloughing of secretory cells and detachment from the HP tubule basement membrane occurs. In the final stages, affected shrimp die from HP dysfunction (Lightner et al. 2012). It is important to note that AHPND is characterized by rapid, mass mortalities of up to 100% within 30–35 days of stocking with PLs or juveniles. Atrophy and discoloration of HP (pale to white), soft shells, guts with discontinuous or no contents, black spots or streaks visible within the HP (OIE 2017).

Colonies of *V. parahaemolyticus* can be isolated in TCBS agar and confirmed with an API 20 E miniaturized identification test strip. Histological samples of the HP should also be observed under the microscope for Level II confirmation. According to the OIE (2017), PCR methods have also been developed to target the toxin genes and can be carried out using 16S rRNA PCR or toxR-targeted PCR.

Prevalence of the disease in *P. vannamei* was studied across the Philippines and was reported to be present in Luzon, Visayas and Mindanao, with the highest incidence in Luzon and the lowest in Mindanao (Dabu et al. 2015). Since its detection in 2015, AHPND cases have been consistently documented based on NACA quarterly reports (NACA-FAO 2022).

The information on the locally-reported bacterial diseases and the corresponding diagnostic methods employed was compiled in Table 2.

4. Fungal and parasitic diseases

4.1 *Larval Mycosis*

Lagenidium spp., Sirolpidium spp., and *Haliphthoros philippinensis* in early postlarval prawns (Brock and Lightner 1990; Hatai et al. 1980) are the fungal strains that cause larval mycosis. The fungi can grow in larval penaeids and eventually replace the shrimp's muscle and soft tissue, after which the fungal hyphae and discharge tubes may appear visible within the body and protrude through the cuticle (Brock and Lightner 1990). Larval mycosis typically has sudden and high mortality rates within 1–2 days. Shrimp larvae affected by the fungi also appear opaque (Alavandi et al. 1995).

Microscopic (Level II) examination of the infected larvae and macroscopic observation of the clinical signs is used as diagnostic methods (Brock and Lightner 1990). It was reported to occur in *P. monodon* larvae (Baticados et al. 1979).

4.2 Microsporidian Infection

The following parasites are the causative agents of microsporidian infection: *Ameson sp., Agmasoma sp.* and *Pleitosphora sp.* (Lightner 1985), *Triwangia caridinae gen, nov., sp. nov.* (Wang et al. 2013). The parasites can infect the muscle tissues, both striated and smooth muscles and the gonads, causing a characteristic white abdomen (Lightner 1985). Muscles and gonads infected by the parasite appear white and hypertrophied with multiple, white tumor-

Table 2. A summary of shrimp bacterial diseases in the Philippines and diagnostic methods used.

Disease	Susceptible host species	Diagnostics	Remarks
Vibriosis	-All cultured penaeids (i.e., *P. monodon* and *P. vannamei*) (Chatterjee and Haldar 2012) -Cultured *Macrobrachium rosenbergii* (Siripornadulsil et al. 2013).	-Microscopic examinations (Level II), API (Level II) -ribotyping, AFLP, FISH, RAPD, PCR, RFLP (Level III) (Chatterjee and Haldar 2012)	-Detected in 11 provinces across the country (dela Pena et al. 2001)
Bacterial Gill Disease	-*P. aztecus, P. stylirostris, P. californiensis, P. vannamei* and *P. monodon* (Lightner 1978).	-Microscopic examinations (Level II), API (Level II), PCR (Level III) (Lightner 1978)	-Reported by Baticados et al. (1990) on a book on Diseases of penaeid shrimps in the Philippines
AHPND	-*P. monodon and P. vannamei (Lightner et al. 2012; OIE 2017).*	-Microscopic examinations (Level II), API (Level II, PCR (Level III) (OIE 2017)	Found prevalent on P. vannamei cultures across Luzon, Visayas and Mindanao region (Dabu et al. 2015)

like swellings in the gills and subcuticular tissues (Kelly 1979). Microsporidiosis is accompanied by low mortality levels reaching about 10–20% of cultured populations if it occurs at all (Lightner 1993).

The spores of microsporidians can easily be identified in unstained wet mounts of infected tissues with a light microscope. They can be distinguished by their size of about 1–8 um, the number of spores produced per sporont and the number of turns made by the polar filament (Lightner 1996).

A microsporidian parasite in *P. merguiensis* has been reported in the Philippines, involving all of its life stages in the ovaries of the host (Baticados and Enriques 1982)

4.3 Gregarine Disease

Nematopsis sp. and *Cephalolobus* sp. (Lightner 1985), *Paraophioidina scolecoides* n. sp. (Jones et al. 1994), *Nematopsis sundarbanensis* (Chakraborti and Bandyopadhyay 2010) are causative agents of Gregarine disease. Spores of the parasite attach to the walls of the gastric filter, midgut, hepatopancreas, stomach and hindgut of host shrimp. Heavy infections display yellow discoloration of the midgut and reduced growth rates (Lightner 1993).

Different life stages of the parasite can be observed with wet mount preparations of the midgut using a light microscope for Level II detection (Overstreet 1973). The disease was reported in *P. monodon* by Baticados et al. (1990) and was last detected in farmed *P. monodon* in Bulacan (Puzon 2003).

4.4 Haplosporidiosis

The disease is linked with haplosporidian parasites with insufficient information regarding the species and taxonomic placement. Poor growth has been associated with infection with the disease with no other characteristic signs and is restricted in the hepatopancreas of infected hosts (Lightner 1996).

Level II diagnosis includes the use of a transmission electron microscope (TEM) using histological tissue samples of hepatopancreas stained with haemotoxylin and eosin (H&E) (Lightner 1996). The occurrence of haplosporidian parasites in P. monodon from the Philippines has been reported by Lightner et al. (1992)

4.5 Hepatopancreatic Microsporidiosis

A microsporidian Enterocytozoon hepatopenaei (EHP) is the lone causative agent of Hepatopancreatic microsporidiosis (NACA 2015). The disease causes growth abnormality in shrimps and displays clinical signs, including lethargy and empty midgut in severe cases (Aranguren et al. 2017). In contrast to other microsporidian infections, EHP only targets the hepatopancreatic tubules (NACA 2015) and susceptibility to infection is increased once coupled with AHPND and septic hepatopancreatic necrosis (SHPN) (Aranguren et al. 2017).

Level III diagnosis includes examination of hepatopancreatic tissue stained with H&E (Tourtip et al. 2009) and molecular detection by PCR and LAMP (Suebsing et al. 2013).

This non-OIE listed disease has been detected in the quarterly monitoring of BFAR for QAAD report since 2016, and recently, it affected cultured *P. monodon* and *P. vannamei* post-larvae in two farms from Luzon and Visayas region (NACA-FAO 2022).

Table 3 compiles data on fungal and parasitic diseases affecting shrimp in the Philippines, presenting details on susceptible species and the diagnostic methods applied for identification.

5. CONCLUSIONS

Aquaculture plays a vital role in the Philippine economy. Hence, the diseases discussed here must be addressed. This review paper highlighted various bacterial, viral, fungal, and parasitic pathogens that have been identified in the Philippine shrimp industry. It included current diagnostics for a variety of shrimp pathogens in the hope of arming the stakeholders with the concepts and tools to keep the industry thriving. It must be emphasized, however, that effective fish health management and biosafety be prioritized. Implementation of prompt diagnosis and early harvest of infected shrimps are the key mitigators of disease outbreaks. Therefore, the development of innovations in tools that provide more sensitive, specific, rapid, and user-friendly diagnostic technology is always relevant to the industry. Additional research for the eradication strategies for these pathogens is also recommended.

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Table 3. A summary of shrimp fungal and parasitic diseases in the Philippines and diagnostic methods used.

Disease	Susceptible host species	Diagnostics	Remarks
Larval mycosis	-Penaeid species, particularly *Penaeus monodon* (Lio-Po and Sanvictores 1985)	-Microscopic examination (Level II) (Brock and Lightner 1990)	-Reported in *P. monodon* larvae samples by Baticados et al. (1979)
Microsporidian infection	-*P. mergiuensis, P. monodon, P. esculentus, P. semisulcatus, P. latisculatus, P. longistylus* (Flegel et al. 1992), *P. douraram* (Kelly 1979) -*Caridinia formosae* (Wang et al. 2013).	-Microscopic examination (Level II) (Lightner 1996)	-Detected in *P. merguiensis* ovaries (Baticados et al. 1985).
Gregarine disease	-*P. monodon* (Baticados et al. 1990; Chakraborti and Bandyopadhyay, 2010) -*P. vannamei* (Jones et al. 1994).	-Microscope examination (Level II) (Overstreet 1973)	-Reported in *P. monodon* by Baticados et al. (1990) -Found in farmed *P. monodon* in Bulacan (Puzon 2003)
Haplosporidiosis	-*P. vannamei* (Dykova et al. 1988), *P. stylirostris* (Lightner, 1996) -*P. monodon* (Lightner et al. 1992).	-Microscopic examination (Level II) (Lightner 1996)	-Detected in P. monodon from the Philippines as reported by Lightner et al. (1992)
Hepatopancreatic microsporidiosis	-*P. monodon* and *P. vannamei* (NACA 2015)	-Microscopic examination (Level), Tourtip et al. 2009) -PCR and LAMP (Level III) (Suebsing et al. 2013).	-Documented in both *P. monodon* and *P. vannamei* cultures in Luzon and Visayas region (NACA-FAO 2022).

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AUTHOR CONTRIBUTIONS

Maningas MB: Conceptualization, Supervision, Writing-Reviewing and Editing. **Tare MV:** Data curation, Visualization, Writing-Original Draft. **Samora VM:** Visualization, Writing - Original Draft.

CONFLICTS OF INTEREST

No animal or human studies were carried out by the authors, and, to the best of our knowledge, no conflict of interest exists.

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