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MINI REVIEW ON EMERGING SHRIMP DISEASES IN SOUTH EAST ASIA

Rocelene Veraann Rukimin, Tamrin M. Lal*

Borneo Marine Research Institute, Universiti Malaysia Sabah,
Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

*Corresponding Author

ABSTRACT

Emerging shrimp disease threats are caused by various etiological agents and are responsible for devastating losses in farmed shrimp around the South Asia and South East Asia. In addition to directly imposing negative economic and ecological effects, these emerging disease outbreaks are also notoriously difficult to prevent and control due to lack of information on the disease itself. The emergence of recent reports in various shrimp diseases are also major concern in shrimp industries and has highlighted some of the difficulties surrounding the diagnosis and control of the diseases. Hence, this paper is intended to provide a review of the literature that focuses on emerging shrimp diseases around South East Asian countries.

Keywords: Early mortality syndrome, hepatopancreatic microsporidiosis, white muscle disease, white feces diseases, shrimp industry

INTRODUCTION

Shrimp aquaculture is a major industry in South Asia and South East Asia. However, disease outbreaks by viruses, bacteria or/and protozoa in shrimp aquaculture can lead to serious economic losses for long period of times. Various type of viral pathogens in shrimp have been identified such as white spot syndrome virus (WSSV), yellow head virus (YHV), covert mortality nodavirus (CMNV), infectious myonecrosis virus (IMNV), taura syndrome virus (TSV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV). Besides these shrimp viral diseases, new threats have been emerged within a few years ago in South Asia and South East Asian Countries. Currently, there are four new popular diseases in shrimp industries which are early mortality syndrome, hepatopancreatic microsporidiosis, white muscle disease and white feces diseases.

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With the recent advances in molecular biology and biotechnology, several new information were collected. Among these, the further details on the epidemiology of these four diseases have been extensively reviewed in Thitamdee *et al.* [1] and will not be discussed further herein. Rather, this review predominantly focuses on the contributing factor, distribution, occurrence, detection method and prevention method of these four shrimp diseases.

EARLY MORTALITY SYNDROME (EMS)

Early mortality syndrome (EMS) or other terms known as acute hepatopancreatic necrosis disease (AHPND), was first detected in China in 2009 and began spreading subsequently to Vietnam, Malaysia, Thailand [2,3] and Mexico [4, 5]. After various studies done regarding this disease, it was suspected that the causative agent of EMS is a type of bacterium—which is a pathogen from the Vibrio genus, most probably *Vibrio parahaemolyticus* [3]. This disease usually affects shrimps during postlarval stage. Postlarvae are infected within 20–30 days after stocking and mortality may reach up to 100% [6]. Signs of infection include slow growth, spiral swimming, loose shells, as well as pale coloration. Affected shrimp also consistently show shrunken and discolored hepatopancreas [7].

There was a report suggesting that the extreme measures taken by totally disinfecting pond bottom and water to kill possible vectors of EMS may have been causing more damage as the procedure promotes the epidemic spread of the EMS disease rather than controlling it. The report further claimed that strategic microbial management is a better approach that can be done to reduce the risks of EMS outbreaks. Pond disinfection removes potential pathogens or their hosts and this causes lack of competition in the microbial community and increase the nutrient availability in the pond [8]. This new condition favours the growth of bacteria *Vibrio* spp, which is a fast growing bacterium [9]. Since it was suspected that *Vibrio* is the causative agent of EMS, this practice possibly will stimulate the multiplication of the EMS causing agent in the pond rather than to reduce it.

Following a good aquaculture practices in pond preparation and management of water supplies before stocking can limit the bacterial growth in culture system. Postlarvae should be screened for the presence of Vibrio before being introduced into the culture pond. Thai farmers have successfully prevented the occurrence of EMS by doing larval rearing in a specific nursery area with tight biosecurity for one month. They released the shrimp to the culture pond once they passed the vulnerable stage and become stronger and achieve higher immunity to diseases. Polyculture of shrimp with Tilapia is also reported to be successful in controlling EMS [10]. Several preventive measures are recommended one of which is the use of clean broodstock and postlarvae which has been made possible by screening using the different PCR detection methods that have been developed [4]. Additionally, the use of healthy broodstock and

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production of healthy and strong PLs were recommended to prevent infection, while improved environmental and feeding management as well as efficient biosecurity measures will prevent outbreaks and spread of the disease [7].

HEPATOPANCREATIC MICROSPORIDIOSIS (HPM)

Hepatopancreatic microsporidiosis (HPM) is caused by microsporodian Enterocytozoon hepatopenaei (EHP) [11]. EHP was initially detected from growth retarded tiger shrimp *Penaeus monodon* back in 2004 in Thailand, where it was identified only as unnamed microsporodian [12]. HPM is not normally associated with shrimp mortality, but information from shrimp farmers indicates that it is associated with significant growth retardation that is not clearly noticeable until 2–3 months of cultivation [13]. EHP is firstly found in Thailand shrimp farm and the microsporodian is normally constrained in the cells of the hepatopancreas of *P. monodon* [14]. Several states in Indonesia have also reported that EHP has been detected in farmed *P. vannamei* populations [15]. In 2010, reports from Vietnam stated that *P. monodon* infected with *E. hepatopenaei* was exhibiting white feces syndrome (WFS) [16]. However, a study on EHP-infected shrimp had suggested that EHP is not the sole causative agent for WFS. According to the results from PCR and in situ hybridization analysis, EHP-infected shrimp did not produce white feces [17]. *E. hepatopenaei* is confined to tubule epithelial cells of the shrimp hepatopancreas and shows no gross signs of disease except retarded growth. It also has much smaller spores (approximately 1 μm in length) and is currently known to infect both *P. monodon* and *P. vannamei* [18]. Reports from shrimp farmers stated that EHP causes severe growth retardation in *P. vannamei*, and this is supported by the massive nature of the some infections in *P. vannamei* as revealed by *in situ* hybridization assays [7, 17].

The actual reason of the alarming rise of EHP as a pathogenic problem in the worldwide shrimp farming industry is obscure, although, environmental stress or decreased immunity caused by inbreeding may have played a role [19].

Cannibalism in shrimps has been found to be a direct transmission mode of EHP from shrimp to shrimp [17]. Cohabitation of infected and non-infected shrimps also causes the increased number of infection in a culture pond [13]. On the other hand, laboratory tests on transmission by cannibalism did reveal that transmission was not accompanied by white feces syndrome (WFS) even though EHP is often found in shrimp exhibiting WFS. Thus, there is some possibility that EHP may be a component cause or pre-disposing agent for WFS [17].

As for today, the most efficient method for preventing infection in culture ponds is to exercise good aquaculture practice in farm or hatchery to maintain biosecurity at highest level. By performing a crucial step by performing preparation of ponds in between culture cycles, residual

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spores that would cause disease outbreak can be eliminated [20]. Another prevention best approach for maturation and hatchery facilities to avoid EHP is to never use wild, captured, live animals (e.g., live polychaetes, clams, and oysters) as feeds for broodstock [21]. Preventive measures that has been used by farmers in Indonesia is by lowering stocking densities (150 – 80 larvae/m^2), proper pond preparation, screening of broodstock and larvae by PCR, as well as adding probiotics in culture ponds [22].

WHITE MUSCLE DISEASE (WMD)

A viral disease on giant freshwater prawn, white muscle disease (WMD), was first reported in the French West Indies [23], then in China [24], India [25], Thailand [26], Taiwan [27] and Australia [28]. This disease caused 100 % mortality within 2 or 3 days in freshwater prawn hatcheries and nursery ponds in different parts of India [29]. Infected postlarvae (PL) exhibited clinical symptoms with lethargy, anorexia and whitening of abdominal muscles and the disease was identified as white muscle disease. Histopathological examination of the infected animals revealed highly necrotic musculature. Degenerated muscle areas showed aggregations of melanized nuclei, many of which looked like inclusion bodies [30]. The moribund PL seriously affected with WMD appeared milky white and the mortalities in hatcheries were reported to be 30-100% [23]. Causative agent of WTD has been identified as *Macrobrachium rosenbergii* nodavirus (MrNV) associated with extra small virus (XSV) [23].

The mode of transmission needs to be studied further, although it is known that the pathogen is spreading through the transport of infected PL and the movement of infected/carrier broodstock can be transmitted by cannibalism, and giant freshwater prawns exhibit a high degree of cannibalism. Bacteria-free inoculum can caused disease in PL either by media immersion or through *Artemia* as a vector [31].

Prevention through improvements in handling and transporting prawns is presently the only available option [32]. To control this disease in the giant freshwater prawn hatchery, the PL, water, broodstocks were disinfected, and all tanks and equipment also were sterilized. The broodstock rearing ponds were disinfected by liming and drying. After hatchery sterilization, MrNV-free larvae were introduced to produce PL. Pond-reared broodtsocks were obtained from selected MrNV-free juvenile giant freshwater prawns MrNV-free juvenile giant freshwater prawns were introduced to be reared to be broodstocks [29].

WHITE FECES SYNDROME (WFS)

White feces syndrome (WFS) is an emerging problem for penaeid shrimp farming industries in Southeast Asia countries, Thailand, Malaysia, Vietnam, Indonesia, China, and in India [33]. This occurrence of this syndrome is usually first evidenced by the appearance of white fecal strings

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floating on surface of the shrimp ponds [15]. The gross signs of affected shrimp include the appearance of a whitish hindgut and loose carapace, and it is associated with reduced feeding and growth retardation [34]. WFS in shrimp arises from transformation, sloughing and aggregation of hepatopancreatic microvilli into vermiform bodies, which superficially resembles like with protozoan Gregarines [35]. Peak mortalities were observed in the case of extremely low dissolved oxygen and low alkalinity [36]. Gregarine protozoans along with huge amount of pathogenic *Vibrio* bacteria may be responsible for WFS [36].

Incidences of WFS is also associated with high stocking densities, poor water quality, poor pond bottom, high plankton blooms and bad feed management and high pollution in pond water were some of other factors which are responsible for white fecal syndrome in *L. vannamei* [33]. The affected shrimp begins to eat less and tend to be darker in colour. In severely affected shrimp hepatopancreas and gut become white and pale in colour [33]. Early disease indications appear in feed trays and at water surface, where abundant floating white feces are observed [35]. The affected shrimp show a loose exoskeleton and protozoan fouling infestation that causes a dark colouration of gills [36].

According to Tang et al. [15], farmers have two main strategies (1) attempting to reduce *Vibrio* spp. populations in the ponds through the frequent addition of probiotics such as *Bacillus* spp. or *Lactobacillus* spp. to the water; and (2) attempting to reduce pathogens in the shrimp digestive system through the use of feed additives. The feed additives being tried include: garlic, in the forms of freshly crushed or processed powders (10–30 g/kg feed); Allicin (a major active component of garlic); vitamin C (2 g/kg feed); and antiprotozoals, such as metronidazole. It is also wise to always remove white feces from the affected shrimp ponds, the white feces contain large quantities of EHP and as they can break down and sink to the pond bottom. The associated EHP can be ingested by shrimp, results in re-infection, and ultimately will increase the severity of the infection [33].

OCCURRENCE AND DISTRIBUTION OF FOUR EMERGING SHRIMP DISEASES

The occurrence and distribution of four emerging shrimp diseases are recorded in Table 1. There are a few factors that affect the occurrence and distribution of shrimp diseases in Southeast Asia countries. One of the main causes is through import of shrimp from infected farm in other country. Besides that, imports of live feeds for cultured shrimp can also contribute to the spreading of the disease. Many Southeast Asia countries which are Malaysia, Thailand and Vietnam suffered from EMS/AHPND disease outbreak. Through detection and examination, it has been confirmed that live polychaetes the infection source of *V. parahaemolyticus* during the disease outbreaks. Indonesia has not been affected with this outbreak up to date. This could be

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because of the country's restriction to import of live shrimp and using domestic live polychaetes instead of live polychaetes from China as feed for broodstock shrimp [21].

Table 1: Occurrence and distribution of EMS, HPM, WFS and WMD in Southeast Asia

Disease	**Distribution in Southeast Asia countries**	**Possible causative agent**	**Species infected**	**Reference**
EMS	Malaysia (2010), Vietnam (2011), Thailand (2012), Philippines (2015)	*Vibrio parahaemolyticus*	*P. monodon, P. vannamei*	Zorriehzahra *et al*. [37] , Hastuti [21], de La Pena *et al*. [39]
HPM	Thailand (2009) Indonesia (2015)	*Enterocytozoon hepatopenaei*	*P. monodon, P. vannamei*	Salachan *et al.* [13], Putth *et al*. [11], Jithendran *et al.* [20]
WFS	Thailand (2010) Indonesia (2014)	*Vibrio parahaemolyticus*	*P. monodon, P. vannamei*	Mastan [33], Limsuwan [36]
WMD	Thailand (2006)	*Microbrachium rosenbergii nodavirus*	*M. rosenbergii*	Hameed & Bonami [29], Yoganandhan *et al*. [26]

CURRENT DETECTION METHOD OF EMS, HPM, WMD AND WFS

The conventional methods for detecting the EMS, HPM, WMD and WFS are through PCR detection (Table 2). PCR detection is the most common method used in routine health monitoring. This method is very sensitive in detecting disease causing pathogens. The failure to detect the etiological agent of EMS, HPM, WMD and WFS would increase the transmission risk of pathogens. Recently, different rapid methods with high sensitivity and specificity have been developed to detect the presence of etiological pathogens. Furthermore, researchers are still developing novel methods with improvements in terms of rapidity, sensitivity, specificity and suitability for in situ analysis. Rapid detection methods using molecular technique are important, particularly in shrimp industry, as they are able to detect the presence of pathogens in the early larval stage, avoid disease outbreak in farm and low the economic loss. These molecular method

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are also sensitive enough to detect pathogens that present in low numbers. Herewith, the recent molecular detection methods for each diseases and their advantages and limitations.

Table 2: PCR detection method of EMS, HPM, WMD and WFS.

Disease	Detection Method	Advantage	Disadvantage	Reference
EMS	PCR	PCR test is able to distinguish between pathogenic EMS-causing *V. parahaemolyticus* isolates and non-pathogenic isolates.	Major drawback of PCR is the high cost and scientific skills needed to perform the test. Small farms might not be able to do PCR test for health screening.	Timwongger *et al.* [39], Soto-Rodriguez *et al.* [5]
HPM	*In –situ* hybridization & PCR	PCR test developed are able to detect specific EHP gene		Salachan *et al.* [13]
WMD	*In-situ* hybridization & PCR	PCR can be applied for routine health monitoring, early virus detection, studying virus–host interaction, detection of carriers and screening of broodstock		Sri Widada *et al.* [40]
WFS	Not available	Not related	Not related	Not available

CONCLUSION

Research on emergence shrimp pathogen is essential due to their role as important disease and their importance in microbial ecology. Awareness of the importance of the emerging shrimp diseases should be raised to local farmer. However, the research on this emerging disease face many challenges such as unknown causative agent to facilitate the proper treatment such as WFS disease, limited option for rapid, sensitive and accurate detection method and the difficulties associated with the experimental challenge models to study the pathogenicity of their causative agents. There are new finding to help improve the technique however, a truly reliable experimental model to assess the virulence of the etiological agent still does not exist. Lack of

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information on the alternative treatment strategies is also challenge to proper disease management strategies for new emerging diseases. This review is hoped to provide insight on the different gap of knowledge that may useful for disease management practices in shrimp cultivation industries.

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