SHRIMP DISEASE MANAGEMENT

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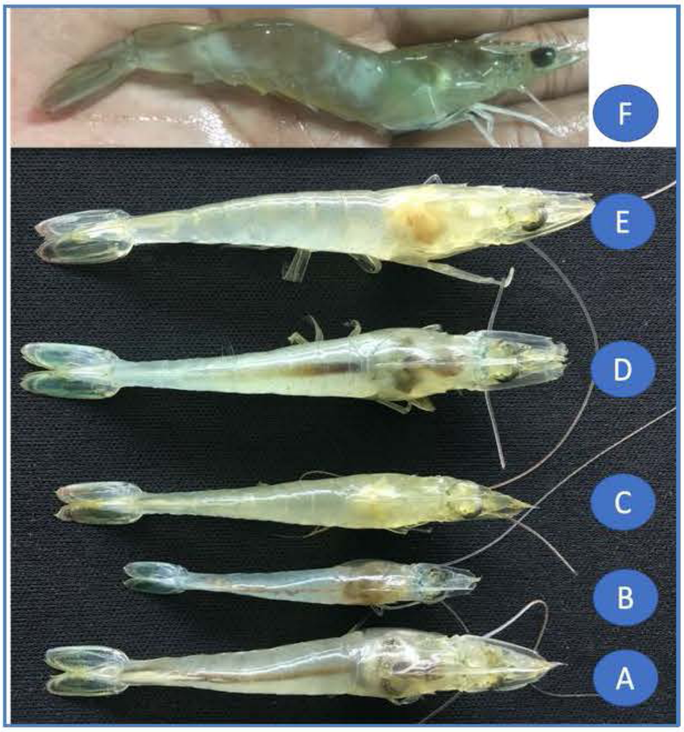
Abstract

Disease remains the most important obstacle of shrimp farming worldwide. ShrimpVet has been working on characterization, diagnosis, and control measures development for important shrimp diseases. At the present time, the most important diseases include WSSV, EMS/AHPND, EHP, White Feces Disease, and muscle necrosis. There are no absolute effective "therapeutics" for shrimp diseases but with better understanding and holistic management, the shrimp farmers can significantly reduce the risk of losing the crop.

Introduction

The intensification of shrimp farming in recent decades has faced many challenges with diseases such as White Spot Syndrome Virus (WSSV) ([1]OIE, Chapter 2.2.8, 2019), Early Mortality Syndrome/Acute Hepatopancreatic Necrosis disease (EMS/AHPND) ([2]OIE Chapter 2.2.1, 2019), Microsporidian *Enterocytozoon hepatopenaei* (EHP) ([3]Chayaburakul, *et al.*, 2004), White Feces Syndrome/Disease (WFS/WFD) ([4]Sriurairatana *et al.*, 2014), and Muscle necrosis caused by *Vibrio harveyi* ([5]Zhou *et al.*, 2015).



(A) Specific pathogen free (SPF) shrimp
(B) EHP-infected shrimp
(C) EMS/AHPND-infected shrimp
(D) WFD-infected shrimp
(E) WSSV-infected shrimp
(F) Muscle necrosis caused by *V. harveyi*

White Spot Syndrome Virus (WSSV)

History

WSSV is among the most devastating disease in shrimp farming. This disease may occur seasonally. It first reported in 1992 in cultured kuruma shrimp (*Penaeus japonicus* Bate, 1888) in the Fujian Province of China and in nearby Taiwan ([6]Zhan *et al*. 1998; [7]Sanchéz-Paz, 2010). In 1995, the first diagnosed case of WSSV in the Americas occurred in a South Texas shrimp farm, and it was suggested that the most probable route for its introduction was through an Asian imported frozen-bait shrimp commodity ([8]Hasson *et al*., 2006). Infection with WSSV has been identified from crustaceans in China, Japan, Republic of Korea, South-East Asia, South Asia, the Indian Continent, the Mediterranean ([9]Stentiford and Lightner, 2011), the Middle East, and the Americas.

Causation

WSSV was assigned by the International Committee on Taxonomy of Viruses (ICTV) as the only member of the genus *Whispovirus* within the *Nimaviridae* family. Virions of WSSV are ovoid or ellipsoid to bacilliform in shape, have a regular symmetry, and measure 80–120 nm in diameter and 250–380 nm in length. A flagella-like extension (appendage) may be observed at one end of the virion ([10]Chakraborty *et al*., 2014; [11]Reddy *et al*., 2013; [12]Walker *et al*., 2009). Today, although various geographical isolates with genotypic variability have been identified, they are all classified as a single species (white spot syndrome virus) within the genus *Whispovirus* ([13]Lo *et al*., 2012).

Symptoms

Clinical signs of WSSV revealed that lethargy, cessation of feeding which leading to empty gut and stomach, pale hepatopancreas. White spots embedded within the exoskeleton are the most commonly observed clinical sign. In most shrimp, these spots range from barely visible to 3 mm in diameter, and they sometimes coalesce into larger plates. The most prominent gross signs were white spots in the carapace, cuticle, and reddish to pink discoloration ([1]OIE, Chapter 2.2.8, 2019).

The affected animals can show lethargy, decreased or absent feed consumption, and abnormal swimming behavior, slow swimming, swimming on side, swimming near water surface, and gathering around edges of rearing units ([14]Corbel *et al*., 2001; [15]Sahul Hameed *et al*., 1998; [16]Sahul Hameed *et al*., 2001). As an acute disease, WSSV infection often results in an 80-100% mortality rate in infected ponds can be expected within a few days of the onset of behavioral signs.

Management

There is no viable treatment at the moment except for exclusion methods ([1]OIE, Chapter 2.2.8, 2019). These include using disease-free or Specific Pathogens Free (SPF) broodstock, testing of postlarvae (PLs) using PCR method, eliminating carriers in shrimp ponds, disinfection of incoming water, and enhancing biosecurity ([17]Withyachumnarnkul, 1999).

Early Mortality Syndrome/Acute Hepatopancreatic Necrosis Disease (EMS/AHPND)

History

Early Mortality Syndrome (EMS) was first reported in China in 2009 as emerging disease-causing mass mortality in cultured shrimp within 30 days of stocking. This syndrome/disease later was reported as spreading to South-East Asia including

Vietnam in 2010, Malaysia in 2011, Thailand in 2012, and the Philippines in 2014 ([18]de la Peña *et al.*, 2015; [19]FAO, 2013; [20]NACA, 2012). In 2013, a group of researchers at the University of Arizona led by Dr. Donald Lightner confirmed that the disease was due to *Vibrio parahaemolyticus* ([21]Tran *et al.*, 2013). Therefore, the EMS was later also named as Acute Hepatopancreatic Necrosis Disease (AHPND) ([18]FAO, 2013). This disease was later confirmed in several other Latin American shrimp farming countries ([22]Nunan *et. al*, 2014). AHPND was also confirmed in the U.S.A in 2017 (USDA's notice reported to OIE, 2017).

Causation

This bacterial disease is caused mostly by *Vibrio parahaemolyticus* ([21]Tran *et al.*, 2013) and later confirmed as carrying a plasmid containing toxin genes ([23]Han *et al.*, 2015). The confirmed toxin genes are *Photorhabdus luminiscens* insect-related (Pir) binary toxin Pir A/B.

Symptoms

Typical signs of AHPND are atrophied hepatopancreas, off-feeding, empty gut and stomach, lethargy, and die-off ([21]Tran *et al.*, 2013). The pathogen can be found at any stage of the shrimp life cycle: eggs, larvae, post-larvae, juveniles, and adults. However, the mortality usually happens during young post-larvae stages, early stocked PLs, animals of 15-45 days old with a mortality rate that could reach 50-100% in a few days without intervention ([24]Joshi *et al.*, 2014; [22]Nunan *et al.*, 2014; [21]Tran *et al.*, 2013).

Histological examinations revealed that the infected shrimp exhibit severe destruction of the hepatopancreas with massive cell sloughing off the tubules during the acute phase. At terminal phase, there is a lot of secondary bacterial (*Vibrios*) colonization and inflammation reactions ([21]Tran *et al.*, 2013).

Management

Post larvae production

Enhancing biosecurity: testing of broodstock and broodstock feed (squid, oyster, and polychaetes), proper treatment of broodstock feed (rinsing in 12 hours of water flow-through, and then freezing at least 24 hours before feeding). There are many trials showing that after freezing of broodstock feed many living pathogens may become inactivated (EHP) or less infective (EMS/AHPND).

Frequent check of broodstock feces using PCR for EMS/AHPND is also important to assess the risk of passing the pathogens to larvae. In practice, this work should be done every 2 weeks.

During the larvae culture, *Vibrio* spp. can proliferate very fast and dominate the larval culture system. This is the reason why applying probiotics before nauplii stocking and daily during the culture is very important to maintain a good microflora system in both water and shrimp larvae gut.

Farming

EMS/AHPND can happen in shrimp ponds as early as a few days after stocking until harvest size. There are a few key steps in disease prevention including PCR test for PLs, proper water disinfection then followed by probiotics inoculation, algae control, proper daily siphoning, and water exchange. Shrimp feed should be added with prophylactic and immune stimulation products. Short-chain fatty acids, mono-glycerides, essential oils, yeast, etc. could be added in feed at appropriate dosages.

Several studies at ShrimpVet also proved that "functional feed" could improve survival rates in EMS/AHPND challenge. Field studies also showed very promising results in terms of survival rate improvement conferred by functional feed.

Enterocytozoon Hepatopenaei (EHP)

History

Infection with *Enterocytozoon hepatopenaei* (EHP) was first discovered in *P. monodon* in Thailand in 2004 ([3]Chayaburakul, *et al.*, 2004) and later described in detail and named ([25]Tourtip, 2005; [26]Tourtip, *et al.*, 2009). EHP was reported to be prevalent to *P. vannamei* cultivated in India ([27]Rajendran *et al.*, (2016), Indonesia ([28]Aranguren *et al.*, 2019; [29]Aranguren *et al.*, 2017), Malaysia ([28]Aranguren *et al.*, 2019; [29]Aranguren *et al.*, 2017), Thailand ([26]Tourtip, *et al.*, 2009) and Vietnam ([30]Ha, *et al.*, 2010). It hampers the digestive and absorptive ability of shrimp leading to growth retardation *P. vannamei* and *P. monodon* ([31]Newman, 2015). Shrimp industry in Southeast Asia has been reported gradually increasing cases of rigorous growth retardation in shrimp populations with high prevalence of microsporidian ([32]Singh and Singh, 2018). Recently, EHP has also been reported in Venezuela, South America ([33]Tang *et al.*, 2017).

Causation

Infection with *Enterocytozoon hepatopenaei* (EHP) is a parasitic disease caused by a microsporidian species. EHP is an obligate, intracellular microsporidian parasite, first characterized and named in 2009 from the giant or black tiger shrimp *Penaeus monodon* from Thailand ([25]Tourtip, 2005; [26]Tourtip, *et al.*, 2009). EHP can be transmitted horizontally among shrimp in a rearing pond ([34]Tangprasittipap *et al.*, 2013) meaning that infections can spread progressively as cultivation continues.

Symptoms

The parasite infects hepatopancreatic cells leading to significantly stunted growth in shrimp. Typical symptoms of EHP include softshells, size variation, very low average daily growth, high FCR, and low survival rate ([35]Tang *et al.*, 2015). EHP may be detected in any stages of shrimp life cycle: broodstock, larvae, postlarvae, juveniles, and sub-adult.

Management

Hatchery Level

Screening: any "live" inputs to the hatchery must be properly screened. This includes PCR tests for broodstock, broodstock live feed, larvae, and postlarvae before dispatching. Water treatment and facility disinfection should be properly done. The most important source of EHP infection is from broodstock live feed. EHP can be found in polychaetes, oysters, and squid. The origin of EHP from live feed could probably from the cross-contamination from water released from nearby shrimp farming activities. In order to avoid this source of contamination, there are some alternatives: SPF-polychaetes, formulated feed for broodstock, proper treatment for live feed (rinsing, freezing, and testing). Freezing has been proven to completely inactivate EHP spores in contaminated feed. Routine testing of broodstock is required. Nauplii can be washed using formalin 200 ppm for 30 seconds.

Farm Level

- Soil: check using PCR. If soil is contaminated, sludge must be removed as much as possible, then the pond bottom will be treated using burnt lime (CaO) at 600 kg/1000 m^2.
- Water: proper treatment including sedimentation, filtration, $KMnO_4$, and Chlorine.
- Liner: should be disinfected prior to new crop using NaOH, $Ca(OH)_2$, citric acid, or formalin.

- During the crop, any potential enteric infection of *Vibrios* should be minimized because Vibrio infection (such as WFD) may increase the susceptibility to EHP.

White Feces Syndrome/Disease (WFS/WFD)

History

White Feces Syndrome (WFS) was reported in farmed shrimp since the early 2000s in Thailand. Its outbreaks have been frequently reported in Thailand which are responsible for a 10 to 15% loss in shrimp production ([4]Sriurairatana *et al.*, 2014).

Causation

The etiology of WFS is quite controversial. Early studies showed that there was a correlation between the presence of *Enterocytozoon hepatopenaei* (EHP) and WFS. However, later on with the expansion of the intensive shrimp farming, the typical presence of WFS was not always linked with EHP but instead the mismanagement and pollution in shrimp farming. In 2017, ShrimpVet Lab managed to induce WFS in laboratory using *Vibrio* isolates obtained from infected shrimp in the field. Challenge studies using enriched bacterial isolates top-coated on shrimp feed could induce identical symptoms as of infected shrimp in the field. This shows the transmissible nature of the disease. Later on, the Lab also worked on the interaction of *Vibrios* and EHP on the occurrence of WFS. Even though EHP is not a direct cause of WFS but the infection of EHP can weaken the integrity of the shrimp hepatopancreas leading to more vulnerability to Vibriosis.

Vibrios alone can cause WFS but it is very likely that the combination of *Vibrios* and EHP will also cause a much more severe irreversible WFS.

Some other factors that are usually associated with WFD occurrence in ponds include algal bloom and crash, overfeeding, high salinity, high temperature, high *Vibrio* counts in water. Those factors favor the bloom of *Vibrios* and in fact *Vibrios* are very likely to be the causation of WFS. Because the nature of WFS is now well understood, we can call it White Feces Disease (WFD).

Symptoms

White feces disease (WFD) usually happens in shrimp ponds of 30+ days of stocking. The disease is characterized by the presence of white fecal strands in shrimp gut and floating on water surface. Shrimp suffered from WFD also exhibit lethargy, reduce feeding, softshells, and mortality. The histology examination of WFD infected animals' reveals that the hepatopancreas is greatly damaged by *Vibrios* leading to a massive sloughing of the tubules and other hepatopancreas cells. The necrotic materials will later deposit into the midgut presenting "white fecal materials" and "white fecal strands" after defecation.

Management

The management strategies for WFD must focus on at least two aspects: biosecurity or microbiota management (feeding, algae and shrimp gut health management) and environmental factors management (intervention).

Biosecurity

Even though EHP is not a direct cause of WFD as discussed earlier, it is still a risk factor and even much dangerous than WFD itself. Therefore, PLs and pond bottom must be checked for EHP using PCR before stocking.

There is a pretty wide range of *Vibrios* capable of inducing WFD. In fact, it is very difficult to keep a *Vibrio*-free shrimp pond because *Vibrio* is part of the natural flora in the system. However, if *Vibrio* is kept under control in the ponds the WFD is not likely to occur.

Feeding Management

It is very likely that the overfeeding is a high-risk factor leading to WFD. This is understandable because the uneaten feed left from overfeeding can be greatly contaminated with *Vibrios* and shrimp can feed on those contaminated materials. It is very helpful that the feeding program must be comprehensive and based on relevant factors such as actual consumption, water quality, algal density, temperature, etc. Shrimp from new genetics lines tends to feed a lot more than previous generations. This is why it could be much more difficult to adjust the feeding rates.

Algae Management

Algae seem to be an important factor for WFD management. In general, a healthy controllable algae population in ponds is needed for better growth, shrimp health, shrimp coloration, etc. However, in intensive ponds, the excessive amount of nutrients released from shrimp feed, feces, molting, excretion, etc. has can favor a very quick bloom of green algae and blue-green algae. The bloom and crash of these algae in turn will favor the proliferation of *Vibrio* utilizing the dead algae as source nutrients. Algae management should be done proactively by minimizing the unneeded nutrients added to water, better feeding management, adequate aeration, appropriate water depth (not less than 1.2 m), and routine application of water bioremediation (or casually called as probiotics) at nighttime.

Shrimp Gut Health Management

This should be done as a must throughout the farming period. Shrimp are constantly attacked by *Vibrios* present in shrimp ponds and this causes a lot of damage to the growth, survivability, and WFD. *Vibrio* control in shrimp gut can be done via prophylaxes added in feed or top dressing. Several prophylaxes including monoglycerides, short-chain fatty acids, essential oils, etc. seem to work against *Vibrio* if added in feed with an appropriate inclusion rate. For pond-site application, probiotics seems to work well but applied appropriately. This could be done via a pre-activation of probiotics before delivering to shrimp gut. There are some suggested protocols for probiotics activation using bananas, sweet potatoes, or cooked soybean crumbles as substrates. The fermented substrates then could be fed to shrimp like a supplemental diet (fermented soybean crumbles) or top-dressed on shrimp feed pellets.

Intervention

This should be done as early as any abnormal signs suggesting WFD appears. WFD can progress very quickly so it is very important to check shrimp health very often and intervene as soon as possible. Those interventions include: reduce feeding, water exchange, increase dosage of probiotics applied in water and in feed, and increase dosing of prophylaxes in feed.

Muscle Necrosis

History

A non-luminescent and highly virulent *Vibrio harveyi* strain is associated with ‘‘bacterial white tail disease’’ of *Litopenaeus vannamei* shrimp was first reported by [36]Zhou *et al*. (2011).

Causation

Histology examination has revealed that the opaque muscle is due to *Vibrios*. *Vibrio* *harveyi* was successfully isolated from the infected muscle (ShrimpVet, 2019, unpublished data; [36]Zhou *et al*., 2011). Subsequent challenge study could produce identical lesions in experimental shrimp (ShrimpVet, 2019, unpublished data).

Symptoms

In intensive farming with plastic-lined ponds, farmers may observe daily mortality in shrimp ponds of 0.1-0.2% daily and may accept this mortality rate. In many cases, the newly death shrimp or even moribund shrimp may exhibit opaque muscle portion in the tails.

Management

Prevention

- Control algal density
- Appropriate density, partial harvest
- High dissolved oxygen (D.O), alkalinity, low NO_2
- Improve shrimp health

Intervention

- Exchange water
- Disinfection (1ppm iodine)
- Prophylaxes
- Follow "Prevention methods"

Functional Feeds

An "all-in-one" functional feed could be formulated to achieve many goals in improving growth performance, shrimp health, disease prevention, and improving shrimp meat quality.

ShrimpVet has been conducting many experiments in order to determine optimal dosages and inclusion rates of different potential compounds and ingredients that have potential effects on disease prevention. Studies show that there are many promising results from organic acids, monoglycerides, yeast, and essential oils in improving shrimp health and disease prevention. Other studies focusing on new feed ingredients such as single-cell protein and fermented protein concentrates from corn and soybean have been conducted. The results showed that with appropriate inclusion rates of these ingredients the shrimp health could improve significantly and the survival rates in challenge studies improved remarkably.

Soybean Fermentation

There were number of studies at ShrimpVet to effectively deliver activated probiotics to shrimp in order to help prevent and treat EMS/AHPND. One of the most effective ways is soybean fermentation.

A simple protocol for soybean fermentation can be as follows:
1. Grind soybean "beans" to crumble sizes of 0.3-0.5 mm in diameter.
2. Cook soybean crumbles in boiling water at 4:1 (water to soybean ratio) for 15 minutes
3. Let the slurry cool down and mix with 50g of molasses and 50mL of young pineapple juice for every kilogram of soybean crumble.
4. Mix 15-30 grams of gut probiotics per kilogram of soybean.
5. Keep the slurry at room temperature for 18-24 hours for fermentation.

The fermented soybean could be fed as a supplemental diet for shrimp at 1-3% of feed volume equivalent.

In case of EMS/AHPND or WFD intervention, the amount of fermented soybean could be fed up to 5% of feed demand equivalent. The fermented soybean has been proven effective in restoring the shrimp healthy gut flora. It also delivers some prophylaxes and thus competes against harmful *Vibrio*. This study also indicates that soybean is not likely to be the cause of WFD as some public concerns. In fact, the application of fermented soybean in industrial feed manufacturing could be beneficial to aquatic animals.

Conclusion

The intensification of modern shrimp farming has brought a lot of new opportunities for aquaculturists worldwide. However, it also brought in many new challenges especially emerging diseases. A disease is usually the result of the combination of the presence of pathogens, environmental/ physical/biological stresses, and shrimp immunity compromise. Therefore, shrimp disease management must be based on a holistic approach of biosecurity, water quality, microbiological management, feed technologies, etc. With more advances in research and the application of new technologies, farming will be more automated, more predictable, with less human and environmental interference, less chemical and antibiotics abuse, and be more environmentally friendly.

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Leading a young team of 80+ people, Loc and his co-workers have been working on various field related to aquaculture including: genetics, diagnostics, aquaculture products testing, disease prevention technologies, hatchery technologies, and farming technologies. ShrimpVet, a private independent research Lab, is now recognized as an international research team that serves the industry in Vietnam and several other aquaculture countries.

Soy In Aquaculture Program

This technical paper was created through the USSEC Soy In Aquaculture (SIA) program and the USSEC Southeast Asian Regional Program. USSEC works with target audiences in Southeast Asia and globally to show the utility and benefits of using United States soybean products in aquaculture diets.

The SIA program replaces the Managed Aquaculture Marketing and Research Program (the AquaSoy Initiative, funded and supported by the United Soybean Board and American Soybean Association) which was designed to remove the barrier to soybean meal use in diets fed to aquaculture species.

The objective of the SIA is to optimize soy product use in aquaculture diets and to create a preference for U.S. soy products in particular, including but not limited to U.S. soybean meal, soybean oil, soybean lecithin, and "advanced soy proteins" such as fermented soy and soybean protein concentrate.

This paper follows the tradition of USSEC to provide useful technical materials to target audiences in the aquaculture industry.

For more information on soybean use in aquaculture and to view additional technical papers, please visit the Soy-In-Aquaculture website at www.soyaqua.org.

References

OIE, 2019, (World Organization for Animal Health), "Manual of Diagnostic Tests for Aquatic Animals - White spot disease," Office International des Epizooties, Paris, Chapter 2.2.8.

OIE, 2019, (World Organization for Animal Health), "Manual of Diagnostic Tests for Aquatic Animals - Acute hepatopancreatic necrosis disease," Office International des Epizooties, Paris, Chapter 2.2.1.

Chayaburakul, K., Nash, G., Pratanpipat, P., Sriurairatana, S., Withyachumnarnkul, B., 2004, "Multiple pathogens found in growth-retarded black tiger shrimp *Penaeus monodon* cultivated in Thailand," Dis Aquat Org. 60, pp. 89-96.

Sriurairatana, S., Boonyawiwat, V., Gangnonngiw, W., Laosutthipong, C., Hiranchan, J., Flegel, T. W., 2014, "White feces syndrome of shrimp arises from transformation, sloughing and aggregation of hepatopancreatic microvilli into vermiform bodies superficially resembling gregarines," PloS one, 9 (6), e99170.

Zhou, J., Fang, W., Yang, X., Zhou, S., Hu, L., Li, X., Qi, X., Su, H., Xie, L., 2012, "A non-luminescent and highly virulent *Vibrio harveyi* strain is associated with "bacterial white tail disease" of *Litopenaeus vannamei* shrimp," PloS one, 7(2), e29961.

Zhan, W.B., Wang, Y.H., Fryer, J.L., Yu, K.K., Fukuda, H., Meng, Q.X., 1998, "White spot syndrome infection of cultured shrimp in China," Journal of Aquatic Animal Health, 10, pp. 405-410.

Sánchez-Paz, A., 2010, "White spot syndrome virus: an overview on an emergent concern," Vet Res., 41(6), pp. 43. Published online 2010 Feb 26. doi: 10.1051/vetres/2010015

Hasson K.W., Fan Y., Reisinger T., Venuti J., Varner P.W., 2006, "*White-spot syndrome virus* (WSSV) introduction into the Gulf of Mexico and Texas freshwater systems through imported, frozen baitshrimp," Dis. Aquat Organ., 71, pp. 91-100.

Stentiford, G.D., Lightner, D.V., 2011, "Cases of white spot disease (WSD) in European shrimp farms," Aquaculture, 319, pp. 302-306.

Chakraborty, S., Ghosh, U., 2014, "*White-spot syndrome virus* (WSSV) in crustaceans: an overview of host–pathogen interaction," J Mar Biol Oceanogr 3(1), pp. 2.

Reddy, A.D., Jeyasekaran, G., Shakila, R.J., 2013, "Morphogenesis, pathogenesis, detection and transmission risks of White Spot Syndrome Virus in shrimps," Fish Aquacult J 1.

Walker, P.J., Mohan, C.V., 2009, "Viral disease emergence in shrimp aquaculture: origins, impact and the effectiveness of health management strategies," Rev Aquacult., 1, pp. 125-154.

Lo., C.F., Aoki, T., Bonami, J.R., Flegel, T.W., Leu, J.H., Lightner, D.V., Stentiford, G., Söderhäll, K., Walker, P.W., Wang, H.C., Xun, X., Yang, F., Vlak, J.M., 2012, "Nimaviridae.In: Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses King A.M.Q.," Adams M.J., Carstens E.B. & Lefkowitz E.J., eds. Elsevier Academic Press, San Diego, California, USA, pp. 229-234.

Corbel, V., Zuprizal, Z., Shi, C., Huang, Sumartono, Arcier, J.-M., Bonami, J.-R., 2001, "Experimental infection of European crustaceans with white spot syndrome virus (WSSV)," J. Fish Dis., 24, pp. 377-382.

Sahul Hameed, A.S., Anilkumar, M., Stephen, Raj, M.L., Jayaraman, K., 1998, "Studies on the pathogenicity of systemic ectodermal and mesodermal baculovirus and its detection in shrimp by immunological methods," Aquaculture, 160, pp. 31-45.

Sahul, Hameed, A.S., Anilkumar, M., Stephen, Raj, M.L., Jayaraman, K., 1998, "Studies on the pathogenicity of systemic ectodermal and mesodermal baculovirus and its detection in shrimp by immunological methods," Aquaculture, 160, pp. 31-45.

Withyachumnarnkul, B., 1999, "Results from black tiger shrimp *Penaeus monodon* culture ponds stocked with postlarvae PCR-positive or -negative for white-spot syndrome virus (WSSV)," Dis. Aquat. Org., 39, pp. 21-27.

de la Peña, L.D., Cabillon, N.A., Catedral, D.D., Amar, E.C., Usero, R.C., Monotilla, W.D., Calpe, A.T., Fernandez, D.D., Saloma, C.P., "Acute hepatopancreatic necrosis disease (AHPND) outbreaks in *Penaeus vannamei* and *P. monodon* cultured in the Philippines," Dis Aquat Organ.116(3), pp. 251-254.

FAO, 2013, "Fisheries and Aquaculture Report No. 1053. FAO/MARD Technical: Workshop on Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Syndrome (AHPNS) of cultured shrimp (under TCP/VIE/3304)," Hanoi, Viet Nam, pp. 25-27.

NACA, 2012, "Report of the Asia Pacific emergency regional consultation on the emerging shrimp disease: early mortality syndrome (EMS)/ acute hepatopancreatic necrosis syndrome (AHPNS)," Published by the Network of Aquaculture Centres in Asia – Pacific, Bangkok, Thailand.

Tran, L., Nunan, L., Redman, R.M., Mohney, L.L., Pantoja, C.R., Fitzsimmons, K. & Lightner, D.V., 2013, "Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp," Diseases of Aquatic Organisms, 105, pp. 45-55b.

Nunan, L., Lightner, D.V., Pantoja, C., Gomez-Jimenez, S., 2014, "Detection of acute hepatopancreatic necrosis disease (AHPND) in Mexico," Dis Aquat Org Vol., 111, pp. 81-86.

Han, J.E., Tang, K.F., Tran, L.H., Lightner, D.V., 2015, "Photorhabdus insect-related (Pir) toxin-like genes in a plasmid of *Vibrio parahaemolyticus*, the causative agent of acute hepatopancreatic necrosis disease (AHPND) of shrimp," Dis Aquat Organ., 113(1), pp. 33-40. doi:10.3354/dao02830

Joshi, J., Srisala, J., Truong, V.H., Chen, I.T., Nuangsaeng, B., Suthienkul, O., Lo, C.F., Flegel, T.W., Sritunyalucksana, K., Thitamadee, S., 2014b, "Variation in *Vibrio parahaemolyticusisolates* from a single Thai shrimp farm experiencing an outbreak of acute hepatopancreatic necrosis disease (AHPND)," Aquaculture, pp. 428-429, 297-302.

Tourtip, S., 2005, "Histology, ultrastructure and molecular biology of a new microsporidium infecting the black tiger shrimp *Penaeus monodon*," Department of Anatomy, Faculty of Science. Mahidol University, Bangkok.

Tourtip, S., Wongtripop, S., Stentiford, G.D., Bateman, K.S., Sriurairatana, S., Chavadej, J., Sritunyalucksana, K., Withyachumnarnkul, B., 2009, "*Enterocytozoon hepatopenaei* sp. nov. (Microsporida: Enterocytozoonidae), a parasite of the black tiger shrimp Penaeus monodon (Decapoda: Penaeidae): Fine structure and phylogenetic relationships," J. Invertebr. Pathol., 102, pp. 21-29.

Rajendran, K.V., Shivam, S., Ezhil Praveena, P., Sahaya Rajan, J.J., Sathish Kumar, T., Satheesha, Avunje, et al., 2016, "Emergence of *Enterocytozoon hepatopenaei* (EHP) in farmed *Penaeus (Litopenaeus) vannamei* in India," Aquaculture, 454, pp. 272-280.

Aranguren, L.F., Mai, H., Pichardo, O., Hanggono, B., Dhar, K.A., 2019, "White Feces Syndrome in shrimp Predictor of EHP?," Global Aquaculture Advocate.

Aranguren, L.F., Han, J.E., Tang, K.F, 2017, "*Enterocytozoon hepatopenaei* (EHP) is a risk factor for acute hepatopancreatic necrosis disease (AHPND) and septic hepatopancreatic necrosis (SHPN) in the Pacific white shrimp *Penaeus vannamei*," Aquaculture, 471, pp. 37-42.

Ha, N.T., Ha, D.T., Thuy, N.T., Lien, V.T.K., 2010, "Occurrence of microsporidia *Enterocytozoon hepatopenaei* in white feces disease of cultured black tiger shrimp (*Penaeus monodon*) in Vietnam," Aquatic Animal Disease.

Newman Stephen G., 2015, "Microsporidian Impacts shrimp production – Industry efforts address control, not eradication," Global Aquaculture Advocate, Volume 18, Issue 2, pp. 16.

Singh, Mukta, Singh, Paramveer, 2018, "Enterocytozoon hepatopenaei: A microsporidian in the midst of serious threat to shrimp aquaculture," Journal of Entomology and Zoology Studies.

Tang, K.F., Aranguren, L.F., Piamsomboon, P., Han, J.E., Maskaykina, I.Y., Schmidt, M.M., 2017, "Detection of the microsporidian *Enterocytozoon hepatopenaei* (EHP) and Taura syndrome virus in *Penaeus vannamei* cultured in Venezuela," Aquaculture, 2017, 480, pp. 17-21.

Tangprasittipap, A., Srisala, J., Chouwdee, S., Somboon, M., Chuchird, N., Limsuwan, C., Srisuvan, T., Flegel, T.W., Sritunyalucksana, K., 2013, "The microsporidian Enterocytozoon hepatopenaei is not the cause of white feces syndrome in whiteleg shrimp *Penaeus (Litopenaeus) vannamei*," BMC Veterinary Research, 9, pp. 139.

Tang, K.F., Pantoja, C.R., Redman, R.M., Han, J.E., Tran, L.H., Lightner, D.V, 2015, "Development of in situ hybridization and PCR assays for the detection of Enterocytozoon hepatopenaei (EHP), a microsporidian parasite infecting penaeid shrimp," J. Invertebr. Pathol., 130, pp. 37-41.

Zhou, J., Fang, W., Yang, X., Zhou, S., Hu, L., et al., 2012, "A Nonluminescent and Highly Virulent Vibrio harveyi Strain Is Associated with ''Bacterial White Tail Disease'' of *Litopenaeus vannamei* Shrimp, PLoS ONE 7(2): e29961. doi:10.1371/journal.pone.0029961.

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