Surveillance and Confirmation of White Spot Disease in Cultured White Leg Shrimp *(Litopenaeus vannamei*) From Port Said and Damietta Governorates

Aya Shoukry[1], Ismail A. M. Eissa[1], Mohamed M. Abd-Eldaim[2], Mohamed Maarouf[2], Amina A. Dessoki[3], and Maather M.M. El-Lamie[1]

[1]Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

[2]Department of Virology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

[3]Department of Pathology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

Abstract

This study was done to detect the epidemiology of the white spot syndrome virus and how to diagnose it by molecular tools. 200 cultured Litopenaus vannamei samples were collected seasonally and randomly from different localities in Egypt such as Damietta and Port Said governorates. Infected shrimp exhibited white spot lesions on the carapace and the external surface. Some cases revealed a generalized pink body color and red coloration of appendages (telson). The overall incidence of white spot disease among collected Litopenaeus vannamei was 11.5% and the seasonal prevalence was 0, 0, 6, and 40% in winter, spring, summer, and autumn respectively. Molecular diagnosis by conventional PCR with primers particularly for WSSV VP28 revealed an expected amplified result at 677 bp fragments using agarose gel electrophoresis. Confirmation of diagnosis was done by histopathological studies, which demonstrated nuclear basophilic inclusion bodies in the subcuticular cells and vacuolation, degeneration with disarrangement in the hepatopancreas of infected shrimp. The current findings call for creating WSSV prevention strategies in white-leg shrimp, especially in seasons with moderate temperatures.

Key words: *Litopenaus vannamei*, PCR, prevalence, WSSV

Introduction:

Aquatic foods provide essential macronutrients and micronutrients, including vitamins and minerals ***(FAO, 2024)***. Shrimp demand is on the rise due to its health benefits, which include high-quality protein, vitamins, minerals, and vital amino acids ***(Watts,* 2024)**. White leg shrimp (*Litopenaeus vannamei)* was

the most produced species in 2020 with 5.8 million tons. *Litopenaeus vannamei* production represented about 51.7% of world production of the major crustacean species in 2020 ***(FAO, 2022)***. The majority of vannamei shrimp represents 83% of all penaeid shrimp culture internationally ***(Amiin et al., 2023)***. In Asia, *Litopenaeus vannamei* is favored for growing over *Penaeus monodon* because of the availability of SPF brood stock, , its low protein requirements and growing costs, its adaptability to a broad range of salinities and temperatures, its special flavor, and its nutritive value ***(El-Saadony et al., 2022)***. In penaeid shrimps, viruses are the primary agents responsible for a disease such as WSSV **(*Arulmoorthy et al., 2020*)**. The most lethal infection is known to be WSSV, which causes white spot disease (WSD), exhibiting a high and rapid mortality rate as reported by OIE **(*OIE*, *2023*)**. WSD is a severe disease resulting in significant financial losses on shrimp farmers and the overall shrimp sector as the virus completes its life cycle within 3–10 days and shrimp farms are susceptible to crop death **(*Talukder et al.*, *2021*)**. As white spot disease is the most lethal disease, cause high and rapid mortality and financial losses in shrimp sector in Egypt. So, this study targeted to record the prevalence & clinical picture of the disease among cultured *L. vannamei* in Port said and Damietta governorates and using molecular and histopathological techniques for its diagnosis.

Material and Methods:

Samples

Two hundred cultured shrimp namely, Litopenaeus vannamei were collected randomly and seasonally from Port Said and Damietta governorates shrimp farms. They were collected between October 2021 and the end of September 2022. Shrimp samples (cuticle, hepatopancreas) used for molecular detection of WSSV and histopathological examination.

Clinical and Postmortem examinations

The clinical and postmortem examination of collected shrimp were performed according to ***Lightner and Redman* (*1998*)** and ***Hafez et al.* (*2019*)** respectively.

Extraction of DNA

Extraction of the entire genomic DNA of WSSV was done according to the manufacturer instructions of (QIAGEN, QlAamp DNA mini kit (50) cat.No.51104,Germany).

PCR amplification of viral DNA

The following is the specific, unique sequence of WSSV primers, as reported by ***van Hulten et al. (2001***): VP28 vp28F 5′ CGA CAT CTT AAT AAC CAA GCA ACG 3′ VP28 vp28R 5′ AAA AGC ACG ATT TAT TTA CTC GG 3′ Primers were designed to target the entire ORFs of WSSV VP28 structural proteins at (677 bp) length.

The PCR reaction mixture contained 2.5 µl genomic DNA, 12.5 µl PCR master mix, 2 µl of forward primer, 2 µl of reverse primer, and 6 µl PCR water in a total volume of 25 µl. The reaction cycle was performed using an Eppendorf thermal cycler. The cycle conditions were pre-denaturation at 95 °C for 3 minutes, followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 65 °C for 30 seconds, extension at 72 °C for 30 seconds, and a final extension at 72 °C for 2 minutes ***(Poulos and Lightner, 2006)***.

Gel electrophoresis and the identification of amplified products

In a flask, 1.5 g of agarose was mixed with 100 ml of 1x TAE electrophoresis buffer to make a 1.5% agarose. To color the sample, 5 µl of ethidium bromide was added. The agarose was then dissolved after heating in the microwave. A comb was put into the electrophoresis bed, and agarose was then added to it. The agarose should be poured with extreme caution to prevent the formation of bubbles. After the gel solidified within 15 minutes and turned cloudy, we removed the comb to create 6 or 10 wells for sample application. The electrophoresis apparatus was filled with the electrophoresis buffer. The electrophoresis bed was put in the electrophoresis apparatus, followed by the application of 5 µl of ladder in the first well and 5 µl of PCR product to the rest of the wells. The power supply was turned on once electrodes were connected to it. It was adjusted for 100 minutes at 80 volts. The gel was taken from its bed and transferred to a UV transilluminator (UVP, USA) for the detection of specific DNA bands to confirm the amplification of the target sequence **(*Aly et al., 2018*)**.

Extracted DNA was also subjected to (The Tsingke Sequencing Company, Kunming, China) for more identification using the same primer.

Histopathological examination

Specimens were freshly taken from cuticle and hepatopancreas of *Litopenaeus vannamei* that showed white spots on their surface. They were prepared for histopathological examination and stained using H&E in accordance with ***Bell and Lightner* (*1988*)**.

Statistical evaluation

IBM SPSS Statistics version 27 (IBM Corp., Armonk, NY, USA) was used for the analysis. Frequency and percentage were used to express qualitative data by One way of variance (ANOVA). P-value <0.001 was considered significant.

Results

Prevalence of white spot syndrome

Figure (1) show the total and seasonal prevalence. Statistical analysis revealed significant differences between infection in different seasons **(P <0.001)**.

Clinical and postmortem examinations

Most of the affected *Litopenaeus vannamei* with WSSV showed

congregation around pond edges, listless, swimming near water's surface and white spot deposits all over the external surface (didn't disappear by scraping them against hard object, ranged from pinpoint dots to spots of 3mm diameter which sometimes coalesce to form large circular patches) (Fig 2-a). Some cases revealed generalized pink coloration of body surface and red coloration of appendages (telson) (Fig 2-b). Internally, hepatopancreas were enlarged and had a brittle texture with a yellowish-white hue. Cuticle was easily detached and branchiostegites was swollen with fluid accumulation.

Molecular identification of WSSV

Conventional PCR utilizing particular primers for the VP28 gene produced an amplified product of expected molecular size 677 bp, as shown in figure (3).

Histopathological findings

Samples of shrimp revealed white deposits on their bodies, as well as obvious basophilic intranuclear inclusion bodies in the cuticular and subcuticular cells (Fig.4-a). Meanwhile, hepatopancreas showed vacuolation, degeneration with disarrangement (Fig.4-b).

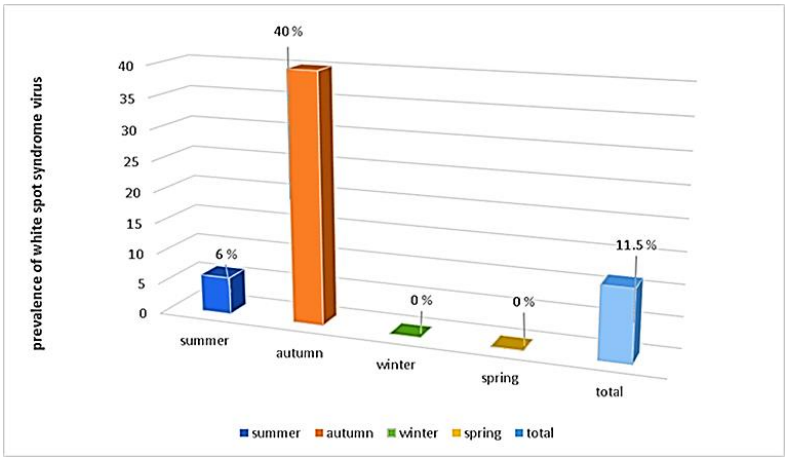

Figure 1: Total and seasonal prevalence of white spot syndrome.

Figure 2: *Litopenaeus vannamei* shows **(a)** white deposits on external surface (arrow)**. (b)** generalized pink coloration of body surface (blue arrow) and red coloration of appendages (Telson) (white arrow).

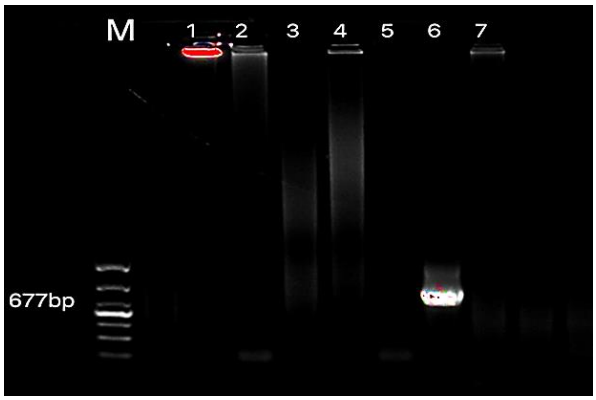

Figure 3: PCR production on Agarose electrophoresis for white spot virus strain. M, molecular weight marker, lanes: 1,2,3,4 and 5: Negative WSSV samples, lanes 6: Positive samples and lane 7: Negative control.

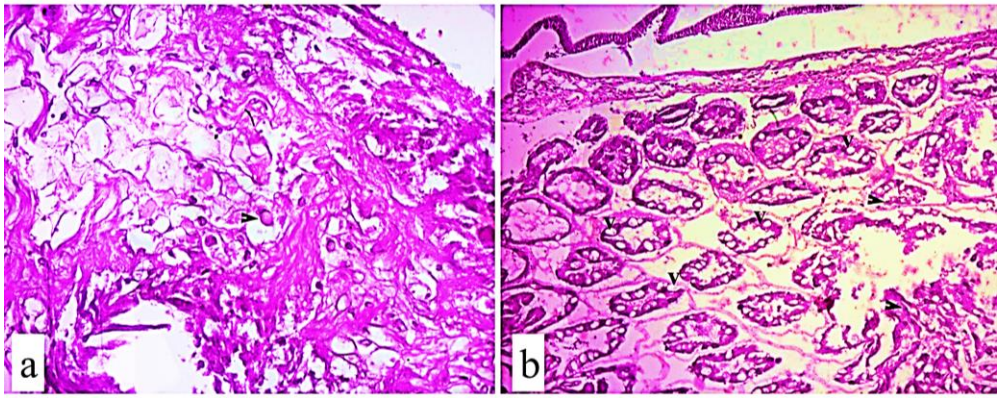

Figure 4: Infected *Litopenaeus vannamei* shows (a) Sub cuticle with intranuclear inclusion bodies (head arrow) H&E, X40. **(b)** hepatopancreas vacuolations (v), degeneration (head arrows), and disarrangement. H&E, X10.

Discussion

The rapid increase in the shrimp industry and intensification of farming methods have coincided with the appearance of deadly diseases caused by complicated interactions between the host, pathogen, and environment. White Spot Syndrome Virus affects a wide variety of hosts, including all types of farmed shrimp and other aquatic invertebrates like crabs and crayfish (***Ahmad et al., 2017***).

In this study, Verbruggen et al. (2016) found that Litopenaeus vannamei infected with WSSV exhibited abnormal behavioral changes, potentially due to metabolic changes during virus replication. They congregate around the pond edges, stop feeding, are listless, and swim near the water surface; these are in agreement with ***Escobedo-Bonilla et al. (2008)*** and ***Varner and Hasson (2008)***, who stated that moribund *Penaeus monodon* can also have chromatophore extension in the early stages of WSSV infection, which causes little to no "white patches" to be visible on the body. As WSD becomes more severe, the cuticle becomes loosened and distinctive in various sizes; white spots (calcium deposits) appear along the inner surface of the carapace; red coloration of the body surface and appendages; a soft shell cuticle that can be separated easily; and circular white spots on the cuticle of the carapace and abdominal segment, which range from minute to large spots that may coalesce together. The WSSV infection, which causes white patches due to the accumulation of calcium salts within the cuticle, may be responsible for these clinical signs. ***Dashtiannasab (2020)*** and ***Wiradana et al. (2019)*** noted that an acute infection typically exhibits occlusions or white patches with a diameter of 0.5–2 mm on the carapace surface, along with

discoloration, pale hepatopancreas, and lesions with white patches on the exoskeleton. These recorded signs, along with PM lesions, serve as distinctive features that aid in the identification of WSSV infection.

White spot syndrome virus (WSSV) had at least 39 structural proteins (***Sánchez-Martínez et al., 2007***). ***Peng et al. (2022)*** stated that VP28 is the major spike protein in the WSSV and is essential for its attachment. ***Park et al. (2013)*** reviewed the conservation of VP-encoding DNA sequences among isolates from various geographic origins. In our study, we used the antigenic spike protein VP28 for the detection of WSSV. As a result, we used primers targeting partial products of VP28 according to ***van Hulten et al. (2001)***, which provides a practical diagnostic PCR methodology applicable to shrimp of different regional origins.

The PCR detection approach involves amplifying the target WSSV genome in shrimp samples using primer sets targeting the VP28 gene in a single-tube reaction. The results of PCR revealed that WSSV yielded an amplification product at an expected 677 bp in contrast to negative findings in non-infected tissues, indicating this gene's existence in infected shrimp tissues. This result was agreed with ***El Shahidy et al. (2015)***.

The total seasonal prevalence was 11.5%. This result was higher than that of ***Kalaria et al. (2022)***, who found the prevalence of WSSV infection as 5.5% in 91 *Litopenaeus vannamei* samples collected from shrimp farms in Karnataka, India, located in Uttara Kannada, Dakshina Kannada, and Udupi districts. Meanwhile, it is lower than that obtained by ***Rathipriya et al. (2019)***, who stated that the prevalence of white spot syndrome virus in the *Litopenaeus vannamei* shrimp farms in Nagapattinam district, Tamil Nadu, India, was 49.12% (84/171) and that of Otta et al. (2014), who found it at 73.33% in the same species of shrimp collected from several farms in India with minimum biosecurity. Also, it's less than what ***Thamizhvanan et al. (2019)*** found when they looked for viral and other pathogen infections in white leg shrimp (Litopenaeus vannamei) from grow-out ponds in Tamil Nadu and Andhra Pradesh states on the east coast of India. They found that 11.7% (28/240) of the shrimp had WSSV (white spot syndrome virus) infection. In addition, ***Dutta et al. (2015)*** recorded that the total prevalence of WSSV infection in wild *P. monodon* was the highest (56.2%) in Chennai, Tamil Nadu, followed by Digha, West Bengal (10.9%), Visakhapatnam, Andhra Pradesh (0.6%), and Chilika, Orissa (0%).

In the current study, there were no infections in the spring and winter seasons (zero%), followed by the summer season (6%) and autumn season (40%). ***De la Luz Vazquez-Sauceda et al. (2016)*** reported that in four sampling sites, the study

area's temperature showed a monthly increase based on the season, with the coldest months being December and January and the hottest months being May through August. In November, wild shrimp tested positive for WSSV using PCR. All other shrimp samples were negative for the virus. Therefore, the temperature directly influences the presence of this virus. Viral positives were acquired at 26 ºC. Similar to this, water temperatures between 26 and 34 °C were associated with WSSV outbreaks in the farmed shrimp *Penaeus monodon*. ***Moser et al. (2012)*** showed the temperature range from 27 °C to 30 °C is the optimum for WSSV (white spot syndrome virus). Infected *Litopenaeus vannamei* shrimp had reduced mortality or been completely prevented at higher (32–33 °C) or lower (<15 °C) water temperatures compared to the optimal temperature range. Reduced replication, apoptosis, and changed WSSV gene expression are some of the hypothesized processes that account for these findings. In the acute stage of infection before clinical signs appear, increasing water temperature from 27–29 °C to 31–33 °C can stop virus replication and death of infected shrimp by WSSV (***Rakhshaninejad et al., 2023***). It has been noted that major WSSV epizootics are less likely to happen during warm seasons, and management techniques that raise pond temperature may present a chance to control this infection. Also, it has been suggested that cold temperatures may protect shrimp from WSSV, most likely by lowering viral replication (***Sánchez-Paz, 2010***).

Regarding the results of histopathological examination, infected hepatopancreas showed vacuolation and degeneration with disarrangement; these results were similar to those recorded by ***Hasan et al. (2024)***, who recorded that hepatopancreas of shrimp infected with WSSV showed signs of necrotic and degraded hepatopancreas, as well as dispersed cellular disintegration. Hepatopancreatic epithelial tissue and tubules also showed signs of degeneration, and ***Tong et al. (2023)*** observed vacuolation of hepatopancreas tissue. ***Megahed (2019)*** observed that the cuticular epithelium showed nuclear hypertrophy of the nucleus and large basophilic intranuclear inclusion bodies, which is similar to our result that there are obvious basophilic intranuclear inclusion bodies in the cuticular and subcuticular cells.

Conclusion:

From the present study, it could be concluded that WSD threaten shrimp cultures. Egyptian shrimp farms could pose a serious risk and have a detrimental effect on the production sector due to infection with WSSV which cause mortalities. Histopathological studies and gross lesions may help in the primary diagnosis of WSSV in *Litopenaeus vannamei*. PCR provide a definitive

diagnosis for WSSV in *Litopenaeus vannamei*. The ongoing worldwide trade may contribute to the spread of viral strains among nations, including Egypt, particularly when there is no certification and no enforcement of laws governing the transfer of free stocks. The current findings call for the creation of WSSV prevention strategies in white leg shrimp, especially in seasons with moderate temperature.

References

Ahmad, T., Sanyal, K. B., Mukherjee, D., Abraham, T. J. & Dash, G. (2017). Detection of white spot virus (WSV) in *Litopenaeus vannamei* from shrimp aquaculture farms in East Midnapore district, West Bengal (India). *Int J Fish Aquat Stud, 5*(2), 200-204.

Aly, S., Mansour, S. & Thabet, R. (2018). Pathology and Molecular Diagnosis of White Spot Syndrome Virus (WSSV) among Vannamei Shrimp in Egypt. *Suez Canal Veterinary Medical Journal. SCVMJ, 23*(2), 127-142.

Amiin, M. K., Lahay, A. F., Putriani, R. B., Reza, M., Putri, S. M. E., Sumon, M. a. A., Jamal, M. T. & Santanumurti, M. B. (2023). The role of probiotics in vannamei shrimp aquaculture performance - A review. *Vet World, 16*(3), 638-649. doi:10.14202/vetworld. 2023.638-649

Arulmoorthy, M. P., Anandajothi, E., Vasudevan, S. & Suresh, E. (2020). Major viral diseases in culturable penaeid shrimps: a review. *Aquaculture International, 28*(5), 1939-1967. doi:https://doi.org/ 10.1007/s10499-020- 00568-3

Bell, T. A. & Lightner, D. V. (1988). A handbook of normal penaeid shrimp histology. *World Aquaculture Society,Baton Rouge,LA.*

Dashtiannasab, A. (2020). Chapter 32 - White Spot Syndrome Virus. In Ennaji, M. M. (Ed.), *Emerging and Reemerging Viral Pathogens* (pp. 717-728): Academic Press.

De La Luz Vazquez-Sauceda, M., Sánchez-Martínez, J. G., Pérez-Castañeda, R., Rábago-Castro, J. L., Aguirre-Guzmán, G. & Vargas-Cruz, D. Y. (2016). White spot syndrome virus (WSSV) and necrotizing hepatopancreatitis (NHP) detection in wild shrimp of the San Andrés lagoon, Mexico. *Revista de Biología Marina y Oceanografía, 51*(2), 455-459.

Dutta, S., Chakrabarty, U., Mallik, A. & Mandal, N. (2015). White spot syndrome virus (WSSV) prevalence associated with disease resistance among wild populations of black tiger shrimp, *Penaeus monodon* (Fabricius). *Aquaculture Research, 46*(2), 453-461.

El-Saadony, M. T., Swelum, A. A., Abo Ghanima, M. M., Shukry, M., Omar, A. A., Taha, A. E., Salem, H. M., El-Tahan, A. M., El-Tarabily, K. A. & Abd El-Hack, M. E. (2022). Shrimp production, the most important diseases that threaten it, and the role of probiotics in confronting these diseases: A

review. *Research in Veterinary Science, 144*, 126-140. doi:https://doi.org/10.1016/j.rvsc.2022.01. 009

El Shahidy, M., El Gamal, R., Dessouki, A., Thabet, R., Abd Elwahab, S. & Abd Eldaim, M. (2015). Detection of White Spot Syndrome Virus in Cultured Penaeid Shrimp using Histopathological Observation and Polymerase Chain Reaction. *Suez Canal Veterinary Medical Journal. SCVMJ, 20*(1), 213-226.

Escobedo-Bonilla, C. M., Alday-Sanz, V., Wille, M., Sorgeloos, P., Pensaert, M. B. & Nauwynck, H. J. (2008). A review on the morphology, molecular characterization, morphogenesis and pathogenesis of white spot syndrome virus. *Journal of fish diseases, 31*(1), 1-18.

Fao. (2022). The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO. . *FAO,Rome*, 266 p.

Fao. (2024). Fishery and Aquaculture Statistics-Yearbook 2021.Rome. doi:https://doi.org/10.4060/cc9523en

Hafez, Y. M., Moustafa, N. Y., Magouz, A. F. & Al-Maria, N. F. (2019). Isolation of white spot syndrome virus (WSSV) in Egyptian shrimp using conventional PCR and real time PCR (qPCR) techniques. *Slovenian Veterinary Research/Slovenski Veterinarski Zbornik, 56*, 457.

Hasan, M. J., Sultana, S., Khan, M. N., Islam, H. M. R. & Islam, M. N. (2024). Molecular diagnosis appended by histopathological signature delineates the white spot syndrome virus (WSSV) infection in penaeid shrimps. *Comparative Immunology Reports, 6*, 200138.

Kalaria, K. K., Girisha, S. K., Nithin, M. S., Puneeth, T. G., Kushala, K. B. & Suresh, T. (2022). Prevalence of disease outbreak from cultured whiteleg shrimp *Penaeus vannamei* farms located in Karnataka, India. *Emergent Life Sciences Research, 8*(2), 240-247.

Lightner, D. V. & Redman, R. M. (1998). Shrimp diseases and current diagnostic methods. *Aquaculture, 164*(1-4), 201-220.

Megahed, M. E. (2019). A comparison of the severity of white spot disease in cultured shrimp (*Fenneropenaeus indicus*) at a farm level in Egypt. I-Molecular, histopathological and field observations. *Egyptian Journal of Aquatic Biology and Fisheries, 23*(3), 613-637.

Moser, J. R., Álvarez, D. a. G., Cano, F. M., Garcia, T. E., Molina, D. E. C., Clark, G. P., Marques, M. R. F., Barajas, F. J. M. & López, J. H. (2012). Water temperature influences viral load and detection of White Spot Syndrome Virus (WSSV) in *Litopenaeus vannamei* and wild crustaceans. *Aquaculture, 326*, 9-14.

Oie. 2023. *OIE (World Organization for Animal Health), 2023. Animal health in the world. OIE-Listed diseases, infections and infestations in force in 2023.*https://www.woah.org/fileadmin/Home/eng/Health_standards/aahm/current/2.2.08_WSSV.pdf [Online]. [Accessed 12 may 2024].

Otta, S. K., Arulraj, R., Ezhil Praveena, P., Manivel, R., Panigrahi, A., Bhuvaneswari, T., Ravichandran, P., Jithendran, K. P. & Ponniah, A. G. (2014). Association of dual viral infection with mortality of Pacific white shrimp (*Litopenaeus vannamei*) in culture ponds in India. *VirusDisease, 25*, 63-68.

Park, J. Y., Kim, K. I., Joh, S. J., Kang, J. Y., Kwon, J. H., Lee, H. S. & Kwon, Y. K. (2013). Development of a highly sensitive single-tube nested PCR protocol directed toward the sequence of virion envelope proteins for detection of white spot syndrome virus infection: Improvement of PCR methods for detection of WSSV. *Aquaculture, 410*(2013 Oct), 225-229.

Peng, W., Bao, Q., Jia, R. & He, P. (2022). Construction of an easily detectable transgenic Synechococcus elongatus PCC 7942 against White Spot Syndrome Virus using vp28 and mOrange Gene and its metabolism in shrimp. *Frontiers in Immunology, 13*, 974014.

Poulos, B. T. & Lightner, D. V. (2006). Detection of infectious myonecrosis virus (IMNV) of penaeid shrimp by reverse-transcriptase polymerase chain reaction (RT-PCR). *Diseases of aquatic organisms, 73*(1), 69-72.

Rakhshaninejad, M., Zheng, L. & Nauwynck, H. (2023). Shrimp (*Penaeus vannamei*) survive white spot syndrome virus infection by behavioral fever. *Scientific Reports, 13*(1), 18034.

Rathipriya, A., Anand, T., Uma, A. & Marx, K. K. (2019). Prevalence of diseases caused by White spot virus and Enterocytozoon Hepatopenaei in *Penaeus vannamei* shrimp farms in Nagapattinam District, Tamil Nadu, India. *Int. J. Curr. Microbiol. App. Sci, 8*(6), 2616-2622.

Sánchez-Paz, A. (2010). White spot syndrome virus: an overview on an emergent concern. *Vet Res, 41*(6), 43.

Sánchez-Martínez, J. G., Aguirre-Guzmán, G. & Mejía-Ruíz, H. (2007). White spot syndrome virus in cultured shrimp: a review. *Aquaculture research, 38*(13), 1339-1354.

Talukder, A. S., Punom, N. J., Eshik, M. M. E., Begum, M. K., Islam, H. M. R., Hossain, Z. & Rahman, M. S. (2021). Molecular identification of white spot syndrome virus (WSSV) and associated risk factors for white spot disease (WSD) prevalence in shrimp (*Penaeus monodon*) aquaculture in

Bangladesh. *Journal of invertebrate pathology, 179*(2021), 107535.

Thamizhvanan, S., Sivakumar, S., Santhosh Kumar, S., Vinoth Kumar, D., Suryakodi, S., Balaji, K., Rajkumar, T., Vimal, S., Abdul Majeed, S. & Taju, G. J. J. O. F. D. (2019). Multiple infections caused by white spot syndrome virus and Enterocytozoon hepatopenaei in pond-reared *Penaeus vannamei* in India and multiplex PCR for their simultaneous detection. *Journal of fish diseases, 42*(3), 447-454.

Tong, Y., Yang, J., Wang, L., Chi, X., Zhu, C., Yin, R., Zhang, L., Li, Y., Zhao, C. & Jia, R. (2023). Effects of dietary supplementation of Anabaena sp. PCC7120 expressing VP28 protein on survival and histopathology after WSSV infection in *Macrobrachium nipponense. Fish & Shellfish Immunology, 139*, 108865.

Van Hulten, M. C. W., Witteveldt, J., Peters, S., Kloosterboer, N., Tarchini, R., Fiers, M., Sandbrink, H., Lankhorst, R. K. & Vlak, J. M. (2001). The White Spot Syndrome Virus DNA Genome Sequence. *Virology, 286*(1), 7-22. doi:https://doi.org/10.1006/viro.2001.1002

Varner, P. W. & Hasson, K. W. (2008). White Spot Syndrome of Crustaceans. In AFS-FHS (American Fisheries Society-Fish HealthSection). FHS blue book: suggested procedures for the detection and identification of certain finfish and shell fish pathogens, 2020 edition. Accessible at: 12 may 2024 https://units.fisheries.org/fhs/fishhealth-section-blue-book-2020/.

Verbruggen, B., Bickley, L. K., Van Aerle, R., Bateman, K. S., Stentiford, G. D., Santos, E. M. & Tyler, C. R. (2016). Molecular mechanisms of white spot syndrome virus infection and perspectives on treatments. *Viruses, 8*(1), 23.

Watts, E. (2024). Seafood handling, processing, and packaging. In Dikeman, M. (Ed.), *Encyclopedia of Meat Sciences (Third Edition)* (pp. 108-124). Oxford: Elsevier.

Wiradana, P. A., Mahasri, G., Sari, R. E. R., Marwiyah, U. C. & Prihadhana, R. (2019). Identification of white spot syndrome virus (WSSV) in pacific white shrimps (*Litopenaeus vannamei*) from ponds postexposure to immunogenic membrane proteins (Zoothamnium penaei). *IOP Conference Series: Earth and Environmental Science, 236*(1), 012085. doi:10.1088/1755-1315/236/1/012085

تقصي وتأكيد مرض البقع البيضاء في الجمبري الأبيض المستزرع في محافظتي بورسعيد ودمياط (*Litopenaus vannamei*)

ايه شكري ابراهيم[1] ، اسماعيل عبد المنعم عيسي[1] ، محمد مصطفي عبد الدايم[2]، محمد معروف[2]، أمينة على الدسوقى[3] ومآثر محمد منير اللمعي[1]

[1] قسم امراض ورعاية الاسماك كلية الطب البيطري جامعه قناه السويس

[2]قسم الفيروسات كلية الطب البيطري جامعه قناه السويس

[3] قسم الباثولوجيا بكلية الطب البيطري جامعة قناة السويس

أُجريت هذه الدراسة للكشف عن وبائية مرض النقط البيضاء وكيفية تشخيصه باستخدام الأدوات الجزيئية. تم تجميع 200 عينة جمبري *Litopenaus vannamei* بشكل موسمي وعشوائي من محافظتي دمياط وبورسعيد.أظهر الجمبري المصاب بقع بيضاء مميزة على الرأس وعلي السطح الخارجي باكمله . كما أظهرت بعض الحالات تغيرًا عامًا في لون الجسم إلى اللون الوردي، بالإضافة إلى احمرار الزوائد (telson). بلغ معدل انتشار فيروس متلازمة البقعة البيضاء بين الجمبري المجمع 11.5%، بينما كان معدل الانتشار الموسمي 0% في الشتاء، 0% في الربيع، 6% في الصيف و40% في الخريف. تم تشخيص المرض جزيئياً باستخدام تقنية تفاعل البوليميراز المتسلسل (PCR) التقليدية مع البرايمرات مخصصة لجين VP28 الخاص بفيروس WSSV، حيث أظهر المنتج المتوقع شريطاً بحجم 677 قاعدة زوجية باستخدام الفصل الكهربائي للجل. تم تأكيد التشخيص من خلال الفحص النسيجي حيث كشف عن تضخم في الانوية خلايا تحت الجلد (القشرة), بالإضافة إلى وجود فجوات وانحلال واضطراب في ترتيب خلايا البنكريا الكبدي للجمبري المصاب. تدعو النتائج الحالية إلى وضع إستراتيجيات للوقاية من فيروس البقع البيضاء في الجمبري الابيض خاصة في المواسم ذات درجات الحرارة المعتدلة.