

Effect of Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) on Growth, Survival Rate and Histopathological Changes of Pacific White Shrimp (*Litopenaeus vannamei*)

Jiraporn Singhapan, Chalor Limsuwan and Niti Chuchird

ABSTRACT

The effect of Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) on the growth and survival rate of Pacific white shrimp (*Litopenaeus vannamei*), shrimp larvae from broodstocks raised in an earthen pond infected with IHHNV according to the results of a polymerase chain reaction (PCR) test were raised in two experimental ponds at the stocking density of 70,000 shrimps per 1,600 square meters, or 44 shrimps per square meter. The results were compared with shrimp larvae from imported specific pathogen free (SPF) broodstocks, raised in two control ponds at the stocking density of 120,000 shrimps per 1,600 square meters, or 75 shrimps per square meter. The salinity in all the ponds was ranging from 3 – 6 ppt. and the shrimps were raised with commercial pellet feed. After 30 days some of the IHHNV-infected shrimps began to show abnormalities such as bent or deformed rostrums, wrinkled walking legs, cuticular roughness, and other cuticular deformities. Most of these shrimps were also smaller than the others in the same pond. As time went on the number of shrimps with these abnormalities in the experimental ponds rose continuously. Those in the IHHNV-infected ponds that did not display clinical signs of abnormalities still grew at a slower rate than the shrimp from SPF broodstocks. The difference was quite apparent after 70 days. When the shrimp in the two IHHNV-infected ponds were harvested after 120 days, the average yield was 1,882.79 kg/ha. The average survival rate was only 44.17 % the feed conversion rate (FCR) was 1.75. The shrimp from SPF broodstocks were harvested after 100 days. The average yield was 7,719.38 kg/ha. The survival rate was 81.99 % and the FCR was 1.35. There was a statistically significant difference between the growth rates and survival rates of the two groups ($p < 0.05$). Histopathological studies and *in situ* hybridization assays of the shrimp that displayed clinical signs revealed Cowdry type A and intranuclear inclusion bodies in the cells of the ectodermal tissues (gills, epidermis and nerve cord) as well as in the mesodermal tissues (hematopoietic organ, antennal gland and lymphoid organ) and the endodermal tissues (hepatopancreas). The shrimp that came from SPF broodstocks did not have any visible abnormalities.

Key words: *Litopenaeus vannamei*, Pacific white shrimp, infectious hypodermal and hematopoietic necrosis virus, IHHNV

INTRODUCTION

Pacific white shrimp (*Litopenaeus vannamei*) have recently become very popular among shrimp farmers as an alternative breed to black tiger shrimp. This is because black tiger shrimp have a slower growth rate and many farmers have lost money in raising them. Also, from March 2002 to February 2003 the Fisheries Department authorized imports of Pacific white shrimp and many farmers who tried raising them found them easy to raise. They grow quickly in the first 90 days and produce high yields, so they are becoming more popular. Since numbers of good quality shrimp larvae from the imported parents are limits, many farmers have smuggled in Pacific white shrimp from Taiwan and China (Tu *et al.*, 1999). Some have also produced larvae from broodstocks raised in earthen ponds, which have a high chance of being infected by various diseases, especially Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV). IHHNV was first observed in the blue shrimp (*Litopenaeus stylirostris*) in Hawaii (Lightner *et al.*, 1983b). This virus has been classified in the Parvoviridae family according to its morphological and physico-chemical features (Bonami *et al.*, 1990). IHHNV is found in black tiger shrimp, but it does not cause any problem. However, IHHNV reduces the growth rate and survival rate of Pacific white shrimp

(Lightner *et al.*, 1983a). This study on the effects of IHHNV in actual shrimp raising ponds can be used as a guideline to find ways to prevent and reduce the problem of the spread of IHHNV, which could affect shrimp production in the future.

MATERIALS AND METHODS

This study was carried out in earthen ponds at a commercial shrimp farm in Nakhon Pathom province. Postlarvae from broodstocks raised in earthen ponds were first checked with a polymerase chain reaction (PCR) test to confirm that they were infected with IHHNV and then they were stocking into two earthen ponds with salinity of 3 - 6 ppt with a density of 43 shrimps per square meter. These shrimps were then compared with postlarvae from imported SPF parents that tested negative for IHHNV in the PCR test, raised in two earthen ponds with a stocking density of 74 shrimps per square meter. Shrimps were fed with commercial pellet feed four times daily. During the culture period the shrimps were observed for signs of abnormalities. They were weighed after 30 days and every week after that until harvest time. The yield, average weight of the shrimps and survival rate in the two groups were then statistically compared. Histopathological studies were made on samples of shrimps with abnormalities caused by IHHNV.

Table 1 Yield, survival rate, weight and feed conversion rate (FCR).

	Pond	Pond size (ha)	Total yield (kg)	Yield (kg/ha)	Survival rate (%)	FCR	Average weight (g)	Days of culture (days)
IHHNV	1	0.645	1,068.1	1,656.48	42.72	1.7	10.65	120
	2	0.541	1,140.6	2,109.10	45.62	1.8	10.17	120
Control	1	0.256	1,943.4	7,591.41	81.66	1.3	11.96	94
	2	0.250	1,958.7	7,847.36	82.32	1.4	12.21	93

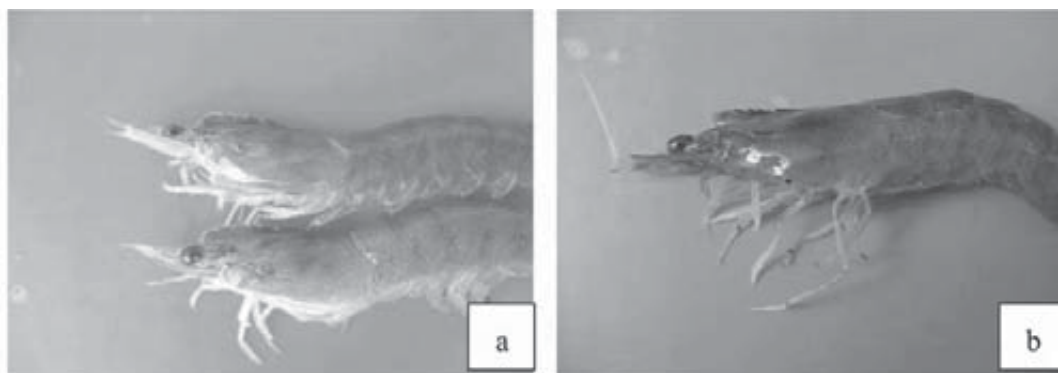


Figure 1 (a,b) Examples of live adult *Litopenaeus vannamei* that show gross signs of IHHNV caused runt deformity syndrome (RDS).

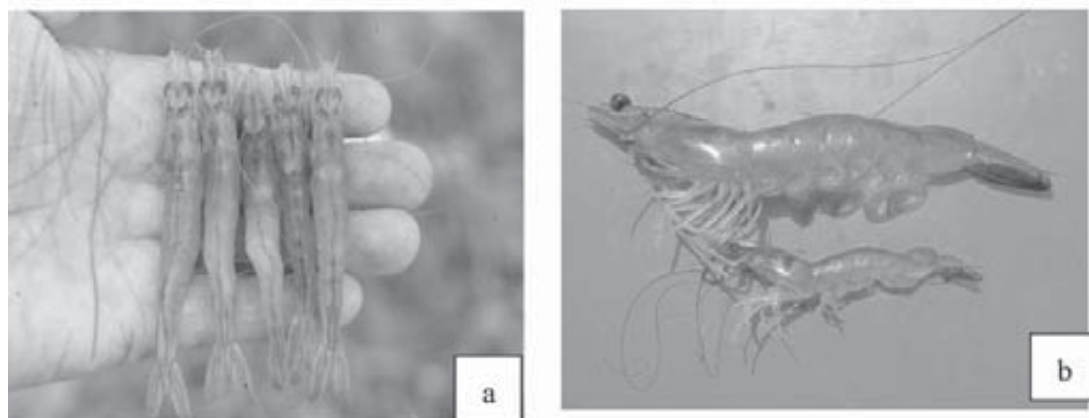


Figure 2 (a,b) Examples of live adult *Litopenaeus vannamei* that show gross sign of IHHNV caused deformed body.

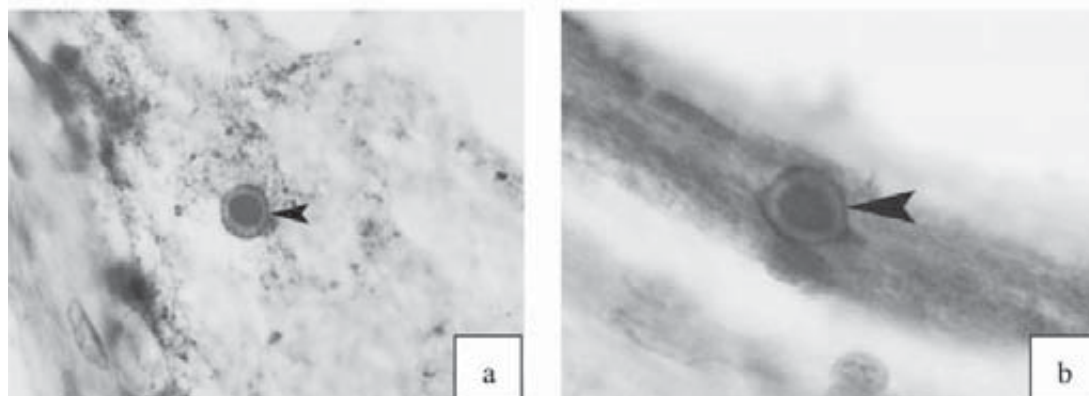


Figure 3 (a,b) A high magnification of nerve cord showing adjacent cell with diagnostic IHHNV intranuclear inclusion bodies in hypertrophical nuclei (Davidson's; H&E; x800).

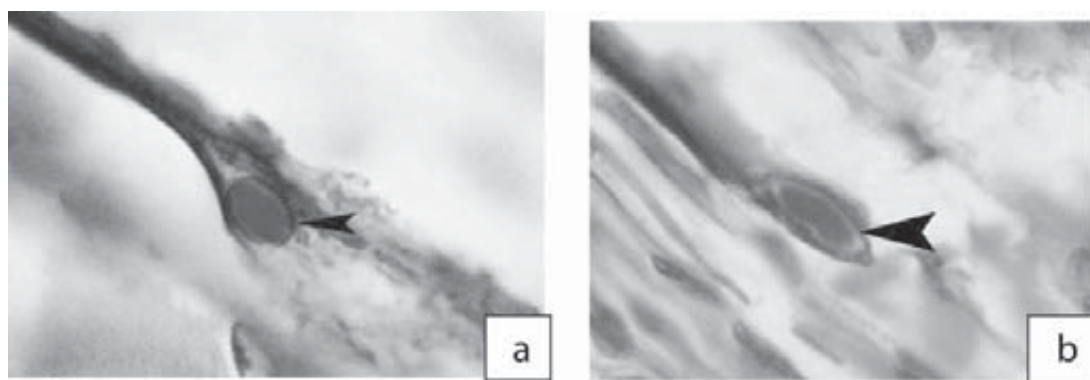


Figure 4 (a,b) A high magnification of nerve cord showing eosinophilic inclusions (Cowdry type A inclusions or intranuclear inclusion bodies) that are pathognomonic for IHHNV infection. (Davidson's; H&E; x800).

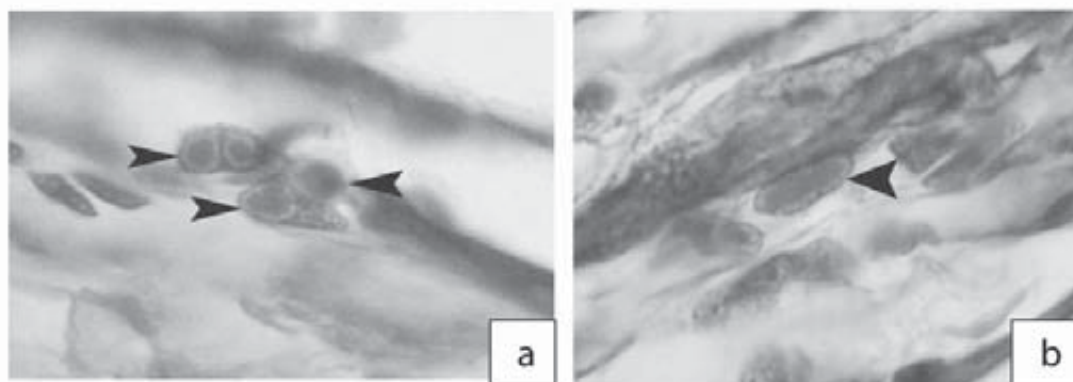


Figure 5 (a,b) Several cells with IHHNV showing adjacent cell with eosinophilic intranuclear inclusions (Cowdry type A inclusions) (Davidson's; H&E; x800).

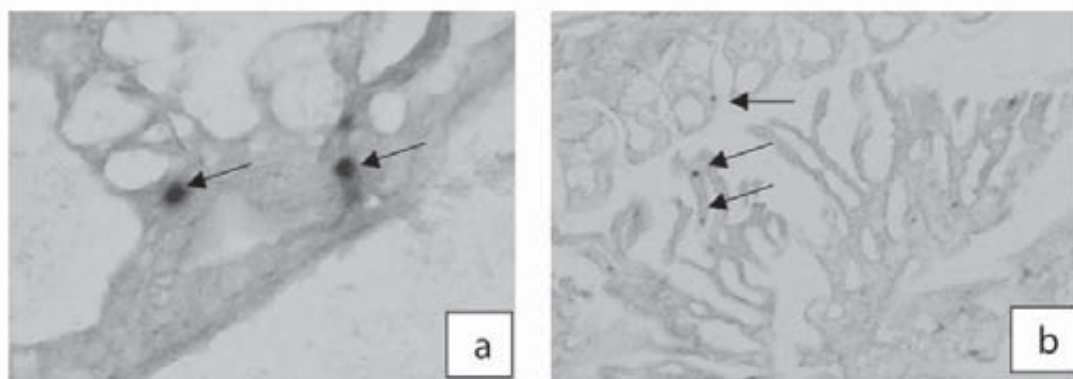


Figure 6 (a,b) *In situ* hybridization assays results from hepatopancreas (a) and gills (b) of *L. vannamei* infected with IHHNV. The infected areas reacted strongly to the IHHNV-specific probe as is visualized by the black precipitate (arrows). (x400).

RESULTS AND DISCUSSION

The yield, survival rate, average weight and FCR of the IHHNV-infected shrimp and the control group are shown in Table 1. When the shrimp in the two IHHNV-infected ponds were harvested after 120 days, the average yield was 1,882.79 kg/ha. The average survival rate was only 44.17 % FCR was 1.75. The shrimp from SPF broodstocks were harvested after 100 days. The average yield was 7,719.38 kg/ha. The survival rate was 81.99 % and the FCR was 1.35 (Table 1). There was a statistically significant difference between the growth rates and survival rates of the two groups ($p < 0.05$). The Pacific white shrimp larvae from broodstocks raised in earthen ponds that were infected with IHHNV began to show signs of abnormalities, i.e., bent or deformed rostrums, wrinkled walking legs, cuticular roughness, and other cuticular deformities, 30 days after stocking (Figure 1a,b). This is in agreement with the results of a study by Lightner (1996), who reported that IHHNV caused 90% mortality of juvenile of blue shrimp *Panaeus stylirostris*. IHHNV has caused significant losses for shrimp farmers (Lightner *et al.*, 1983a,b; Tang *et al.*, 2000). It causes slow growth, small size, a low survival rate and runt-deformity syndrome (Figure 2a,b) or RDS (Kalagayan *et al.*, 1991). Histopathological studies of IHHNV-infected shrimp showed Cowdry type A and intranuclear inclusion bodies in the cells of the ectodermal tissue (gills, epidermis and nerve cord) (Figure 3a,b; 4a,b; 5a,b) as well as in the mesodermal tissue and the endoderm tissues (Figure 6a,b). The shrimp that came from SPF broodstocks did not have any abnormalities. Result from this study indicated that shrimp farmers should choose postlarvae from SPF broodstocks that have tested negative for IHHNV in PCR tests in order to achieve a good yield.

ACKNOWLEDGEMENTS

The authors would like to thank the National Research Council of Thailand for financial support.

LITERATURE CITED

- Bonami, T.A., B. Trumper, J. Mari, M. Brehelin and D.V. Lightner. 1990. Purification and characterization of the infectious and hypodermal and hematopoietic necrosis virus of penaeid shrimps. **J. Gen. Virol.** 71: 2637-2664.
- Kalagayan, G., D.Godin, R. Kanna, G. Hagino, J. Sweeney, J. Wyban and J. Brock. 1991. IHHN virus as an etiological factor in runt-deformity syndrome of juvenile *Penaeus vannamei* cultured in Hawaii. **J. World Aquacult. Soc.** 22: 235-243.
- Lightner, D.V. 1996. **A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp**, World Aquaculture Society, Baton Rouge, LA. 304 p.
- Lightner, D.V., R.M. Redman and T.A. Bell. 1983a. Infectious hypodermal and hematopoietic necrosis, a newly recognized virus disease of penaeid shrimp. **J. Invertebr. Pathol.** 42: 62-70.
- Lightner, D.V., R.M. Redman, T.A. Bell and J.A. Brock. 1983b. Detection of IHHN virus in *Penaeus stylirostris* and *P. vannamei* imported into Hawaii. **J. World Maric. Soc.** 14: 212-225.
- Tang, K.F.J., S.V. Durand, B.L. White, R.M. Redman, C.R. Pantoja and D.V. Lightner. 2000. Postlarvae and juveniles of a selected line of *Penaeus stylirostris* are resistant to infectious hypodermal and hematopoietic necrosis virus infection. **Aquaculture.** 190: 203-210.
- Tu, C., H. Huang, S. Chaung, J. Hsu, S. Kuo, N. Li, T. Hsu, M. Li and S. Lin. 1999. Taura syndromes in pacific white shrimp *Penaeus vannamei* cultured in Taiwan. **Dis. Aquat. Org.** 38: 159-161.