Could Acquired Defense Ability Against Yellow-Head Virus be Induced in *Penaeus monodon* ?

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Abstract

Penaeus monodon, juveniles and postlarvae (PL), were administered with yellow-head virus (YHV) by injection and immersion, respectively. The juvenile shrimps survived low-concentration administration of YHV, as well as a subsequent injection with lethal concentration of the virus. PL 20 that were previously exposed to YHV showed a relative resistance to YHV infection at one month after the last exposure. The results suggests that YHV exposure could induce resistance to YHV infection in *P. monodon.*

Introduction

Outbreaks of yellow-head disease (YHD) in giant tiger shrimp *Penaeus monodon* began in 1992 (Boonyaratpalin et aI., 1993; Chantanachookin et aI., 1993); and its prevalence, although having decreased, has continued up to the present. In 1993 alone, the economic loss from this disease was estimated at 30 millions US $ (from Asian Shrimp News, Second Quarter, No. 14). The disease is caused an RNA-type virus (Wongteerasupaya et aI., 1995), commonly known as yellow-head virus (YHV). Clinically, YHD-shrimps swim to the water surface and dike during the day. Their exoskeletons appear paler than in normal shrimps, with distinctive yellowish hepatopancreases and gills. Mass mortality, usually 100%, occurs in a few days after the first symptom appears.

Among several preventive measures against YHD, vaccination against YHV has been one approach. Before the successful vaccine could be developed, studies on its possibility should be carried out. The report herein suggests that induction of defense ability against YHV is possible.

Materials and Methods

Crude extract of YHV was prepared from moribund juvenile *P. monodon* (15-20 g BW) following YHV injection. Original sample of YHV was isolated from *P. monodon* taken from an YHD outbreak. Gills from the moribund shrimps were homogenized with lobster haemolymph buffer (LHB) at a ratio of 1:10

(W/V). The homogenate was centrifuged at 3,000 rpm, at room temperature, for 5 min. The supernate was isolated and centrifuged again for another 5 min. The supernate was then passed through a 0.45 μm filter. The filtrate was kept at -70 °C until use. At time of experiment, the YHV stock was diluted and injected intramuscularly into the shrimp. A few days after the injection, the shrimp became moribund and YHV was isolated from its gill, according to the mentioned procedure. This practice has been serially repeated and carried on for more than three years in the laboratory.

This report is composed of two studies; one with juvenile *P. monodon* and the other with the postlarvae 20 (PL 20). In the first study, the shrimps were divided into three groups, 350 of each. Group 1 received LHB injection, 0.1 ml per piece. Group 2 were injected with 1:100,000 diluted YHV. Group 3 received a series of YHV injection, with interval of 5 days between each injection. The first injection was with 1:500,000 dilution; the second with 1:250,000 dilution; and the third with 1:100,000 dilution. Survival rate was observed throughout the whole period and was extended 10 days after the last injection. The shrimps of each group were reared in one-ton fiber-glass tanks. Water was exchanged 20% daily and commercial feed provided.

In the study with PL 20, the shrimp were divided into four groups: Group 1 received no treatment; Groups 2 to 4 were serially immersed with YHV from low to high concentrations, at 4 days interval. The immersion schedule was as in Table 1.

Table 1. Immersion of PL 20 *P. monodon* with yellow-head virus in four groups of animals. The numbers indicate the dilution of the virus.

Group	1st Immersion	2nd Immersion	3rd Immersion	4th Immersion
1		None		
2	1:500,000	1:100,000		
3	1:500,000	1:100,000	1:75,000	
4	1:500,000	1:100,000	1:75,000	1:50,000

Each group consists of 500 pieces with 3 replicates. The shrimps in each replicate were reared in 300 l fiber-glass tanks. Water was exchanged 20% daily and commercial feed provided. Survival rate was determined at PL 43 or 23 days after the first immersion.

The shrimp were reared after PL 43 for another one month. They were then sampled for YHV challenge. The sample size was 10 pieces x 3 replicates. YHV was injected at 1:10,000 dilution and survival rate determined.

Moribund shrimp after YHV challenge were randomly checked for YHV infection, using rapid diagnostic technique (Nash et al., 1993).

Results

In the first study, all the shrimp in Group 1 which had not received YHV survived 100%. The shrimps in Group 2 that received YHV 1:100,000 dilution began to develop YHD at day 3 post-injection and 100% mortality occurred at day 10 post-injection (Fig. 1). Group 3 survived 100% after the first and the second injection. But, in contrast to Group 2 shrimps, 100% of them survived following the third injection with 1:100,000 dilution (Fig. 2).

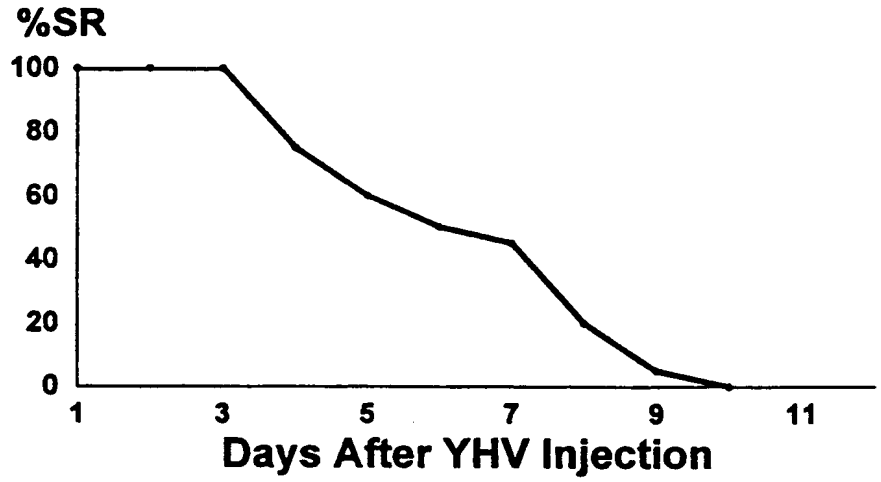

Figure 1. Survival rate of Group 2 (see text) *P. monodon* following an injection of yellow-head virus at 1:100,000 dilution.

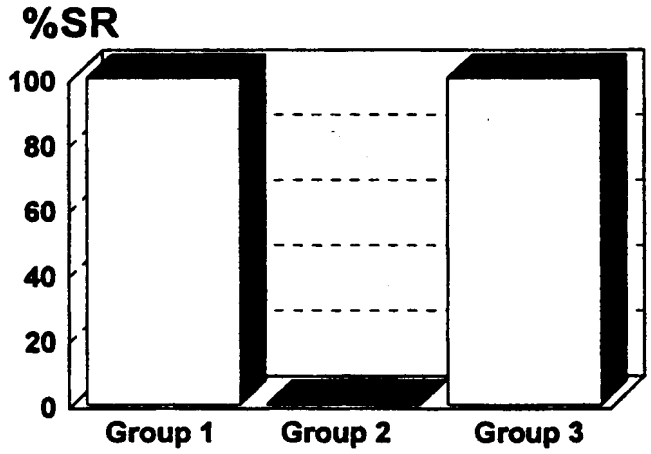

Figure 2. Survival rate of *P.monodon* in the first study (see text). Group 1 was control, and not challenged; Group 2 had never been exposed to YHV and was challenged; and Group 3 had been exposed to YHV and was challenged.

In the second study, the survival rate determined at intervals was shown in Table 2. A decrease in the survival rate was observed after the first immersion at 1:500,000 dilution in Group 3 and Group 4 animals, but not in Group 2 animals. Significant decrease in the survival rate of Group 2 animals (compared to the Group 1 control animals) did not occur until at PL 32, when one replicate was totally lost.

Table 2. Survival rate of *P. monodon* postlarvae at different stages after immersion with yellow-head virus. Each number is an average of three replicates.

Group	At PL 20	At PL 24	At PL 28	At PL 32	At PL 43
1	100%	51%	36%	31%	26%
2	100%	55% (4 days after the 1st immersion)	31% (4 days after the 2nd immersion)	22%	19%
3	100%	38% (4 days after the 1st immersion)	16% (4 days after the 2nd immersion)	15% (4 days after the 3rd immersion)	13%
4	100%	32% (4 days after the 1st immersion)	25% (4 days after the 2nd immersion)	22% (4 days after the 3rd immersion)	7% (4 days after the 4th immersion)

After being challenged with YHV at one month later, the animals in Group 4 showed a relatively longer period of survival (Fig. 3). Half of the animals in the first three groups died around day 4 post-injection, whereas, half of the animals of Group 4 died around day 7.

Histology from rapid diagnosis method revealed YHV infection in moribund shrimps following YHV challenge (figure not shown).

Discussion

The result of the first study suggests that *P. monodon* could become resistant to lethal concentration of YHV if they received sub-lethal concentrations earlier. The fact that Group 3 animals survived 100% before receiving the final 1:100,000 dilution is an important point. Had the shrimps survived less than 100% after the first or the second injection, the interpretation of the result might be that the stronger shrimps that survived the injection were resistant to YHV. And that

means the increased defense ability was due to the innate property of the shrimp, not the acquired one. This study clearly showed that the shrimps of the same source, being administered the same amount and same lot of YHV, could respond differently. And the difference depends on whether the shrimps were previously exposed to the virus.

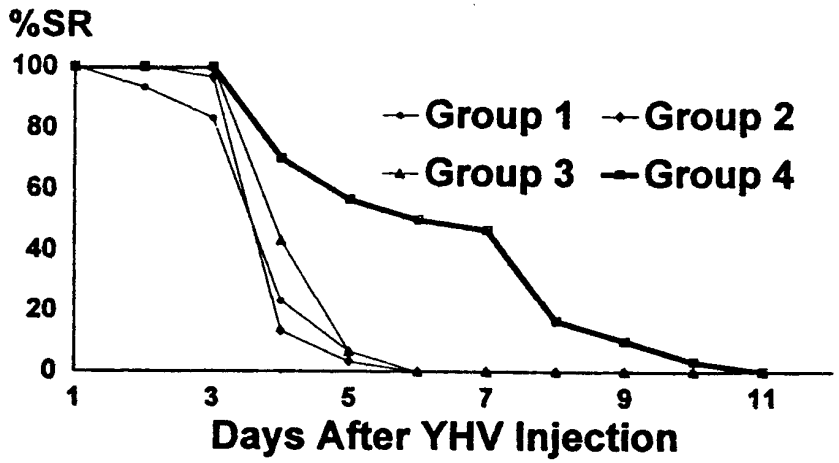

Figure 3. Survival rate of *P. monodon* after being challenged with yellow-head virus at one month after PL 43. See text for group description.

In the second study, the difference in the survival rate of postlarvae at various stages is difficult to interpret. In general, survival rate of larvae and postlarvae in hatcheries is extremely variable, depending on water qualities, infections, etc. The cause of mortality in this study could not be precisely advocated since histological diagnosis was not done. However, the higher mortality in Group 2 to 4 with progressive manner suggests that the mortality was likely caused by YHV infection; and YHV at 1:100,000 dilution was able to kill the shrimps. When being challenged with YHV at one month after PL 43, resistance to the infection was again observed in the shrimps previously exposed to YHV. In this case, the possibility of being due to the innate property of the shrimp can not be ruled out. Group 4 shrimps that survived the best could possibly be the strongest shrimp that survived earlier infection, and thus tended to survived better in the challenging test. The finding that the shrimp died 100% at day 12 post-injection does not necessarily mean that the shrimp had no resistance to the virus. Had the challenged dose of YHV been lower, the difference in the survival rate of Group 1 and Group 4 shrimps could be wider.

According to most investigators, crustaceans possess a primitive, non-specific internal defense system (Sinderman, 1971; Ratcliffe et al., 1985; Smith

and Chrisholm, 1992). Although this study suggests that there might be some sort of defense mechanism similar to vertebrate immune system, the non-specificity type of response can not be ruled out. The crude YHV extract as prepared contained several other cellular components from shrimp tissues and other foreign substances that might act as non-specific inducer. But the fact that immersion or feeding *P. monodon* with some commercial immuno-enhancers, extracts from bacterial cell wall, yeast cell wall, etc. did not bring out any protection against the virus (Withyachumnarnkul, unpublished) suggests that not many agents could induce this kind of response. These findings, therefore, raise the possibility of vaccination the shrimp with specific pathogen as a means of inducing protection in *P. monodon* against yellow-head disease.

References

Boonyaratpalin, S., Supamattaya, K., Kasornchandra, J., Direkbusarakom, S., Aekpanithanpong, U. and Chantanachookin, C. (1993) Non-occluded Baculo-like virus, the causative agent of yellow-head disease in the black tiger shrimp *(Penaeus monodon). Gyobyo Kenkyu* **28(3):** 103-109.

Chantanachookin, C., Boonyaratpalin, S., Kasornchandra, J., Direkbusarakom, S., Ekpanithanpong, U., Supamataya, K., Sriurairatana, S. and Flegel, T.W. (1993) Histology and ultrastructure reveal a new granulosis-like virus in *Penaeus monodon* affected by "yellow-head" disease. *Dis. Aqua. Org.* **17**: 145-157.

Nash, G., Akarajamorn, A. and Withyachumnarnkul, B. (1993) Rapid diagnosis of yellow-head disease in black tiger shrimp. *AAHRI Newsletter* Vol.2, No. July 1993.