Occurrence of Lymphoid Organ Spheroid Cells in Domesticated Pacific White Shrimp (*Litopenaeus vannamei*) Broodstock in Thailand

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ABSTRACT

The mortality rate and histopathology of unilaterally ablated female Pacific white shrimp (*Litopenaeus vannamei*) broodstock from six commercial hatcheries in Thailand were studied. Hatcheries A, B, and C used shrimp broodstock that were domesticated in Thailand. These shrimp were reared in earthen ponds without proper biosecurity systems. Meanwhile, hatcheries D, E, and F imported shrimp broodstock from the United States of America (USA) and reared them in closed recycling facilities with biosecurity systems in order to investigate the health of shrimp broodstock used in the Thai shrimp industry. The mortality rate was monitored for two months, and 10 female broodstock with similar lengths (18-21 cm) from each hatchery were sampled for histopathological studies of the hepatopancreas and lymphoid organ. The results revealed that the mortality rate of shrimp domesticated in Thailand was 43.47±9.51%, significantly higher (P>0.05) than that of the imported group, which was 5.93±1.93%. The histopathology of the hepatopancreas revealed that no differences existed in the appearance of the lipid cells in the hepatopancreas of the broodstock from the two studied groups. The number of lipid cells in the shrimp hepatopancreas ranged from 77 to 93%. For the lymphoid organ, a significant difference (P<0.05) was found between the number of spheroids in the lymphoid organ of the shrimp in the group that was domesticated in Thailand (42.00±18.83%) and in the imported group (0.17±0.91%). We suspect that the presence of numerous spheroids in the lymphoid organ of the shrimp in the domesticated group is probably related to the contamination of the infectious antigens during grow out since these shrimp were cultured in earthen ponds without proper biosecurity systems. The practice of only stocking farms with shrimp that are free of diseases, the implementation of effective disease management, better management of land and water usage, and the treatment of effluents can reduce loss due to diseases.

Keywords: Pacific white shrimp broodstock, Lymphoid organ, Spheroid cell

INTRODUCTION

The Pacific white shrimp, *Litopenaeus vannamei*, is the major shrimp species cultured in China, Taiwan, and Thailand (Limsuwan and Chanratchakool, 2004). Since 2012, shrimp farmers in Thailand have experienced the early mortality syndrome (EMS), which has caused major economic losses in many cultivation areas throughout the country. Affected shrimp show signs of pale coloration due to pigment loss as well as atrophy of the hepatopancreas. These signs may become apparent as early as four days post stocking (Munkongwongsiri *et al*., 2013). *V. parahaemolyticus* is reported to be suspected of causing mass mortality (Tran *et al*., 2013). During the outbreak of EMS on farms, an abnormally high mortality of shrimp broodstock was observed in some hatcheries in Thailand. These broodstock died soon

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after eyestalk ablation, with the mortality rate up to 40-50%. Eyestalk ablation is a common technique used to stimulate gonadic maturation by removing the X-organ sinus gland complex located in the eyestalk of female shrimp. This organ secretes a variety of hormones, including the gonad-inhibiting hormone (GHH) (Fingerman, 1997). This method can increase the total egg production and increase the percent of females ready for reproduction. However, eyestalk ablation can cause severe physiological stress and the lowering of the immunological resistance of shrimp. Still, the degree of the physiological stress, the decrease in the immunological responses, and the mortality of the shrimp could be limited by removing only one eyestalk of each shrimp (unilateral ablation) Maggioni *et al*., 2004; Sainz-Hernández *et al*., 2008).

The hepatopancreas is the primary digestive organ of shrimp. It plays the dual role of secreting digestive enzymes and absorbing nutrients (Rosas *et al*., 1995). Johnson *et al*. (1998) reported that the hepatopancreas is highly sensitive to physiological and environmental changes; thus, it can be used to monitor the shrimp's health condition. Meanwhile, the lymphoid organ, which is located dorso-anterior to the ventral hepatopancreas, is believed to play an important role in immune-defense against pathogens (Anggraeni and Owens, 2000). This organ has a filtering function to remove foreign materials from the haemolymph by forming lymphoid organ spheroids (Rusaini and Owens, 2010). Since lymphoid organ spheroids are mostly found in the lymphoid organ of shrimp infected with viruses or bacteria (Angraeni and Owens, 2000; Burgents *et al*., 2005; Pongsomboon *et al*., 2008), the number of lymphoid organ spheroids in the lymphoid organ of shrimp can be used to determine the shrimp's health status.

The present study monitored the mortality rate of shrimp broodstock from two sources (domesticated in Thailand and imported from USA) after unilateral eyestalk ablation. The histopathology of the hepatopancreas and lymphoid organ of the shrimp were examined. The completeness of the hepatopancreas cells and the number of spheroids in the lymphoid organ of the shrimp were used to determine the health condition of the shrimp broodstock used in Thailand.

MATERIALS AND METHODS

Six commercial hatcheries located in the eastern and southern parts of Thailand were studied (Table 1). Hatcheries A, B, and C used shrimp broodstock that were domesticated in Thailand. These shrimp were reared in earthen ponds without proper biosecurity system (inadequate reservoir and treatment ponds, no tire bath at the farm entrance, no footbath and hand disinfection at the pond entrance, no crab fence, and no bird scaring device). Meanwhile, hatcheries D, E, and F imported shrimp broodstock from the USA. These shrimp were reared in closed recycling facilities with biosecurity systems. Female shrimp broodstock with similar lengths (18-21 cm) from six hatcheries were sampled and tested for white spot syndrome virus (WSSV), yellow head virus (YHV), Taura syndrome virus (TSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), and infectious myonecrosis virus (IMNV), following the guidelines of OIE (2012). All broodstock that were free of those viruses then underwent unilateral eyestalk ablation following the method that Wyban and Sweeney (1991) described. After 48 h, they were transferred to reproduction tanks. The

Table 1. Location, pedigree of broodstock, and rearing system of the six hatcheries where shrimp samples were obtained

Hatchery	Location	Pedigree	Rearing system
A	Chantaburi	Domesticated	Open system
B	Rayong	Domesticated	Open system
C	Phuket	Domesticated	Open system
D	Chonburi	Imported	Closed system
E	Phuket	Imported	Closed system
F	Phuket	Imported	Closed system

mortality rate was recorded for two months, and 10 female shrimp from each hatchery were collected for the histopathological study of the hepatopancreas and lymphoid organ.

Histopathological study

Ten female shrimp from each hatchery were fixed in Davidson's fixative and processed for histological study, as described by Bell and Lighter (1988), to investigate the completeness of the hepatopancreas and the number of lymphoid organ spheroids in the lymphoid organ. The results were calculated to percent/unit.

Statistical analysis

Mortality rate and histological data are presented as means±standard deviation. One-way analysis of variance (ANOVA) and Duncan's multiple range test were used to compare the data among six hatcheries. The exception was the completeness of the hepatopancreas study, where the Kruskal-Wallis one-way ANOVA was applied. The differences were considered to be significant at $P<0.05$.

RESULTS AND DISCUSSION

Mortality study

The mortality of the shrimp broodstock after a reproduction period of 60 days is shown in Table 2. Mortality rates of broodstock from hatcheries A, B, and C were 42, 35 and 54%, respectively. Meanwhile, mortality rates of the imported broodstock from hatcheries D, E, and F were 6, 8 and 4%, respectively.

The results revealed that the mortality rate of Thai domesticated broodstock was high, at around 43.47±9.51%, which is significantly different ($P>0.05$) from that of the imported broodstock (5.93±1.93%) (Table 3).

Histopathological study

Based on histology, the cell structure of each organ, the number of lipid cells in the hepatopancreas, and the number of spheroids in the lymphoid organ were used for classifying shrimp health condition. No differences were found in the appearances of the lipid cells in the hepatopancreas of the broodstock

Table 2. Mortality rates of shrimp broodstock from six hatcheries

Hatchery	Number of shrimp	Number of dead shrimp	Mortality (%)
A	242	101	42%
B	186	65	35%
C	268	144	54%
D	304	18	6%
E	280	22	8%
F	350	14	4%

Table 3. Mortality rates between shrimp from domesticated group and imported group

Treatment	Mortality (%)
Domesticated group (Hatcheries A, B, and C)	$43.47±9.51^{a}$
Imported group (Hatcheries D, E, and F)	$5.93±1.93^{b}$

Data are presented as means±standard deviation of 10 shrimp. Means in the same column with different superscripts are significantly different from one another ($P<0.05$).

from different sources. The number of lipid cells in the hepatopancreas of the shrimp ranged from 77-93%. Shrimp in the group that was domesticated in Thailand had an average number of lipid cells in the hepatopancreas of 80.67±22.58%. Meanwhile, shrimp in the imported group had an average number of lipid cells in the hepatopancreas of 83.67±16.29% (Tables 4-5 and Figures 1-2). No sign of bacterial infections, such as nodule formation or melanization, was observed in the hepatopancreas of the shrimp from all of the studied groups. All broodstock used in this study showed a healthy hepatopancreas with a high number of lipid cells. Felgenhauer (1992) reported that the hepatopancreas is a digestive organ composed of blind tubules containing primarily four types of cells (E, F, B, and R), which synthesize, transport, and secrete digestive enzymes and store lipids, glycogen, and minerals. Tubule formation and the size of this organ can be used as indicators of nutritional quality in shrimp (FAO, 2004) because the hepatopancreas is where most of the enzymes required for metabolism are produced (Cuźon *et al*., 2004). Manan *et al*. (2015) reported that a healthy hepatopancreas showed a well-organized glandular tubular structure; intact E, B, and R epithelial cells; rounded epithelial tubules; and a high number of lipid cells. Since all studied shrimp were broodstock, they were fed with high nutritional feeds, such as polycheate, mollusk, and squid, to enhance their reproductive performance. Thus, no difference was found in the histopathological changes of the hepatopancreas of the shrimp from all groups.

For the lymphoid organ, significant differences were found in the number ofspheroids in the lymphoid organ of the shrimp among the six hatcheries. The number of spheroids in the lymphoid organ of the shrimp ranged from 0-63% (Figures 3-6). The broodstock that were domesticated in Thailand had a significantly higher number of spheroids in the lymphoid organ, 42.00±18.83% ($P<0.05$), compared with the 0.17±0.91% of spheroids in the lymphoid organ of the broodstock from the imported group (Tables 4-5). The lymphoid organ consists of folded tubules with a central hemal lumen, and a wall layered with cells is a site of bacterial uptake and phagocytosis by hemocytes (Van de braak *et al*., 2002). The lymphoid organ is also proposed to have a bacteriostatic

Table 4. Lipid cells in the hepatopancreas and spheroids in the lymphoid organ of broodstock from difference sources

Hatchery	Lipid cells in hepatopancreas (%)	Spheroids in lymphoid organ (%)
A	78.00±22.01^{a}	43.00±6.75^{b}
B	80.00±32.66^{a}	20.00±6.67^{c}
C	84.00±8.43^{a}	63.00±4.83^{a}
D	77.00±9.49^{a}	0.50±1.58^{d}
E	81.00±18.53^{a}	0.00±0.00^{d}
F	93.00±16.36^{a}	0.00±0.00^{d}

Data are presented as the means±standard deviation of 10 shrimp. Means in the same column with different superscripts are significantly different from one another ($P<0.05$).

Table 5. Lipid cells in the hepatopancreas, and percent of spheroids in the lymphoid organ between shrimp from domesticated group and imported group

Treatment	Lipid cells in hepatopancreas (%)	Spheroids in lymphoid organ (%)
Domesticated group (Hatcheries A, B, and C)	80.67±22.58^{a}	42.00±18.83^{a}
Imported group (Hatcheries D, E, and F)	83.67±16.29^{a}	0.17±0.91^{b}

Data are presented as the means±standard deviation of 10 shrimp. Means in the same column with different superscripts are significantly different from one another ($P<0.05$).

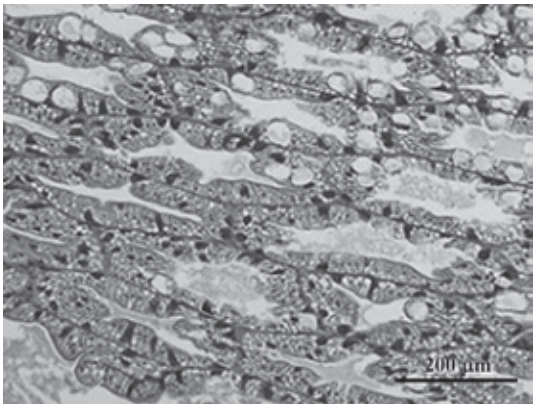

Figure 1. Many lipids collected from the hepatopancreas of domesticated broodstock (H&E: bar = 200 µm)

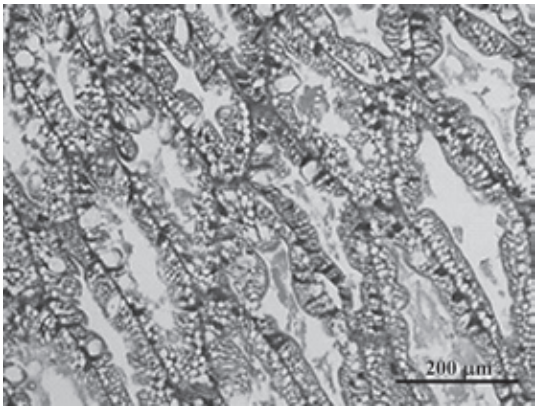

Figure 2. Many lipids collected from the hepatopancreas of imported broodstock (H&E: bar = 200 µm)

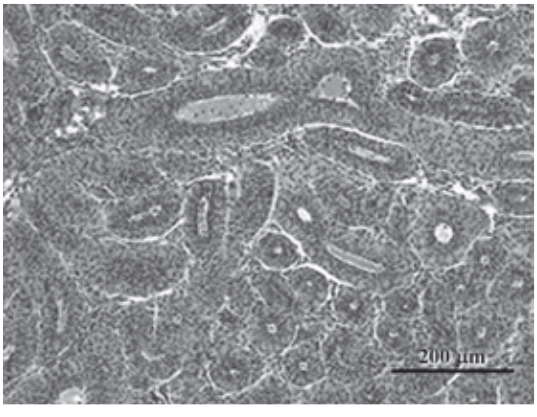

Figure 3. No lymphoid organ spheroids in broodstock from hatcheries D, E, and F (H&E : bar = 200 µm)

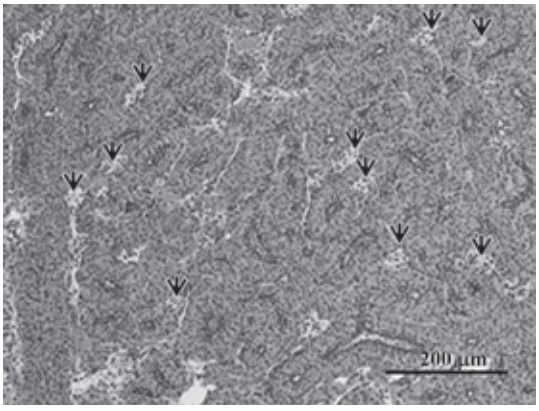

Figure 4. 20 percent of lymphoid organ spheroids (arrow) in broodstock from hatchery B (H&E : bar = 200 µm)

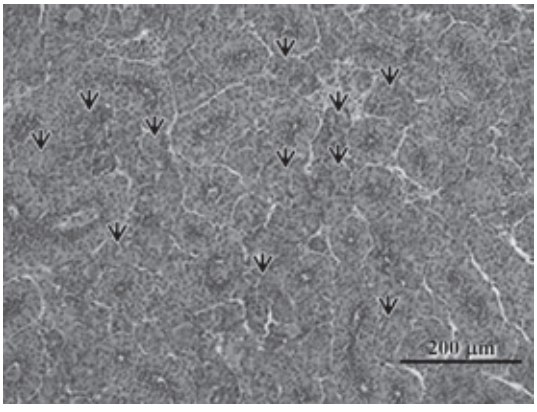

Figure 5. 40 percent of lymphoid organ spheroids (arrow) in broodstock from hatchery A (H&E : bar = 200 µm)

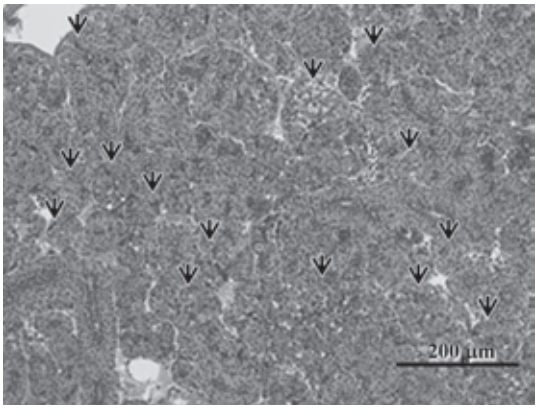

Figure 6. 60 percent of lymphoid organ spheroids (arrow) in broodstock from hatchery C (H&E : bar = 200 µm)

effect and is a major site for antiviral defense by forming lymphoid organ spheroid cells (Anggraeni and Owens, 2000; Burgents *et al*., 2005). The presence of spheroid cells within the lymphoid organ has been observed in many naturally or experimentally inflected penaeid species. The lymphoid organ duct and tubules show similar morphologies and immune reactivity and therefore probably have the same function in the formation of spheroids from the outer layers of the walls as well. The formation of spheroids in the lymphoid organ has been described in many infectious diseases of penaeid shrimp, including Oka organ hypertrophy and metastasis (OHM) syndrome, mid-crop mortality syndrome (MCMs) in Australian penaeid shrimp, monodon slow growth syndrome (MSGS), and many viral diseases of the penaeid species (Lightner *et al*., 1987; Anggraeni and Owens, 2000; Anantasomboon *et al*., 2006; Rusaini and Owens, 2010). Anggraeni and Owens (2000) reported that the spheroid cells in the lymphoid organ of penaeid shrimp have characteristics of degranulated haemocytes, contain phagocytosed material, and show phenoloxidase and peroxidase activity.

Van de braak *et al*. (2002) reported that spheroids are mainly found in shrimp obtained from field situations and are seldom in shrimp that have been raised in recirculation systems. Since the spheroids are associated with many systemic viral and bacterial infections in penaeid shrimp, the very active spheroid cells might indirectly develop from circulating haemocytes and contribute to the degradation of both viral and bacterial material. Thus, the presence of the spheroids is probably related to the history of the infectious antigens of the shrimp. In this aspect, the more shrimp that come into contact with pathogens, the greater the number of spheroids found in the lymphoid organ of shrimp. This coincides with our results, where numerous spheroid cells were found in broodstock that were domesticated in Thailand. These shrimp were cultured in earthen ponds on a farm where effective disease management had not been implemented. Thus, they might have been susceptible to infection by some pathogens that were endemic throughout the culture period. The broodstock that were imported from the USA and cultured in closed recirculation facilities showed a significantly lower number of spheroid cells in the lymphoid organ. Although the impact of spheroids on the mortality rate of shrimp was found in this study, the mechanism explaining why shrimp with numerous spheroids died remains unclear. Future studies focusing on determining the effect of lymphoid organ spheroids on the health of shrimp are recommended. We suspect that environmental impacts and pathogen contamination in cultural systems may affect the shrimp's immune defense against invading pathogens before spheroids cells are formed. The avoidance of environmental conditions that induce stress and effective pathogen exclusion practices by using a closed recycling system in biosecurity facilities could reduce the occurrence of spheroids in the lymphoid organ of shrimp. The practice of only stocking farms with shrimp that are free of diseases of concern and using controlled water sources can reduce the risk of diseases during grow out.

Lightner (2005) reported that *Litopenaeus vannamei* specific-pathogen-free (SPF) stocks bred in captivity under effective biosecure conditions are available in the USA and in some countries in Asia. However, the implementation of effective pathogen exclusion practices at most broodstock production farms in Thailand has not been achieved. Thitamadee *et al*. (2016) reported that white spot syndrome virus, Taura syndrome virus, yellow head virus, and other pathogens continue to impact shrimp farmers in Thailand severely. To reduce losses due to the pathogens of concern, biosecurity concepts must be applied to many existing types of shrimp farming (Walker and Mohan, 2009). Lightner (2003) reported that biosecurity has been defined as the practice of the exclusion of specific pathogens from cultured aquatic stocks at broodstock facilities, hatcheries, and farms, or from entire regions or countries for the purpose of disease prevention. The latter issue of controlled water sources is being accomplished through better farm siting, farm design, and water management through the use of such strategies as zero-water-exchange inland shrimp farming and the use of water treatment devices that remove potential vectors from the source water (Browdy *et al*., 2001; Fegan and Clifford, 2001; Samocha *et al*., 2001).

CONCLUSION

This study demonstrated that shrimp broodstock cultured in earthen ponds in Thailand had a significantly higher mortality rate and number of lymphoid organ spheroids compared with shrimp broodstock that were imported from the USA and cultured in closed biosecurity facilities. To improve the quality of shrimp broodstock in Thailand and to reduce losses due to disease, the development of production systems based on the cultivation of SPF stocks in relatively biosecure environments is necessary.

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