

## Effect of *Vibrio* spp. in White Feces Infected Shrimp in Chanthaburi, Thailand

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### ABSTRACT

The shrimp culture sector in Thailand has been facing the threat of white feces syndrome which severely affected the area throughout the cultivation period in 2010. This study investigated the cause of white feces disease in farm-reared Pacific white shrimp (*Litopenaeus vannamei*) in Chanthaburi province, eastern Thailand from June to December 2010. A total of 12 grow out ponds were studied, of which 6 were affected by white feces syndrome and 6 were unaffected. Estimations of *Vibrio* spp. and total count of bacteria were conducted from the haemolymph and intestine of diseased and healthy shrimp using thiosulfate citrate bile salts sucrose agar (TCBS agar) for *Vibrio* spp., and tryptic soy agar (TSA) for total bacteria. Identification of *Vibrio* spp. was conducted by using API-20E test strips. Histological examination was also conducted on the hepatopancreas and intestine of shrimp. Results showed that total bacteria and *Vibrio* spp. found in haemolymph and intestine were significantly higher in diseased shrimp ( $P < 0.05$ ) than in healthy shrimp. Seven species of *Vibrio* spp. were identified: *V. vulnificus*, *V. fluvialis*, *V. parahaemolyticus*, *V. alginolyticus*, *V. mimicus*, *V. cholerae* (non01) and *Photobacterium damsela* (*V. damsela*). Gregarine parasites were found in only 2% of all sampled shrimp (diseased and healthy). Histopathological examination in severely affected shrimp revealed diffused haemocyte encapsulation and dilated hepatopancreatic tubules accompanied by necrosis.

**Keywords:** White feces syndrome, *Vibrio* spp., Pacific white shrimp

### INTRODUCTION

Since January 2010, shrimp farmers in Thailand have been suffering from white feces syndrome. This disease has been reported from both farm-reared black tiger shrimp (*Penaeus monodon*) and Pacific

white shrimp (*Litopenaeus vannamei*) which caused slightly to moderate losses in some cultivated areas (Chuchird *et al.*, 2008; Prompamorn *et al.*, 2007). This disease is the most widespread occurrence causing major losses in farms especially during the unusually high water temperature in ponds

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(reaching 33-34°C in the afternoon) from February to June 2010. Although the severity of white feces occurrence decreased to some degree after continuous raining from July onwards, most farms still encountered white feces. Strings of white feces floating on the water surface is the first sign of this disease, followed by a significant decrease in feed consumption (Limsuwan, 2003). Shrimp size of 7-12 g or 50-70 days post-stocking are the most commonly affected in intensive *L. vannamei* farms regardless of salinity conditions. Solving the problem through the use of probiotics, immunostimulants or organic acids together with a reduced feeding rate gave some satisfactory results. A delayed response could cause significant losses because infected shrimp would develop loose shell followed by mortality. Most shrimp farmers had to harvest their stock before the shrimp reach marketable size with a higher feed conversion ratio than normally harvested shrimp.

The objective of this study was to investigate the cause of white feces disease outbreak in farm-reared *L. vannamei* located in Chanthaburi province, eastern Thailand in 2010.

## MATERIALS AND METHODS

### *Study site*

This research was conducted in an intensive shrimp farm in Chanthaburi province, eastern Thailand. White feces infestations have been reported in cultured *L. vannamei* from this farm. Six grow-out ponds (1 ha. each) with white feces

infestation, and six grow-out ponds (1 ha. each) without white feces infestation were investigated. Ten day post larvae (PL10) of *L. vannamei* were stocked into each pond at 120 PL/m<sup>2</sup> with adequate aeration. Shrimp were fed commercial pelleted feed (36% protein) 4 times/day (0600, 1000, 1400 and 1800 hours) throughout the experimental period. Salinity during the study period was 25-30 ppt.

### *Parasitic observation*

Fifty shrimp from each pond were collected and examined for parasitic infection. The midgut glands of sampled shrimp were removed and gut contents were spread on a glass slide to prepare a wet-mount. The wet-mount was examined by direct microscopy (by light microscope) with 10x and 20x objectives for parasite infection such as gregarine sporozoites, trophozoites, or gametocysts (identification followed Lom and Dykova, 1992).

### *Bacteria study*

Fifty shrimp from each pond were collected and analysed for bacterial infection. Shrimp intestines were dissected making sure they were not contaminated, then weighed and homogenized with vortexing in sterile saline (1.5% NaCl). The supernatant was diluted ten-fold of which, 0.1 ml was spread on Tryptic Soy Agar (TSA) and Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS Agar) by spread plate method. The haemolymph was extracted by insulin syringe and then spread on TSA and TCBS agar. The plates were incubated at 32°C for 24 hours.

### *Bacterial Identification*

The morphology and the number of colonies on TCBS were recorded. A type of each colony was subcultured on TCBS and TSA (supplemented by 1.5% NaCl) until a single colony was achieved. The colony was identified by API20E (BioMÉRIEUX INDUSTRY).

### *Histopathological study*

Fifty diseased shrimp from each pond were fixed in Davidson's solution. Processing of samples was done following Bell and Lightner (1998) and sagittal sections of 3-4  $\mu$  were fixed on glass slides. The slides were stained using hematoxylin and eosin (H&E) stain recommended by Sheehan and Hrapchak (1980). Histological slides were examined by light microscopy.

## RESULTS AND DISCUSSION

The first sign of white feces disease was the string of white feces floating on the water surface after 60 days of culture period. Diseased shrimp showed a white intestine compared with the normal shrimp (Figs. A and B). Observation of white feces using light-microscope revealed numerous lipid cells as shown in Fig. C. Parasitic observation of shrimp sampled from diseased ponds revealed gregarine infection in one pond having 2% gregarine infection (Fig. D). This result is similar to the report by Chuchird *et al.* (2008) which found gregarine infection only in a few sample of *Penaeus monodon* collected from ponds with white feces during the 2007 outbreak in Thailand. This result indicated that gregarine might not be the major cause of white feces disease, as they are common intestinal parasites of both wild and cultured shrimp (Ball, 1959; Kruse, 1959; Feigenbaum, 1975).

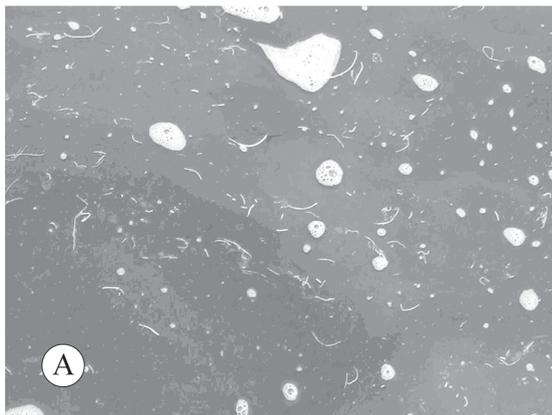


Figure A. Strings of white feces floating on water surface.

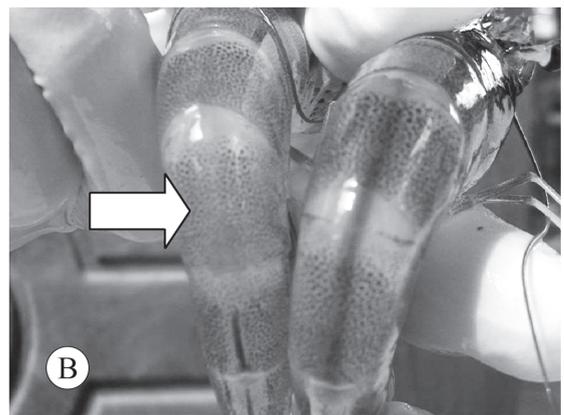


Figure B. White color of intestine on infected shrimp (arrow).

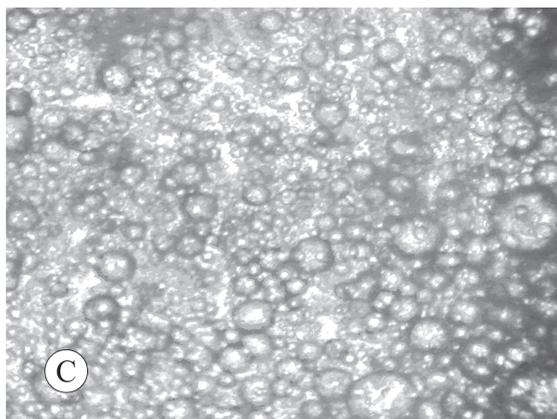


Figure C. Lipid cell within the white intestine of diseased shrimp.

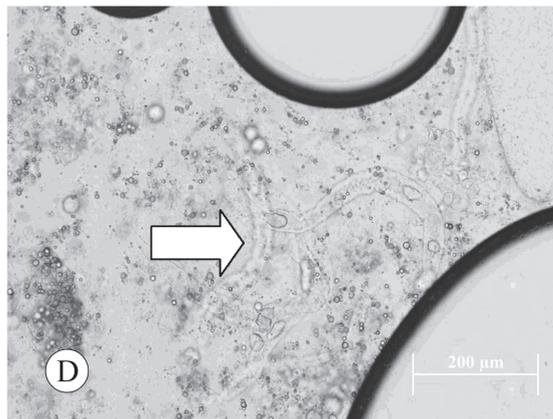


Figure D. Gregarine parasites in intestine of infected shrimp (arrow).

The average total bacteria from haemolymph and intestine of shrimp from white feces ponds were  $1.774 \pm 0.810 \times 10^5$  cfu/ml and  $6.136 \pm 4.291 \times 10^7$  cfu/g, respectively. This result was significantly ( $P < 0.05$ ) higher than total bacteria from haemolymph and intestine of shrimp from the normal (uninfected) ponds which were  $2.701 \pm 0.282 \times 10^4$  cfu/ml and  $1.803 \pm 0.117 \times 10^7$  cfu/g, respectively. Meanwhile

the average number of *Vibrio* sp. from haemolymph and intestine of shrimp from diseased ponds were  $8.505 \pm 6.187 \times 10^4$  cfu/ml and  $3.508 \pm 0.728 \times 10^7$  cfu/g, respectively. This result was significantly ( $P < 0.05$ ) higher than the average number of *Vibrio* sp. from haemolymph and intestine of shrimp from normal or uninfected ponds which were  $6.338 \pm 1.444 \times 10^3$  cfu/ml,  $3.487 \pm 0.731 \times 10^6$  cfu/g, respectively (Table 1).

Table 1. Total bacteria in the haemolymph and intestine of normal (uninfected) ponds and white feces ponds.

Pond	Total bacteria			
	Haemolymph (cfu/ml)		Intestine (cfu/g)	
	Normal	White feces	Normal	White feces
1	$2.534 \times 10^4$	$1.582 \times 10^5$	$1.823 \times 10^7$	$5.107 \times 10^7$
2	$2.323 \times 10^4$	$2.750 \times 10^5$	$1.962 \times 10^7$	$14.208 \times 10^7$
3	$2.523 \times 10^4$	$1.412 \times 10^5$	$1.790 \times 10^7$	$3.758 \times 10^7$
4	$2.892 \times 10^4$	$2.775 \times 10^5$	$1.888 \times 10^7$	$7.368 \times 10^7$
5	$2.872 \times 10^4$	$0.785 \times 10^5$	$1.727 \times 10^7$	$3.997 \times 10^7$
6	$3.062 \times 10^4$	$1.342 \times 10^5$	$1.631 \times 10^7$	$2.378 \times 10^7$
Mean±SD	$2.701 \pm 0.282 \times 10^4$ <sup>a</sup>	$1.774 \pm 0.810 \times 10^5$ <sup>b</sup>	$1.803 \pm 0.117 \times 10^7$ <sup>a</sup>	$6.136 \pm 4.291 \times 10^7$ <sup>b</sup>

a, b values within the same column sharing different superscripts are significantly different at  $P < 0.05$

Gomez-Gil *et al.* (1998) reported that the mean numbers of *Vibrio* spp. found in the intestine of healthy shrimp were  $2.10 \times 10^6$  cfu/g (median  $5.32 \times 10^5$ , max.  $1.03 \times 10^7$ , min.  $1.01 \times 10^4$ ,  $n=25$ ) which is similar to the present study. Lightner (1997, 1998) reported that the presence of a high amount of bacteria in the haemolymph indicates septicaemia which is commonly found in diseased animals.

Seven species of *Vibrio* were isolated from infected shrimp in this study. These

species were *V. vulnificus*, *V. fluvialis*, *V. parahaemolyticus*, *V. alginolyticus*, *Photobacterium damsela* (*V. damsela*), *V. mimicus* and *V. cholerae* (non01). The species of *Vibrio* were found in haemolymph and intestine of the shrimp (Table 3) though the *Vibrio* count varied according to species (Table 2). This is similar to the report by Inthusai (2006) which found three species of *Vibrio* including *V. fluvialis*, *V. alginolyticus* and *V. parahaemolyticus* from black tiger shrimp infected with white feces in Thailand in 2007.

Table 2. The number of *Vibrio* spp. in the haemolymph and intestine of the control and white feces groups.

Pond	<i>Vibrio</i> spp.			
	Haemolymph (cfu/ml)		Intestine (cfu/g)	
	Control	White feces	Control	White feces
1	$4.446 \times 10^3$	$7.978 \times 10^4$	$3.448 \times 10^6$	$3.853 \times 10^7$
2	$6.183 \times 10^3$	$18.955 \times 10^4$	$4.441 \times 10^6$	$3.956 \times 10^7$
3	$8.811 \times 10^3$	$3.078 \times 10^4$	$4.242 \times 10^6$	$2.049 \times 10^7$
4	$5.915 \times 10^3$	$12.504 \times 10^4$	$2.918 \times 10^6$	$3.923 \times 10^7$
5	$5.817 \times 10^3$	$3.822 \times 10^4$	$3.302 \times 10^6$	$3.638 \times 10^7$
6	$6.855 \times 10^3$	$4.692 \times 10^4$	$2.573 \times 10^6$	$3.628 \times 10^7$
Mean±SD	$6.338 \pm 1.444 \times 10^3$ <sup>a</sup>	$8.505 \pm 6.187 \times 10^4$ <sup>b</sup>	$3.487 \pm 0.731 \times 10^6$ <sup>a</sup>	$3.508 \pm 0.728 \times 10^7$ <sup>b</sup>

<sup>a, b</sup> values within the same column sharing different superscripts are significantly different at  $P < 0.05$

Table 3. The percent of disease shrimp infected with each species of *Vibrio* spp. in this study

Species of <i>Vibrio</i>	Percent of disease shrimp infected with each species of <i>Vibrio</i> spp.	
	Haemolymph	Intestine
<i>V. vulnificus</i>	80	80
<i>V. fluvialis</i>	44	44
<i>V. parahaemolyticus</i>	26	26
<i>V. alginolyticus</i>	20	20
<i>Photobacterium damsela</i> ( <i>V. damsela</i> )	18	18
<i>V. mimicus</i>	8	8
<i>V. cholerae</i> (non01)	6	6

*Vibrio* species are part of the natural microflora of wild and cultured shrimps (Sinderman, 1990) and they become opportunistic pathogens when natural defence mechanisms are suppressed (Brock and Lightner, 1990). They are usually associated with multiple etiological agents. However, some *Vibrio* species, or strains of certain species, have been identified as primary pathogens (Owens and Hall-Mendelin, 1989; Owens *et al.*, 1992). Pathogenic strains of *V. harveyi*, *V. vulnificus* and *V. parahaemolyticus* have caused massive epidemics in Thailand (Nash *et al.*, 1992) and the Philippines (Lavilla-Pitogo *et al.*, 1990). *V. anguillarum*, *V. campbelli*, *V. nereis*, *V. cholerae* (non 01) and *V. splendidus* have also been reported in association with disease outbreaks in shrimps (Chen 1992; Lavilla-Pitogo, 1990; Sahul-Hameed *et al.*, 1996). According to Jayasree *et al.* (2006) occurrence of five types of diseases: tail necrosis, shell disease, red disease, loose shell syndrome (LSS) and white gut disease

(WGD) are caused by *Vibrio* spp. in *Penaeus monodon* from culture ponds in coastal Andhra Pradesh. Among these, LSS, WGD, and red disease caused mass mortalities in shrimp culture ponds. Six species of *Vibrio* i.e. *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *V. vulnificus* and *V. splendidus* are associated with the diseased shrimp.

Histological sections of shrimp with white feces disease typically show bacterial infection including haemocytes forming capsule (nodule formation) (Fig. E), atrophy in hepatopancreas, (Fig. F) melanization (Fig. G) and necrosis in gill and the detachment of epithelial cells from the basal lamina of mid gut (Fig. H). This result is similar to the report by Sindermann (1971) which found a mass of haemocytes and multi-layer arrangement that prevented bacteria from spreading to other parts which confirmed the process of nodule formation (Smith and Ratcliffe, 1976) and melanization (Lightner and Redman, 1977; Johnson, 1980)

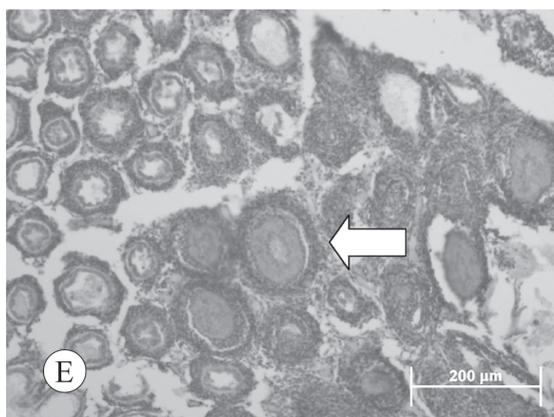


Figure E. Histopathology by H&E staining of hepatopancreas of infected shrimp showed a sign of nodule formation and hemocytic infiltration (arrow)

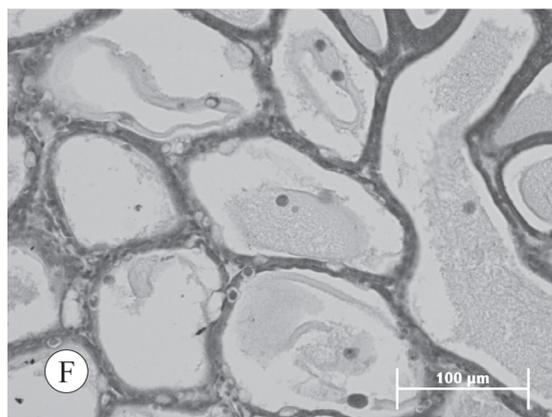


Figure F. Histopathology by H&E staining of hepatopancreas of infected shrimp showing a sign of cell atrophy.

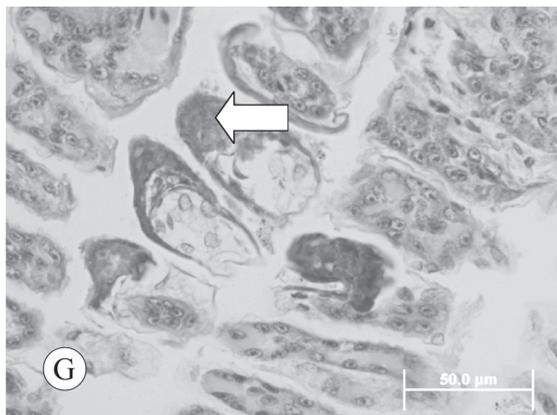


Figure G. Histopathology by H&E staining of gills of infected shrimp showed melanization. (arrow)

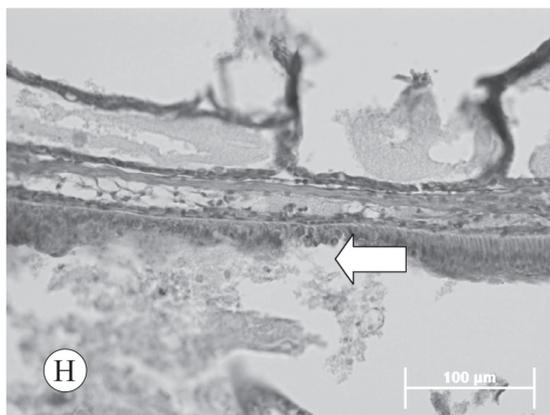


Figure H. Histopathology by H&E staining of infected shrimp showed a sign of the detachment of epithelial cells from the basal lamina of mid gut. (arrow)

## CONCLUSION

This study indicated that most of the shrimp that had white feces had large amounts of *Vibrio* bacteria in their haemolymph and intestine, consisting of *Vibrio vulnificus*, *V. fluvialis*, *V. parahaemolyticus*, *V. alginolyticus*, *V. damsela*, *V. mimicus* and *V. cholera* (non01) at the proportions of 80, 44, 26, 20, 28, 8 and 6 %, respectively. Only 2% of gregarine protozoa were detected from all sampled shrimp (diseased and healthy).

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