



## The identification of *Vibrio* Spp. Bacteria from *Litopenaeus Vannamei* infected by White Feces Syndrome

Maskar Jayadi\*, Arief Prajitno, Maftuch

Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Jl. Veteran, Malang  
65145, Jawa Timur Province, Indonesia

**Abstract :** The vannamei shrimp ponds in Indonesia have infected by White Feces Syndrome (WFS) intensively in Sumatra, Java, Sulawesi, Bali, Lombok and Sumbawa. This research is aimed to know the density and type of *Vibrio* spp. from hemolymph, hepatopancreas and intestines of vannamei shrimp which is infected by WFS. The bacteria identification test was performed in the Microbiology Laboratory of Balai Besar Perikanan Budidaya Air Payau Jepara (a brackish water aquaculture office in Jepara, Central Java). The research method in this study is an explorative descriptive research by using a simple random sampling. Conventional method is used for the identification of the bacteria. The indication of the shrimp which is infected by WFS is already exposed to the *Vibrio* spp. genus bacteria, it consists of *V. Parahaemolyticus*; *V. Vulnificus*; *V. cholerae* and *V. anguillarum*. The total average of *Vibrio* spp. in hemolymph is  $2,9 \times 10^4$  CFU/ml, while in hepatopancreas is  $4,4 \times 10^7$  CFU/g and in intestines  $3,9 \times 10^7$  CFU/g.

**Keywords:** WFS, *Vibrio*, Vannamei Shrimp.

### Introduction

The case of White Feces Syndrome (WFS) on the vannamei shrimp cultivation is characterized by a decreased appetite levels, abnormal growth, and white feces<sup>1</sup>. The level of vannamei shrimp life which is severely infected by WFS is 20-30%. The daily weight gain (ADG) of the infected vannamei shrimp is  $\leq 0.1$  g while the ADG of normal vannamei shrimp is 0.2 g. The value of the feed conversion ratio (FCR) on the infected vannamei shrimp is 1.7-2.5 while on the normal vannamei shrimp is 1,5<sup>2</sup>. The symptoms of WFS occurred after the cultivation above 50 days since the first day of stocking.

The vannamei shrimp which is infected by WFS is due to *Vibrio* spp. type *V. vulnificus*, *V. fluvialis*, *V. parahaemolyticus*, *V. alginolyticus*, *V. mimicus*, *V. cholerae* and *V. damsela*. WFS is caused by the *V. Cholerae* bacteria, it is based on the comparison between the genetic and phenotype characteristics of the *V. Cholerae* bacteria which is isolated from the infected vannamei shrimp and the other isolated *V. cholerae* bacteria<sup>3</sup>.

This research is aimed to find the density and type of the *Vibrio* bacteria from hemolymph, hepatopancreas and intestines of the infected vannamei shrimp. The research is located in the Microbiology Laboratory of Balai Besar Perikanan Budidaya Air Payau Jepara (a brackish water aquaculture office in Jepara Regency, Jawa Tengah Province, Indonesia).

## Methods

The research method in this study is an explorative descriptive research, and the sampling was done by using a simple random sampling. Conventional method is used for the identification of the bacteria<sup>4</sup>. The main parameter observed in this study is the density and type of the *Vibrio* bacteria in hemolymph, hepatopancreas and intestines.

## Sample

The vannamei shrimp samples are obtained from the WFS investigation results at the vannamei shrimp cultivation area in Kragan, Rembang Regency, Jawa Tengah Province. Vannamei shrimp cultivation technologies are developed at the location of the semi-intensive and intensive sampling. The average cultivation area is 2000 up to 2500 m<sup>2</sup>. The stocking density is between 60-100 tail/m<sup>2</sup> and the feed protein is about 36%. The water quality at the moment of sampling is 29.8°C up to 31.8°C and it has a dissolved oxygen of 6.2-7 mg/L; water pH of 7.5-8.4; and 23-30 ppt for the salinity.

## *Vibrio* spp. Total Identification

There are 30 samples of vannamei shrimp. The hemolymph of the shrimp is taken by using *syringe*, and then disseminated on TCBS to be incubated at 32°C for 24 hours. The surgery of the vannamei shrimp is performed aseptically in order to take the hepatopancreas and intestines. Each hepatopancreas and intestines is weighed as much as 1 gram, then it mashed with sterile mortar and added with 9 ml of tri-salt solution. Next, 1 ml samples are taken by using a 1 ml micropipette and poured on the reaction tubes. The samples in reaction tubes are noted for 10<sup>-1</sup>, they are homogenized with the help of *vortex*. This step is done until the 6<sup>th</sup> reaction tubes and recorded as 10<sup>-6</sup>. The samples of 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> are taken by using 0.1 ml micropipette and poured on the petri dish that already contains TCBS, then it gets flattened by using a spatula. The petri dish which has the bacteria within is coated with plastic wrap and stored in the incubator for 24 hours, so that the bacteria can live and grow maximally.

## Result and Discussion

Based on the observation results of the infected ponds, white and long feces which has similar appearance with threads is floating on the surface of the water (Figure 1A) and the water color of the ponds is thick-green (Figure 1B). The results also showed about the clinical symptoms of the infected vannamei shrimp such as decreased appetite, white hepatopancreas (Figure 1C), and the average cultivation time (age) which is >50-75 days. The white feces which floats on the surface of the water is the result of the shrimp hepatopancreas peeling which is issued by the shrimp through the intestines in the form of *Aggregated Transformed Microvilli* (ATM)<sup>2</sup>.

The intestines of the shrimp looks empty and white due to the lack of food (Figure 1D). The peeling of the hepatopancreas makes the intestines full, it caused the appetite of the shrimp decreased and the process of nutrients absorption interrupted<sup>5</sup>.

The color of the water which is used for the cultivation is thick-green due to the high organic material which comes from the unconsumed feed. Organic materials such as detritus and mineral can be used as a food source to heterotrophic organisms like plankton to stimulate the autotrophic production<sup>6</sup>. There are only about 22% nitrogen of the feed which are used to form the body of the shrimp and the rest are disposed into the environment in the form of organic nitrogen and ammonium (NH<sub>4</sub><sup>+</sup>). Those materials are good for the growth of plankton<sup>7</sup>.

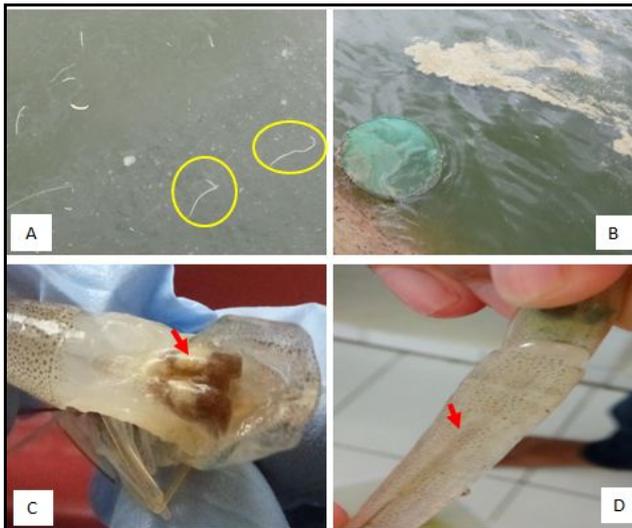


Figure 1. Clinical signs of WFS infection. (A) Long-white feces which looks like threads floating on the surface of the water, (B) the water color which is thick-green, (C) pale-white hepatopancreas, (D) empty intestines.

Table 1. *Vibrio* spp total on the hemolymph, hepatopancreas and intestines of the infected vannamei shrimp.

| Replication | Fish Pond            | <i>Vibrio</i> spp. total            |                                     |                                     |
|-------------|----------------------|-------------------------------------|-------------------------------------|-------------------------------------|
|             |                      | Hemolymph (CFU/ml)                  | Hepatopancreas (CFU/g)              | Intestines (CFU/g)                  |
| I           | 1                    | $2,5 \times 10^3$                   | $8,3 \times 10^7$                   | $3,3 \times 10^5$                   |
|             | 2                    | $1,3 \times 10^3$                   | $4,24 \times 10^7$                  | $3,36 \times 10^7$                  |
|             | 3                    | $8,0 \times 10^4$                   | $5,8 \times 10^7$                   | $9,2 \times 10^7$                   |
| II          | 1                    | $1,5 \times 10^3$                   | $3,3 \times 10^7$                   | $5,1 \times 10^7$                   |
|             | 2                    | $1,1 \times 10^3$                   | $2,5 \times 10^7$                   | $2,3 \times 10^7$                   |
|             | 3                    | $8,59 \times 10^4$                  | $2,3 \times 10^7$                   | $4,0 \times 10^7$                   |
|             | <b>Average Value</b> | <b><math>2,9 \times 10^4</math></b> | <b><math>4,4 \times 10^7</math></b> | <b><math>3,9 \times 10^7</math></b> |

The average total of the *Vibrio* spp. in hemolymph is  $2.9 \times 10^4$  CFU/ml, whereas in hepatopancreas is  $4.4 \times 10^7$  CFU/ml and intestines is  $3.9 \times 10^7$  (Table 1). The *Vibrio* spp. total on the infected vannamei shrimp on average is  $8.505 \times 10^4$  CFU/g for hemolymph and  $3.508 \times 10^7$  CFU/g for intestines. It is possible that the number and types of the *Vibrio* spp. bacteria can be found in *Litopenaeus vannamei* shrimp which is normal and healthy<sup>8</sup>. Various population of *Vibrio* spp are found in the intestines, stomach and hepatopancreas, ranging from  $2 \times 10^2$ –  $3 \times 10^3$  CFU/ml.  $2 \times 10^6$  CFU/g<sup>8</sup>. It is also reported that the high amount of bacteria in hemolymph shows a common septicemia which is found on a sick animal<sup>9</sup>.

**Table 2. Bio chemical test on hemolymph isolates, intestines and hepatopancreas of the infected vannamei shrimp.**

| Biochemical Test        | HM.1 | HM.2 | SS.1 | SS.2 | HP.1 | HP.2 |
|-------------------------|------|------|------|------|------|------|
| TCBS                    | +    | +    | +    | +    | +    | +    |
| Shape                   | btg  | btg  | btg  | btg  | btg  | btg  |
| Cat Gram                | —    | —    | —    | —    | —    | —    |
| Swarming                | —    | —    | —    | —    | —    | —    |
| Growth with 0% NaCl     | —    | —    | —    | —    | +    | —    |
| Arginine decarboxilase  | —    | +    | —    | —    | —    | —    |
| Lysine decarboxilase    | +    | —    | +    | +    | +    | +    |
| Ornithine decarboxilase | +    | —    | —    | +    | +    | +    |
| Nitrat reduced          | +    | +    | +    | +    | +    | +    |
| Oxidase                 | +    | +    | +    | +    | +    | +    |
| Gas from Glucose        | —    | —    | —    | —    | —    | —    |
| Indol                   | +    | +    | +    | +    | +    | +    |
| ONPG                    | +    | +    | +    | —    | +    | —    |
| MR                      | +    | +    | +    | +    | +    | +    |
| VP                      | —    | —    | —    | —    | —    | —    |
| Simmon citrat           | +    | +    | +    | —    | +    | —    |
| Gelatinase              | +    | +    | +    | +    | +    | +    |
| Resisten to :           |      |      |      |      |      |      |
| 0/129 10 µg             | +    | +    | +    | +    | +    | +    |
| 0/129 150 µg            | —    | —    | —    | —    | —    | —    |
| ampicillin 10 µg        | —    | —    | —    | —    | —    | —    |
| Starch Hydrolysis       | —    | +    | —    | +    | +    | +    |
| Urea Hydrolysis         | —    | —    | —    | —    | +    | —    |
| Acid from :             |      |      |      |      |      |      |
| L-arabinose             | —    | —    | —    | +    | —    | —    |
| Arbutin                 | —    | —    | —    | —    | +    | —    |
| Salicin                 | +    | —    | +    | —    | +    | +    |
| Sucrose                 | +    | +    | +    | —    | +    | —    |
| Xylose                  | —    | —    | +    | —    | —    | —    |
| Growth on :             |      |      |      |      |      |      |
| Ethanol                 | —    | —    | —    | —    | —    | —    |
| Propanol                | —    | —    | —    | —    | —    | —    |

The identification result of the *Vibrio* spp. bacteria from 6 isolates (Table 2) is HM1 and HM2 isolates, it is close with the strains of *V. vulnificus* and *V. anguillarum*, the isolates from SS1 and SS2 is identified as *V. vulnificus*, *V. parahaemolyticus*, while Hp.1 and Hp.2. has the similarities with *V. parahaemolyticus* and *V. cholera*. It is reported that *V. parahaemolyticus* once attacked the vannamei shrimp in China and Thailand<sup>10</sup>. *Vibrio parahaemolyticus* is a species of shrimp pathogenic bacteria which is injected with *v. parahaemolyticus* with the concentration of 10<sup>4</sup> CFU/ml to kill half of the shrimp population in 7 days<sup>11</sup>. It is also reported that *V. parahaemolyticus* has infected the vannamei shrimp with the clinical symptoms such as black stripes on the side of the *chephalothorax*<sup>12</sup>. *Vibrio parahaemolyticus* is the cause of the intestinal epithelium damage of the penaeidae shrimp (Martin *et al.*, 2004). Cao *et al.*, (2015) concluded that the White Feces Syndrome is caused by the *v. cholerae* bacteria, it is based on the comparison between the genetic and phenotype characteristics of the *v. cholerae* bacteria which is isolated from the infected vannamei shrimp and the other isolated *v. cholerae* bacteria. There are three (3) types of *Vibrio* spp which are found from the bacteriological analysis on the hemolymph of the tiger prawn which is infected by white gut disease, such as *V. harveyi*, *V. alginolyticus*, and *V. Anguillarum*. The average total density of the *Vibrio* is 0,2 x 10<sup>5</sup> CFU/ml (Jayasree *et al.*, 2006).

To conclude, these studies provide such indication that the infected vannamei shrimp is due to the *Vibrio* spp. bacteria which consists of *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae* and *V. anguillarum*. The average total of *Vibrio* spp. on hemolymph is  $2.9 \times 10^4$  CFU/ml, whereas in hepatopancreas is  $4.4 \times 10^7$  CFU/g and in intestines is  $3.9 \times 10^7$  CFU/g.

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