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IDENTIFICATION OF VIBRIO SP. BACTERIAL POPULATIONS IN INTENSIVE VANNAME SHRIMP (LITOPENAEUS VANNAMEI) FARMS AS A PREVENTIVE MEASURE FOR VIBRIOSIS INFECTION

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ABSTRACT
The majority of vannamei shrimp aquaculture activities are carried out intensively with very high stocking densities, leading to a decline in water quality and triggering a series of disease outbreaks. Vibriosis disease outbreaks due to poor aquaculture environmental conditions are characterized by accumulated waste loads. Identification of the causative agents of vibriosis is crucial to ensure disease diagnosis and appropriate therapy as a preventive strategy for vibriosis disease and to maintain production stability. Based on the results of Vibrio sp. bacterial colony counts from 14 observed vannamei shrimp ponds, 6 shrimp ponds still show counts above the maximum threshold of ≥1.0x103 cfu/ml according to the quality standards set by the Ministry of Marine Affairs and Fisheries. However, if the density of Vibrio sp. bacteria reaches 104, it will cause massive shrimp mortality in aquaculture ponds. This is undoubtedly influenced by the water quality of the observed ponds, which remains stable or non-fluctuating and optimal for vannamei shrimp aquaculture activities.

KEY WORDS
Intensive cultivation, *L. vanname*, *Vibrio sp.*

A frequent impact of intensive aquaculture systems is a decline in water quality in ponds, which results in a number of disease attacks and large economic losses. The majority of vaname shrimp farming activities in Indonesia are carried out with very high stocking densities (Ariadi *et al*. 2020). High stocking density can reduce water quality, this is due to the accumulation of various wastes in the shrimp farming ecosystem (Cheong *et al*. 2020). This agrees with Wafi *et al.* (2021), that the increase in waste is parallel to the increase in cultured aquaculture organisms. The decrease in water quality that can be caused, due to the accumulation of waste, includes a decrease in the amount of *dissolved oxygen* and an increase in the colony of pathogenic bacteria as disease agents (Wafi *et al*. 2020). Pathogenic bacteria have the potential to cause disease in the host through damage to organ constituent cells through genetic changes (Suharni *et al*. 2008). Another factor that can trigger disease in aquaculture systems is environmental pollution by human waste, and it is possible that the source of pond water as a maintenance medium already contains pathogenic bacteria, which can contaminate the shrimp being reared.

One of the pathogenic bacteria that often infect shrimp is *Vibrio* sp. This bacterium has been identified as the cause of early mortality syndrome (EMS) and acute hepatopancreatic necrosis disease (AHPND) in vaname shrimp (Culot *et al*. 2021). The emergence of these bacteria is generally due to the poor quality of the culture environment and the accumulation of waste. Waste accumulation can arise from untreated seawater, because the sea is the final disposal site for various wastes from land activities, including domestic waste. This is very dangerous because domestic waste has the potential to carry various types of pathogenic bacteria.

Identification of the causative agent of disease is an important step to ascertain the type of disease and appropriate treatment, so as to prepare disease prevention measures and stabilize production figures. Proper treatment can also reduce the negative impact caused by the use of chemicals, such as avoiding the emergence of drug-resistant bacterial strains (Nurjanah *et al*. 2014). The category of isolates with resistance from the sensitivity test of the appropriate drug concentration is addressed by not being able to show the optimal inhibition zone based on specifications.

The purpose of this study was to identify the presence and determine the population of *Vibrio* sp. in vaname shrimp ponds in intensive farming systems, so as to effectively overcome *Vibriosis* disease and minimize the spread of the disease. The research method was conducted exploratively by conducting sampling in a number of intensive vaname shrimp ponds.

METHODS OF RESEARCH

This study focuses on the identification and calculation of the number of *Vibrio* sp. bacteria colonies, as well as measuring water quality and survival rates of vanname shrimp in the Intensive Aquaculture Area.

Calculation of *Vibrio* sp. bacterial colonies was carried out one by one from the visible colonies and grouped *Vibrio* sp. bacterial colonies based on the color reaction of the culture media caused. Calculation of bacteria is done using the *total plate counter* (TPC) method. If the number of bacterial colonies is more than 250, it is declared as Too Many To Count (TBUD). Calculation of total colonies according to Ambat *et al*. (2022), which is calculated by a *colony counter* or manually then multiplied by the amount of dilution done and uses CFU / ml units. Here is the formula for total vibrio.

$$N = \frac{\Sigma C}{[(1x\ n1) + (0{,}1\ x\ n2)]\ x\ (d)}$$

Where: N: Number of colonies, in colonies/ml or colonies/gram; ΣC: Number of colonies in all counted cup; n1: Number of cups on the first dilution that calculated; n2: Number of cups on second dilution that calculated; d: The first dilution that calculated.

Survival rate (SR) is the percentage of test fish at the end of rearing compared to the number of test fish at the beginning of rearing. Supporting fish survival when influenced by feeding with sufficient quality and quantity and good environmental conditions. *Survival rate* (SR) is used to determine the mortality rate of test fish during the study, which is based on the following formula (Effendi, 2002):

$$SR = \left(\frac{N_t}{N_0}\right) x100\%$$

Where: SR: Survival rate of fish (%); Nt: Number of test fish at the end research (tail); N0: Number of test fish at baseline research (tail).

One of the most important aspects of aquaculture is to pay attention to the living medium of aquatic organisms. This is because the quality of water or living media greatly determines the life of cultivated aquatic organisms, all important aspects of the waters include physical, chemical and biological parameters that also need to be known and understood. The water quality parameters reviewed in this study include DO, temperature, pH and salinity, which are the most important parameters for aquatic organisms:

- DO was measured using a DO meter;
- Temperature is measured using a thermometer;
- pH was measured using a pH meter;
- Salinity was measured using a Salinometer.

RESULTS AND DISCUSSION

Based on the results of bacterial isolates using specific planting media, it shows that there are two forms of Vibrio sp. bacterial colonies that can be observed, namely green-blue colonies and white-yellowish colonies, as shown in Figure 1. Green Vibrio sp. bacterial colonies indicate the inability to ferment sucrose, while yellow colonies indicate the ability to ferment sucrose and reduce the pH level in TCBSA media. (Ihsan and Retnaningrum 2017).

Then, Rahmanto and colleagues (2014) have identified the type of Vibrio based on the color of the colonies that appear. Green Vibrio colonies that grow on TCBSA media have a similarity of 86% with V. fischeri and V. mimicus, while yellow colonies have a similarity between 90-95% with V. alginolyticus and V. harveyii.

Data on the number of bacterial colonies counted from samples taken from several vaname shrimp farming ponds can be found in Table 1.

Table 1 – Colony count results of *Vibrio* sp.

Pond	Vibrio (CFU/ml)		Total Vibrio (CFU/ml) ($<3.0x10)^3$
	Yellow ($<1.0x10)^3$	Green ($<1.0x10)^2$	
K1	420	10	430
K2	20	0	20
K3	250	10	260
K4	560	0	560
K5	470	0	470
K6	1020	110	1130
K7	20	0	20
K8	1160	0	1160
K9	240	20	260
K10	1240	0	1240
K11	1550	150	1700
K12	200	0	200
K13	1240	0	1240
K14	1630	0	1630
K15	530	10	530
K16	440	0	440

Data on the total number of *Vibrio* bacteria from analyzed water samples of vaname shrimp ponds in intensive culture systems are listed in Table 1. All ponds found *Vibrio* colonies with varying amounts. This phenomenon indicates that Vibrio bacteria have a wide tolerance to pond water conditions, as illustrated in Table 2.

The total number of samples that have been calculated for total *Vibrio* colonies is 16 samples, showing the abundance of Vibrio colonies in some vaname shrimp farming ponds has reached the maximum threshold (≥1.0x103 cfu/ml), such as in ponds K6, K8, K10, K11, K13, and K14. According to Ambat *et al.* (2022), *Vibrio* abundance that has the potential to harm the environment and cultured shrimp occurs when the abundance reaches 10^3 CFU/ml which is the maximum threshold or quality standard set (No.75/PERMEN-KP/2016).

The pathogenic ability of bacteria is highly dependent on the population size of the bacteria; the higher the density, the greater the pathogenic potential. (Zafran and Roza 1998). According to Taslihan *et al*. (2004), if the number of Vibrio sp. bacteria in water exceeds 10^4 CFU/ml and the number of general bacteria exceeds 10^6 CFU/ml, it can cause mass mortality of farmed shrimp in ponds.

Based on the observation of the survival of vaname shrimp in a number of ponds observed, the survival of vaname shrimp has an average value of 81%. Vaname shrimp survival is classified as good, this is because the abundance of vibro bacteria in a number of ponds does not exceed the maximum threshold value of vibrio bacteria abundance. In addition, there were no fluctuations in pH, dissolved oxygen, temperature, and salinity that could stress the shrimp and trigger disease development.

According to Yusuf (2014), bacteria of the genus Vibrio can develop rapidly if the concentration of organic matter in the culture medium is relatively high. If the *Vibrio* spp population exceeds the population of non-pathogenic bacteria, it can result in a decrease in the survival rate of cultured shrimp. According to Aras and Faruq (2024), through the introduction of probiotics that can reduce the level of pollutants in the aquaculture environment, this can also support the growth of natural bacteria that can inhibit the growth of harmful bacteria such as *Vibrio* sp.

Good seed quality is another important factor that can affect shrimp survival. Currently, many importers have brought in top quality vaname shrimp broodstock that can compete with local broodstock. According to Dara *et al*. (2023), vaname shrimp broodstock imported from Hawaii, USA, has been able to produce vaname shrimp seeds that are free of specific pathogens (SPF) and resistant to specific pathogens (SPR), making them more resistant to disease.

In this study, pond water quality was measured through physical and chemical parameters, such as temperature, pH, salinity, and dissolved oxygen levels. Pond water quality has a significant impact on shrimp growth. These water quality measurements also aim to evaluate the suitability of pond water for shrimp farming. Table 2 below illustrates the range of pond water quality values observed.

Table 2 – Range of water quality values for vaname shrimp ponds

Water Quality Parameters	Range
Temperature	27 - 28 C^o
pH	8,1 - 8,2
Salinity	32 - 33 ppt
DO	6.24 - 6.14 mg/l

The temperature in most ponds remains stable and optimal for vannamei shrimp farming activities, in accordance with the findings of Haliman and Adijaya (2005), which state that the ideal temperature for optimal growth of vannamei shrimp ranges from 26°C to 31°C. If the temperature is below the standard, it will result in a decrease in metabolic rate, while high temperatures even exceeding the limit can cause oxygen to become saturated and limit oxygen intake for shrimp (Maknun and Sumsanto, 2023).

pH in some ponds is still in the optimal range, in accordance with the research of Rakhmatun and Mujiman (2003), which recommends a good pH range for shrimp ponds between 6.8 to 8.7. According to Wyban and Sweeny (1991), the ideal pH for intensive shrimp farming is between 7.8 to 8.9, with an optimum value of about 8.0 ppt. The high content of organic matter in water can cause a decrease in pH, due to increased levels of CO_2 due to microbial activity in decomposing organic matter (Sari 2007).

Based on the data collected, salinity in all three ponds is still favorable for culture, in line with the findings of Briggs et al. (2004), which highlighted one of the advantages of vanamei shrimp, namely its very wide tolerance to various parameters of the aquatic environment. Wyban and Sweeney (1991) noted that vannamei shrimp have a wide salinity tolerance range, ranging from 15 to 35 ppt.

Dissolved oxygen levels in the three ponds are still within the maximum limit. This is supported by the views of Soetomo (2002) which states that the optimal range of dissolved oxygen for shrimp culture ranges from 4 to 8 ppm. Zavala and Espino (2000) explain that low oxygen levels in the aquatic environment is caused by the high content of organic matter and the rate of decomposition. As a result, when organic matter accumulates in the area, microorganisms will work aerobically in its decomposition causing a decrease in dissolved oxygen concentration.

CONCLUSION

Sampling results in several ponds in the Bulukumba Vannamei Shrimp Farming Area, South Sulawesi, identified two types of Vibrio sp. The colonies were divided into two colors, namely green-blue and white-yellowish, which were found in several sample ponds with abundances reaching 10^3 CFU/ml, which is the maximum limit. However, the number did not reach 10^4 CFU/ml, so it did not cause mass mortality of shrimp in aquaculture ponds.

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