Review

Vibriosis Management in Indonesian Marine Fish Farming

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Abstract. Vibriosis is a bacterial disease that has been reported in Indonesian marine fish culture since the 1990s. The disease was reported mostly in grouper and shrimp (monodon and vanname) farming, although the infections in snapper (*Lates calcarifer)* and abalone (*Haliotis squamata)* aquaculture were also occurred. Causative agents of vibriosis in Indonesia marine fish is involving 14 species of vibrio namely *Vibrio harveyi, V. anguillarum, V. alginoluticus, V. parahaemolyticus, V. fluvialis, V. furnisii, V. methcnikovii, V. vulnificus, V. ordalii*, *V. cincinnatiensis*, *V. carchariae, V. azureus*, *V. mimicus* and *V. damsela*. Control of vibriosis is conducted with water quality management, applications of vaccines, antibiotics, probiotics and immunostimulants. Most vaccines developed and commercially available in Indonesia are in the form of inactive-whole cell types. The vaccine product is effective enough to protect fish from vibriosis. Probiotics have been widely studied since the 2000s and have been shown to increase fish and shrimp growth and resistances against vibriosis. Immunostimulants began to be developed since 2010 based on the extracts of terrestrial plants, seaweeds and chitosan. It is therefore concluded that the continuous existence of pathogenic strains in aquaculture farm requires further development of the control methods, including periodic updating of the vaccines, probiotics and immunostimulant formulas for more potent efficacies.

1 Introduction

Vibriosis is a bacterial disease caused by a member of the genus Vibrio. The bacteria, *Vibrio* spp. has a wide range of hosts including wild and cultured fish [1]. The causative agent of vibriosis can survive and attack both freshwater and marine fish [2]. The symptoms of vibriosis are usually hemorrhagic septicemia, the presence of ulcers and abscesses on the skin. *Vibrio* spp. The causative agent vibriosis is gram-negative bacteria with straight or curved cells with key metabolic characters including fermentative and motile with polar flagella [3].

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Vibriosis of Indonesian marine fish farming has been reported since the 1990s. It is the main bacterial disease of cultured marine fish and shrimps in Indonesia [4]. The disease was most predominantly reported in grouper [5, 6]. A summary of the species found as causative agents of vibriosis in Indonesian marine culture is shown in table 1. In the earlier report, *V. carchariae* was known to correlated with vibriosis in shrimp [7] and *V. anguillarum, V. alginolyticus, V. parahaemolyticus,* and *V. marinus* were associated with vibriosis in grouper [8]. Since then, between 2000-2010, more species were found to contribute to vibriosis in fish and shrimps, including *V. alginolyticus, V. parahaemolyticus, V. anguillarum, V. vulnificus, V. metchnikovii, V. Furnisii, V. Fluvialis, V. ordalii*, and *V. cincinnatiensis* [5, 9,10]. Thereafter, Vibriosis in Indonesian fish farming were associated with the infection of *V. harveyii, V. alginolyticus, V. vulnificus, V. anguilarum, V. parahaemolyticus, V. azureus, V. damsela* (*Photobacterium damsela* subspecies piscicida), *V. mimicus* and *V. fluvialis* [11, 12, 13, 6, 14, 15]. It is interesting that there are species that continue to survive in the mariculture fish, species that are no longer found at the present mariculture, and new species that have only been discovered in the last decade. It is challenging to overcome the disease caused by new isolated species in the last decade such as the *V. azureus*, *V. mimicus* and *V. damsela* (*Photobacterium damsela* subspecies *piscicida*).

2 Susceptible host and predisposition factors

Outbreak of vibriosis have been reported in seven species of marine fish farming in Indonesia (Table 1). The susceptible fish are snapper [15] and grouper [5]. Outbreak of vibriosis was also reported in tiger shrimp *Penaeus monodon* [7, 16], abalone [17], dan pacific white shrimp *Lithopenaeus vannamei* [14].

Tabel 1. Infection of *Vibrio* spp in Indonesian marine fish farming.

No	Susceptible host	Pathogens	Source
1	Tiger shrimp (*Penaeus monodon*)	*V. carchariae*	[7]
		V. harveyii, V. alginolyticus, V. vulnificus, V. anguilarum, V. parahaemolyticus	[11]
2	Humpback grouper (*Cromileptes altivelis*)	*V. anguillarum, V. alginolyticus, V. parahaemolyticus, V. marinus*	[8]
		V. alginolyticus, V. parahaemolyticus, V. anguillarum, V. vulnificus, V. metchnikovii, V. Furnisii, dan V. Fluvialis	[5]
3	Tiger grouper (*Ephinepelus fuscoguttatus*)	*V.anguillarum, V. ordalii, V. fluvialis dan V. alginolyticus*	[9]
4	Abalone (*Haliotis squamata*)	*V. cincinnatiensis*	[10]
5	Hybrid grouper cantang (*E. lanceolatus* × *E. fuscoguttatus*)	*V. alginolyticus, V harveyi, V. azureus, V. damsela* (*Photobacterium* damsela subspecies *piscicida*)	[6]
6	Pacific white shrimp (*Lithopenaeus vannamei*)	*Vibrio vulnificus, V. mimicus, V.damsella, V. parahaemolytics, V. fluvialis*	[13]
		Vibrio sp.	[14]
7	Seabass (*Lates calcarifer*)	*V. alginolyticus*	[12]
		V. vulnificus	[15]

Vibrio is an opportunistic pathogen that always present in the water. The disease outbreak only occurred in the disease supporting environmental condition. [2] explained that environmental factors have a great influence on vibriosis outbreaks. Some influential water quality parameters are water temperature, dissolved oxygen, ammonia, organic matter and heavy metal content. Some other factors outside the water quality factor are density, fish handling in fish transportation, adaptation and acclimatization. [2] also suggested that vibrio attacks can be malignant or non-malignant depending on the type of strain. If the fish is attacked by a less virulent strain, the onset of symptoms of the disease and the inflammatory response will be slow. But if the attack is carried out by a vicious strain the symptoms and death will take place quickly. In acute attacks, mortality happens with no symptoms of disease, such as those experienced by chronically attacked fish. The degree of malignancy of a bacterium is encoded in genetic determinants located in the plasmid [18]. Vibriosis can be transmitted vertically through friction with fish experiencing vibriosis or through water [2].

3 Pathology and Diagnosis

Vibrio infects and causes diseases in shrimps under such favorable environmental conditions. [13] stated that clinical symptoms of shrimp that are attacked by vibriosis are characterized by the present reddish carapace, melanosis of the skin, necrosis of the tail, and redness of the swimming legs and walking legs.

Vibriosis of groupers occurred in eggs, larvae, juvenile and broodstock stages. Some Vibrio species can cause serious epizootics, but some species are opportunistic that cause disease when fish suffered in a stress condition, such as injured due to handling, parasites and other factors [2]. Vibriosis in tiger grouper is characterized by decreased appetite, weak and tilted swimming behavior, pale kidneys and darker body color [9]. Infection of *V. fluvialis* in humpback grouper was associated with pathological symptoms such as atrophy, infiltration of leukocytes and plasma cells in gill tissue, bacterial colonization of the intestinal epithelial tissue, degeneration of vacuoles in the liver, bacterial cell colonization and leukocyte and plasma cell infiltration in the skin tissue of the abdominal fin bases [19] (Figure 1).

Vibrio alginolyticus infection in snapper (*L. calcarifer*) causes epithelial tissue rupture, hyperplasia and necrosis of the gills, necrosis, and vacuolization of the kidney tissue [12]. The existence of these pathogenic bacteria are usually confirmed by bacterial isolation using thiosulfate citrate bile salts sucrose (TCBS) agar. The medium contains bile which is only tolerated by most members of the genus Vibrio. Most Vibrio species will grow on that medium within 24 hours of incubation to form green or yellow colonies. *Vibrio parahaemolyticus, V. alginolyticus, V. furnisii, V. vulnificus* and *V. anguillarum* are likely to form greenish yellow colonies, while *V. methcnikovii* and *V. fluvialis* formed yellow colonies [5]. For routine maintenance, Vibrio bacteria grow well on marine agar, zobell, 50% seawater complex agar medium [20], TSA trisalt [19] and Nutrient Agar [1].

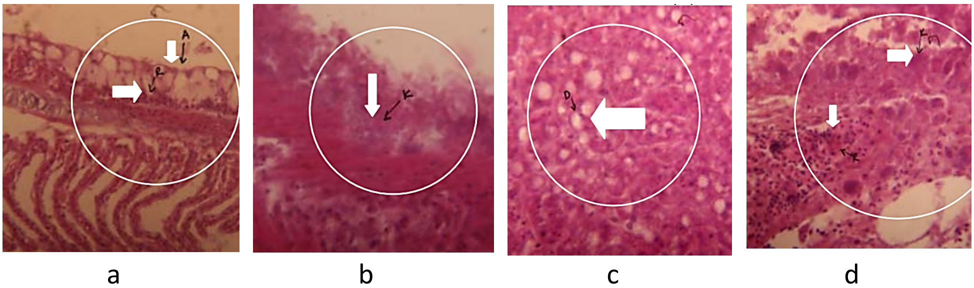

Fig 1. Pathology of vibriosis in humpback grouper (*Cromileptes altivelis*): a. atrophy and infiltration of leukocytes and plasma cells in the gills tissue, b. bacterial colonization of intestinal epithelial tissue, degeneration of vacuoles in the liver, d. bacterial cell colonization, infiltration of leukocyte and plasma cells in the skin tissue of the abdominal fin base [19].

Table 2. Pathogenicity of *Vibrio* spp. in Indonesia marine fish farming industry.

Species (size)	**Pathogen**	**Infection Methods**	**Temperature (ºC)**	**LD50 (cfu/ind or cfu/ml)**	**Source**
Cromileptes altivelis (7 cm)	*V. fluvialis*	Injection	29	$(1.1 \pm 0.5) \times 10^7$	[19]
Cromileptes altivelis (5 cm)	*V. alginolyticus, V. parahaemolyticus, V. anguillarum, V. vulnificus, V. metchnikovii, V. Furnisii, V. Fluvialis*	Injection	nd	$< 10^6$	[5]
Penaeus monodon (larvae)	*V. harveyii, V. alginolyticus, V. vulnificus, V. anguilarum, V. parahaemolyticus*	Imersion	nd	$<10^7$	[11]
Lates aclcarifer	*V. vulnificus*	Injection	nd	10^7	[15]
E. lanceolatus × *E. fuscoguttatus* (8 cm)	*V. alginolyticus* *V. harveyi* *V. damsela* (*P. damsela* subspecies *piscicida*)	Injection	29	7×10^6 6×10^4 2×10^4	[21]
Abalone (Haliotis squamata)	*V. cincinnatiensis*	Injection	nd	$>10^6$	[10]

nd: no data

4 Treatment or medication

Water quality management is the key to the success of fish farming. Disinfection of shrimp cultivation media using Cobalt-60 gamma ray irradiation was carried out by [22]. The results showed that the static irradiation method with a dose of 20 kg was able to sterilize the media from *Vibrio harveyi* up to 100% after 1 hour of irradiation process. While in the circulation method, irradiation for 2 hours was able to sterilize the media from *V. harveyi* up to 98.75%.

Based on the Decree of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia Number 52/KEPMEN-KP/2014 regarding Fish Medication Classification, there are three classes of antibiotics that are permitted to be used during the cultivation process, namely the quinolone (enrofloxacin) group, the tetracycline and erythromycin group. These three types of drugs are still permitted for use with supervised use procedures.

Treatment of vibriosis in snapper was done by [15]. The study was conducted by infecting snapper seeds with *V. vulnificus* at the DL50 dose (10^8 cells/ml) to develop vibriosis in fish. After disease symptoms appear, the drug treatment was done by immersion and injection. Results of the study indicated that immersion with 50 ppm dose of Oxytetracycline (OTC) for 5 days was able to treat snapper from vibriosis.

5 Vaccination

Research on vaccine development in Indonesian mariculture industry is presented in Tabel 3. Vaccine for vibriosis was established in Indonesia since 2005 in humpback grouper [23]. An outer membrane vaccine was developed by [24] in tiger grouper *Ephinepelus fuscoguttatus* and a formalin killed cells was developed for vibriosis in abalone [17]. Thereafter, research on vaccination for vibriosis is developing in Indonesia.

5.1 Vaccination of humpback grouper

Vaccination for vibriosis in humpback grouper was conducted using a polyvalent vaccine that consisted of inactivated whole cells of four *Vibrio* spp. strains [23]. Efficacy of the vaccine was confirmed by oral, injection and immersion application in grouper with relative percent survival (RPS) of 100. The vaccine product has been patented in 2010 with patent ID P0026256, and is commercially produced by PT Caprifarmindo with the product name "CapriVac Vibrio". Trial with immersion of the Vibrio polyvalent vaccine in humpback grouper juvenile was subsequently carried out by [25].

5.2 Vaccination of tiger grouper

Vaccination in tiger grouper was carried out by [24]. In this research, some outer membrane proteins were injected into groupers of sorts. The immunogenicity of each membrane protein was evaluated based on antibody titers. The results showed that the 73.4 kDa size protein was the most immunogenic protein and thus became a potential vaccine candidate. The efficacy of the immunogenic protein in protecting groupers from vibriosis need to be evaluated.

Vaccination of tiger grouper seeds was also carried out by [26] in floating net cages. Polivalent Vibrio vaccine consisted of antigen from *Vibrio harveyi, Vibrio alginolyticus,* and *Vibrio parahaemolyticus* with a final density of 10^{10} cells / mL were evaluated. The vaccine was given to the tiger grouper larvae in the hatchery by soaking for 1 hour with a concentration of 1 mL vaccine / L of sea water and followed by a booster at 1 week later. Revaccination is given after the fish are stocked in floating net culture for 60 and 120 day post immunization. The results showed that the tiger grouper responded positively to the polyvalent vaccine. The value of vaccinated fish antibody titers was higher than the control groups. Upon challenge infection, the survival rate in the vaccination group was 77.11% but the control groups were 44.56%, resulting RPS value of 58%. These results indicate that the polyvalent bacterial vaccine was immunogenic in groupers and able to provide protection against vibriosis.

5.3 Vaccination in abalone

Vaccination in abalone to cope with vibriosis was carried out by [17]. The whole inactivated vaccine of *V. harveyi* cells was immersed in juvenile abalone with a concentration of 10^7 cells/ml of sea water for 3 hours. Booster was given on the 15th day after the initial vaccination. On the 60th day, challenge test was conducted on both abalone groups with *V. harveyi*. The results showed that the survival rate of vaccinated and unvaccinated abalone was 90% and 52%, respectively. These results indicated that the vaccine was protective in abalone against *V. harveyi* infection.

Table 3. Vibriosis vaccine developed in Indonesia.

No	Type of vaccine	Fish species	RPS*	Source
1	Vaksin polivalen vibrio (formalin and/or heat killed cells)	Humpback grouper (*Cromileptes altivelis*)	100	[23]
2	Vaksin protein outer membran *V. Alginolyticus*	Tiger grouper *(Ephinepelus fuscoguttatus*)	nd	[24]
3	Vaksin *Vibrio harveyi* (formalin killed cells)	Abalone (*Haliotis squamata*)	79	[17]
4	Vaksin polivalen Vibrio (formalin killed cells)	Tiger grouper *(Ephinepelus fuscoguttatus*)	58	[27]

*RPS: relative percent of survival; nd: no data

6 Probiotics

Probiotic bacteria have been widely used as biocontrol agents in tiger shrimp hatchery. Biocontrol of *V. harveyi* infection in shrimps was done by using non-pathogenic strain of *V. metchnikovii* [20]. In these trials, researchers used a bacterial adherence test to shrimp. The existence of *V. methcnikovii* can reduces adherence ability of pathogenic *V. harveyi*.

[28] examined the effects of different doses of skt-b vibrio probiotic bacteria in addition to survival and growth rates of tiger shrimp (*Penaeus monodon*) larvae. Research was conducted with 5 treatments: probiotic application with the doses of 10^3, 10^4, 10^5, 10^6 CFU/ml) and control (non-probiotic). The results showed that probiotics given to tiger shrimp with a dose of 10^4 CFU / ml produced the best survival rate of 94.67%. However, there was no difference in shrimp growth performance between treatments. Thereafter, [29] he evaluated the effect of molasses addition on the survival and growth of tiger prawns given the probiotic Vibrio Skt-b. The study was conducted in vitro and in vivo. In vitro test results

showed that Vibrio SKT-b can grow on molasses media with a population density of 4.1 × 10^7 CFU/mL. In vivo test results showed that MB3 treatment (addition of 3 ppm molasses and bacteria) resulted in the shrimp survival and weight growth of 93.3% and 35.94%, while the control shrimp obtained survival and growth of 83.3% and 30.38% respectively.

[30] examine probiotics, prebiotics and symbiotic to enhance the immune responses of vannamei shrimp (*Litopenaeus vannamei*) to *Vibrio harveyi* infection. In the study, the Probiotic SKT-b (10^6 CFU/ml) and oligosaccharides from sweet potatoes as a prebiotic were used results from the study revealed that higher immune responses were observed in symbiotic group (1% probiotic+2%prebiotic) with total hemocytes of 3.4 – 9.3 x 10^6 cells/ml, phagocytic activity of 16.5 – 77,55%, and phenol oxidase activity of 0.13 – 0.59. the study concluded that the addition of symbiotic in the feed resulted in better immune response compared to the addition of probiotics and prebiotics.

[31] screened lactic acid bacteria (BAL) from the digestion tract of Tiger Grouper (*Epinephelus fuscoguttatus*) based on tolerance to pH and bile salts, pathogenicity and their ability to suppress the growth of *Vibrio alginolyticus* in co-culture tests. The results showed that there were 3 selected 3 selected isolates of fish probiotic candidates (KSBU 12C, KSBU 5Da, KSBU 9) with important characters, such as resistance to acid and bile salts, non-pathogenic to tiger grouper and able to suppress the growth of *V. alginolyticus* at probiotic concentrations of 107 cfu/ml.

Screening of marine bacteria as a source of probiotics for controlling vibriosis in marine aquaculture has been carried out by [32]. In the study, isolates of marine bacteria *Pseudoalteromonas* sp. S2V2 with the ability to kill various strains of pathogenic Vibrio that attack fish and sea shrimp. S2V2 strain is a potential candidate for probiotics for use in Indonesia because it is originated from Indonesian waters.

[33] examined the effect of feeding supplementation of probiotics (*Bacillus* sp.) and prebiotics (*Ipomoea batatas* extract) on growth performance, survival rate, immune response and disease resistance humpback grouper (*Cromileptes altivelis*) (weight 3 g ± 0.48). The results obtained showed that the addition of probiotic and prebiotic in feed significantly increased the daily growth rate (SGR), feed conversion ratio (FCR), total erythrocytes, total leukocytes, total hemoglobin, phagocytosis activity, respiratory burst grouper activity (*Cromileptes altivelis*).

7 Immunostimulants

Screening of anti-Vibrio compounds from seaweed *Halimeda renchii* and *Eucheuma cottonii* was carried out by [34]. The results of the research showed that *Halimeda cenchii* contained anti-Vibrio compounds with the highest inhibitory activity against *V. alginolyticus* (16.7 mm of inhibition zone), while *Eucheuma cottonii* has the highest inhibitory power against V. *parahaemolyticus* (24.1 mm of inhibition zone). MIC value of the active extracts from the seaweeds was 0.05%.

The study on the use of chitosan as a vibriosis control in vaname shrimp was conducted by [35]. The results showed that the addition of chitosan to feed for 14 days of maintenance and for 24 hours after the salinity stress test had a significantly effect on the total number of vaname shrimp hemocytes. The highest total hemocytes obtained in the treatment of 250 mg chitosan/kg of feed. This results suggested that chitosan is promising source of shrimp immunostimulant.

[36] screened sponges and mangroves from Kei Island Indonesia to know the potential to cure *Vibrio parahaemolyticus, V. harveyi* and *V. alginolyticus*. The results showed that the

bark extract of a coastal plant, *Diospyros maritima* has a very strong anti-Vibrio activity with MIC value of <0.098 to 3.125 μg /mL. Further research on the structure of active compounds in these plants is interesting.

8 Future Prospects

The study of vibriosis in fish and shrimp in Indonesian aquaculture has been carried out for almost three decades, and has resulted in disease control technologies that has benefited many fish and shrimp farmers. However, the research on vibriosis need to needs to be continued in a more comprehensive and advanced studies considering that this disease still existed in farms and continues to grow until now.

Management of vibriosis through water quality control, vaccination, application of probiotics and immunostimulants needs to be maintained. Vibriosis vaccine that has been available today is mostly in the form of inactivated whole-cell vaccine that the application in fish still requires booster injections. The challenge going forward is to develop a vaccine that is effective enough without requirement of booster so that disease control becomes more efficient. New vaccines also need to be considered and developed to obtained better efficacies. The discovery of a variety of potential probiotic candidates based on various in vitro and in vivo tests that have been carried out shows the great potential of establishment of Indonesian original probiotics to be used by fish and shrimp farming communities. The use of native probiotic strains in Indonesia will be more environmentally friendly compared to the probiotic products originating from abroad. Finally, the study of immunostimulant products for fish farming has a pretty good opportunity to be developed. This is because the immunostimulatory power of immunostimulant in inducing the natural immunity of fish and shrimp is very good for the management of fish and shrimp health against various types of diseases that continue to develop.

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