The Effectiveness of Vaccines in Gurame (Osphronemus goramy) and Challenged Aeromonas hydrophila

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Abstract. Gurame (Osphronemus goramy) is one of the most commodities cultivated by the people in Indonesia, but there is a production constraint due to gurame that attacked Motile Aeromonas Septicemia (MAS) disease caused by Aeromonas hydrophila. One of the most effective precautions that can be used is vaccination. Based on the above description, it is necessary to research the effectiveness of Aeromonas hydrophila vaccine in gurame. This research aims to determine the effect of Aeromonas hydrophila vaccine and the amount of bacterial density in an effective vaccine for preventing MAS on gurame (Osphronemus goramy). This study used an experimental method with a complete randomized design consisting of 5 treatments and 4 replications. Parameters observed were survival rate (SR) and antibody titre of carp. The results of this research indicate that giving Aeromonas hydrophila vaccine to gurame can give an effect for increasing the antibody titer and survival rate (SR). The results showed that the amount of 104 CFU/ ml of bacterial density in the Aeromonas hydrophila vaccine was the effective vaccine density used as the MAS prevention and showed a high survival rate of 95%. The conclusion of this research is by giving Aeromonas vaccine can prevent MAS and the density of Aeromonas hydrophila vaccine 104 CFU/ml.

1. Introduction
Motile Aeromonas Septicemia (MAS) or red spot disease caused by Aeromonas hydrophila. These bacteria are a cause of acute bacterial disease, can infect all ages of fish and various types of fresh fish, and can cause death up to 100% [1]. The use of antibiotics or other chemicals for the treatment of fish has begun to be abandoned. This is caused by negative impacts such as residues in the body of fish, environmental pollution, and bacterial resistance to certain types of antibiotics [2].

Vaccination is an environmentally friendly technology because it comes from living microorganisms, does not pollute the environment and is right on target [3]. Its use does not cause negative impacts both on fish, the environment, and consumers and can be done on various sizes of fish from seed to parent [3]. Giving vaccines to aquaculture is one of the preventive measures in tackling fish diseases especially those caused by Aeromonas hydrophila [4].
The effectiveness of vaccine administration is not only seen from the value of antibody titer, it can also be seen from the percentage of Survival Rate (SR). A vaccine can be declared effective if it reaches SR more than equal to 73.50 [5].

This research was conducted with intramuscular injection method with the aim to determine the concentration of effective vaccine administration in the prevention of Motile Aeromonas Septicemia (MAS) in carp (Osphronemus goramy) after the challenge test.

2. Materials and Methods

2.1 Materials

The materials used in this study were a culture of *Aeromonas hydrophila*, Tryptone Soya Agar (TSA) medium, Tryptone Soya Broth (TSB) medium, Physiological NaCl solution, Mac Conkey Media, gouramy fish with 10-15 cm length, ion, aquades, hot water, alcohol chlorine, and fish feed.

2.2 Methods

This study used an experimental method with a completely randomized design (CRD) consisting of treatments (A, B, C, D, E) and 4 replications. The treatments in this study are as follows: A, Negative control (Fish injected with physiological NaCl and challenged by *Aeromonas hydrophila*). B, Positive control (Fish injected with vaccines and not challenged with *Aeromonas hydrophila* bacteria). C = Vaccine *A. hydrophila* 10⁴CFU / mL. D = Vaccine *A. hydrophila* 10⁵ CFU / mL. E = Vaccine *A. hydrophila* 10⁶CFU / mL.

2.2.1 Making Vaccines

The virulence of *Aeromonas hydrophila* which has been enhanced is grown on the NA medium and incubated at 27°C for 18-24 hours. Remove one bacterial colony to TSB and re-incubate for 15-18 hours. The bacterial suspension is harvested by centrifuge 3000 rpm for 10 minutes the supernatant is removed, the sediment is washed with physiological NaCl and centrifuged 3 times. Preparation of the Heat killed vaccine is done by heating the bacteria to a temperature of 100°C for 10 minutes. Furthermore, a viability test is performed on the isolate to ensure the antigen is inactive.

2.2.2 Vaccination

Vaccination is carried out on carp measuring 7-9 cm. The vaccine is injected intramuscularly at a dose of 0.1 ml/head with bacterial density according to treatment. Each treatment was injected on 10 fish with 4 replications. Booster (re-vaccination) with the same dose carried out in the next two weeks. The fish were then reared for 24 days and observed every day and taken blood samples every week to test the antibody titer. Blood samples are taken just before vaccination, after vaccination, after the booster, and after challenge testing. Blood sampling with a sterile syringe (syringe) through the caudal artery.

2.2.3 Antibody Titer Test

Antibody titer test can be done by taking the blood serum of the test fish. Blood sampling was carried out on caudal veins using a syringe and then placed on a microtube and stored in a tilted condition at 40°C for 24 hours. If it has settled then the supernatant is taken while the data is discarded, but if there is still a mixture of the denatan with the supernatant, it can be separated by centrifuge. Then do the observation with dilution plates.

2.2.4 Survival Rate (SR)

Survival Rate (SR) or percentage of fish survival is a comparison of the number of fish that are still alive until the end of rearing with the initial number of rearing fish.
2.3 Analysis data
The data obtained were analyzed for the Analysis of Variance Analysis (ANOVA) test with the F test at 95%, if it has a significant effect, to see the difference between treatments is further tested by the Duncan test.

3. Results and Discussion
Based on the results of the antibody titer I test (before vaccination) found an average range of antibody titer of 5.00-7.00. This means that the test fish have antibodies against *A. hydrophila* because of the possible emergence of an immune response due to exposure to pathogens in the environment of the test fish. As stated by [6] that fish health is influenced by several main components namely the environment, host, and pathogen. The presence of pathogens in the waters and supported by low immunity in the host can trigger a pathogen attack on the host.

### Table 1. Antibody titers before the challenge test

| Treatment | Observation of antibody titers (*mean ± SD*) |
|-----------|---------------------------------------------|
|           | D7  | D15 | D29  |
| A (K-)    | 5.00\(^b\)±2.00 | 6.00\(^a\)± 2.39 | 22.00\(^a\)± 12.00 |
| B (K+)    | 6.00\(^a\)±2.31 | 12.00\(^a\)± 4.62 | 24.00\(^a\)± 9.24 |
| C (10^4) cfu/ml | 7.00\(^a\)±2.00 | 40.00\(^c\)± 16.00 | 96.00\(^b\)± 36.95 |
| D (10^5) cfu/ml | 5.00\(^a\)±2.00 | 22.00\(^b\)± 12.00 | 80.00\(^b\)± 55.43 |
| E (10^6) cfu/ml | 6.00\(^a\)± 2.31 | 56.00\(^c\)± 16.00 | 72.00\(^b\)± 44.22 |

Note: Different superscript notations in one column indicate a significant difference p <0.05.
- D7: observation on the 7th day;
- D15: observation on the 15th day;
- D29: observation on the 29th day.

### Table 2. Survival Rate (SR)

| Treatment | Replication | Mean ± SD |
|-----------|-------------|-----------|
|           | 1  | 2   | 3  | 4  |
| A (K-)    | 80 | 60  | 60 | 60 | 65.00\(^a\)± 10.00 |
| B (K+)    | 100 | 80  | 80 | 80 | 85.00\(^b\)± 10.00 |
| C (10^4) cfu/ml | 100 | 100 | 100 | 100 | 95.00\(^b\)± 10.00 |
| D (10^5) cfu/ml | 80  | 60  | 100 | 100 | 95.00\(^b\)± 10.00 |
| E (10^6) cfu/ml | 80  | 100 | 100 | 100 | 85.00\(^b\)± 19.15 |

Note: different superscript letters notation in one column shows a significant difference (p<0.05).

One week after booster the value of the test fish antibody titer increased, but a significant increase occurred in treatment C, namely vaccination with a density of 104 CFU / ml. The booster is done on the 14th day after the first vaccination. As stated by [7] that one week after the first vaccination, antibodies will be formed and are still in an exponential phase, whereas on the 10-15th day fish antibodies are in a stationary phase, ie the number of antibodies produced is equal to the amount of damage occur. So that it can be re-vaccinated (booster) which can increase the value of antibody titer.
This is confirmed by [8] that booster vaccination is a booster vaccination that is carried out within a few days after the first vaccination, 1-2 weeks or as needed in the same or different ways to increase vaccine efficacy. The booster can also increase the production of antibody titers because the test fish have immunity memory and the process of recognition of antigens a second time [3]. As stated by [9] High antibodies produced by fish due to stimulation of antigens into the body of the fish, then phagocytes by macrophages. Macrophages will send signals to the lymphocytes and then respond by proliferating and differentiating to form specific antibodies in accordance with the incoming antigen.

Based on the results of existing antibody titers, it can be seen that treatment C can provide good protection in the test fish. In the first vaccination, all treatments experienced an increase, but a significant increase occurred in treatments C and E. Besides, antibody production at the first vaccination not as high as after the booster [10]. So a booster needs to be done so that the antibodies that are formed become higher and can fight the infecting pathogens. Antibody titers after booster in treatments C, D and E were not significantly different, so it can be said that booster vaccination in each treatment was equally able to increase antibody titers. The high antibodies produced by vaccination can increase the immune response to infectious diseases. This happens because of the increased protection of the fish's body. This can be proven by looking at the survival rate of the test fish and clinical symptoms after the challenge test that is not too severe.

The survival rate of fish in each treatment is higher than the control that is 95% for treatments C and D and 85% for treatment E. Based on these results indicate that the vaccine used is able to provide good protection for carp. As noted by [5] that vaccination is said to be good if the survival rate (SR) of fish is on average more than 73.50%.

4. Conclusion

*Aeromonas hydrophila* vaccine can increase antibody titer (6.00 to 40.00 and after booster to 96.00) and the survival rate of carp (*Osphronemus goramy*) which reaches 95% compared to controls which only reach 65% in the density of the *Aeromonas hydrophila* 10^4 CFU / ml.

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