The variances of hematology of gurami (*Osphronemus gouramy*) which is vaccinated and challenged by *Aeromonas hydrophila*

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Abstract

Freshwater fish that became the leading commodity of gourami. Fish cultivation is often experienced constraints of infection from motile aeromonas septicaemia (MAS), also known as red spotting disease caused by bacteria *Aeromonas hydrophila*. A vaccine is an effective preventive measure because in this way immune can be obtained only once or twice vaccine administration until the fish is harvested. This study aims to determine the hematological picture of gourame (*Osphronemus gouramy*) that vaccinated and challenged bacteria *Aeromonas hydrophila*. This research is an experimental research using completely randomized design which consists of 5 treatments and 4 replications. The treatment of this study was the difference of vaccine dose ie A (control +) fish injected vaccine without any challenge test, B (control -) fish injected NaCl Physiological and tested challenge, C vaccine dose 10⁴ CFU/mL, D vaccine dose 10⁵ CFU/mL, and E vaccine dose of 10⁶ CFU / mL. The results of this study showed vaccinations and tests of bacteria *Aeromonas hydrophila* in gouramy can give effect to total erythrocytes, hemoglobin, leukocyte total, and leukocyte differential. The 10⁶ cell/mm³ (E) vaccine dose provides the best (responsive immune response) effectiveness compared to the other two doses.

1. Introduction

Prevention and control of diseases in fish must be done as early as possible so that no major economic losses occur. Until now, the method that has been widely used to treat diseases in cultured fish is treated with chemicals or antibiotics. This method is very risky because it can cause resistance to bacteria, requires quite expensive costs, and can pollute the environment [1]. Besides, it can cause residues on the body of fish and endanger human health.

Vaccination is an effort to prevent fish diseases by stimulating the immunity of fish vaccinated against certain diseases in fish. Disease control by vaccination is very efficient because in this way immunity can be obtained only by once or twice giving the vaccine until the fish are harvested. Besides vaccination does not cause side effects for fish as does the use of antibiotics [2].
One way to find out the health condition of fish is by looking at the picture of the blood. Physiological deviations of fish will cause blood components to also experience changes. Changes in the picture of blood and blood chemistry both qualitatively and quantitatively can determine the condition of his health. Therefore, it is necessary to make further observations on fish hematology to see differences in the picture of fish blood that is immunized and not vaccinated.

This study aims to determine the changes in hematology of gouramy (*Osphronemus gouramy*) fish that are vaccinated and challenged by *Aeromonas hydrophila*. The benefit of this research is to find out the influence of vaccine use on gouramy (*Osphronemus gouramy*) through blood changes so that it can be used as a prevention of MAS in vaccine cultivation.

2. Materials and Methods

2.1. Materials

The materials used in this study were 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, Turk solution, Hayem solution, 0.1 N HCl, and distilled water.

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* 10⁴ CFU/mL. D = Vaccine *A. hydrophila* 10⁵ CFU/mL. E = Vaccine *A. hydrophila* 10⁶ CFU/mL. 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.

2.2.1. Making vaccines

The virulence of *Aeromonas hydrophila* which has been enhanced is grown on 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 [3]. Preparation of the Heat killed vaccine is done by heating the bacteria to a temperature of 100°C for 10 minutes [4].

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. 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. Blood sampling

In taking blood, fish are placed with the head on the left, before the syringe has been rinsed with EDTA 0.01 M as an anticoagulant. Blood collection is carried out on the vein caudalis. The blood that has been taken, is inserted into a tube to immediately observe the blood profile. The parameters observed included measurement of total erythrocytes, leukocytes, differential leukocytes, and hemoglobin levels.

2.3 Data analysis

The data obtained were analyzed for the analysis of variance (ANOVA) test with the F test at 95%, if it has significant effect, to see the difference between treatments is further tested by the Duncan’s test.
3. Results and discussion
The highest erythrocytes, when tested about treatment E, were not significantly different from treatments C and D. It can be said that vaccination can stimulate the body’s defense response and maintain the number of erythrocytes during the bacterial infection *Aeromonas hydrophila*. Low erythrocytes in treatment A (negative control) indicate the presence of pathogens that enter the body of fish will lyse erythrocytes and reduce total erythrocytes. *Aeromonas hydrophila* has hemolysin which can lyse erythrocytes. Hemolisyn is an endotoxin that causes hemorrhage and frees hemoglobin and as a major virulence factor of *Aeromonas hydrophila* [5].

Table 1. Total erythrocytes.

| Treatment | Average Total Erythrocytes (x10⁶ cells / mm³) ± SD |
|-----------|---------------------------------------------------|
| H+7 (Normal) | H+11 (Vaccination) | H+25 (Booster) | H+33 (Challenge test) |
| A (K-) | 1.28 ± 0.06 | 2.22 ± 0.04 | 2.45 ± 0.03 | 1.44 ± 0.01 |
| B (K+) | 1.16 ± 0.03 | 1.42 ± 0.03 | 2.01 ± 0.01 | 2.82 ± 0.03 |
| C (10⁴) | 1.17 ± 0.04 | 1.21 ± 0.03 | 2.46 ± 0.01 | 3.15 ± 0.00 |
| D (10⁵) | 1.23 ± 0.03 | 2.10 ± 0.07 | 2.48 ± 0.02 | 2.75 ± 0.04 |
| E (10⁶) | 1.28 ± 0.02 | 2.01 ± 0.01 | 2.82 ± 0.01 | 3.31 ± 0.02 |

Note: Notations shown with different superscript letters in the same column indicate significantly different (p <0.05). A (K-): fish not vaccinated and challenged, B (K+): fish vaccinated and not challenged, C: bacteria 10⁴ cells/mL, D: bacteria 10⁵ cells/mL, and E: bacteria 10⁶ cells/mL.

Table 2. Haemoglobin levels (g%).

| Treatment | Haemoglobin levels ± SD |
|-----------|-------------------------|
| H+7 (Normal) | H+11 (Vaccination) | H+25 (Booster) | H+33 (Challenge test) |
| A (K-) | 7.05 ± 0.05 | 7.25 ± 0.34 | 7.20 ± 0.28 | 6.38 ± 0.45 |
| B (K+) | 6.95 ± 0.86 | 7.28 ± 0.15 | 7.20 ± 0.32 | 7.45 ± 0.31 |
| C (10⁴) | 6.75 ± 0.61 | 7.20 ± 0.58 | 7.40 ± 0.28 | 7.55 ± 0.52 |
| D (10⁵) | 6.70 ± 0.80 | 7.25 ± 0.47 | 7.35 ± 0.35 | 7.45 ± 0.31 |
| E (10⁶) | 6.70 ± 0.23 | 7.08 ± 0.15 | 7.55 ± 0.11 | 7.63 ± 0.20 |

Note: Notations shown with different superscript letters in the same column indicate significantly different (p <0.05). A (K-): fish not vaccinated and challenged, B (K+): fish vaccinated and not challenged, C: bacteria 10⁴ cells/mL, D: bacteria 10⁵ cells/mL, and E: bacteria 10⁶ cells/mL.

Table 3. Total leukocytes.

| Treatment | Average Total Leukocyte Count (x 10⁴ cells / mm³) ± SD |
|-----------|-------------------------------------------------------|
| H+7 | H+11 | H+25 | H+33 |
| A (K-) |  |  |  |  |
| B (K+) |  |  |  |  |
| C (10⁴) |  |  |  |  |
| D (10⁵) |  |  |  |  |
| E (10⁶) |  |  |  |  |
A (K-): fish not vaccinated and challenged, B (K+): fish vaccinated and not challenged, C: bacteria $10^4$ cells/mL, D: bacteria $10^5$ cells/mL, and E: bacteria $10^6$ cells/mL. 

### Table 4. Percentage of lymphocytes.

| Treatment | H+7 (Normal) | H+11 (Vaccination) | H+25 (Booster) | H+33 (Challenge test) |
|-----------|--------------|-------------------|----------------|----------------------|
| A (K-)    | 85.25 ±1.25  | 84.25 ±1.70       | 83.00 ±2.16    | 82.25 ±0.95          |
| B (K+)    | 86.25 ±2.06  | 89.25 ±0.95       | 88.25 ±0.95    | 87.00 ±0.81          |
| C (10^4)  | 85.00 ±1.82  | 86.50 ±1.29       | 82.75 ±2.62    | 82.00 ±0.81          |
| D (10^5)  | 85.75 ±2.19  | 88.25 ±0.83       | 84.50 ±1.14    | 84.00 ±0.83          |
| E (10^6)  | 84.75 ±1.15  | 88.25 ±1.15       | 86.00 ±1.52    | 85.00 ±1.00          |

Note: Notations shown with different superscript letters in the same column indicate significantly different (p <0.05). A (K-): fish not vaccinated and challenged, B (K+): fish vaccinated and not challenged, C: bacteria $10^4$ cells/mL, D: bacteria $10^5$ cells/mL, and E: bacteria $10^6$ cells/mL.

### Table 5. Percentage of monocytes.

| Treatment | H+7 (Normal) | H+11 (Vaccination) | H+25 (Booster) | H+33 (Challenge test) |
|-----------|--------------|-------------------|----------------|----------------------|
| A (K-)    | 8.75 ±0.95   | 5.25 ±1.52        | 7.25 ±0.95    | 8.50 ±1.15          |
| B (K+)    | 7.25 ±2.06   | 6.50 ±1.29        | 7.50 ±1.00    | 8.00 ±1.15          |
| C (10^4)  | 8.75 ±2.36   | 7.25 ±0.95        | 10.25 ±1.52   | 8.75 ±0.89          |
| D (10^5)  | 9.00 ±3.04   | 8.00 ±1.22        | 12.00 ±1.51   | 8.50 ±1.15          |
| E (10^6)  | 9.75 ±1.15   | 9.75 ±1.52        | 9.50 ±2.08    | 8.50 ±1.15          |

Note: Notations shown with different superscript letters in the same column indicate significantly different (p <0.05). A (K-): fish not vaccinated and challenged, B (K+): fish vaccinated and not challenged, C: bacteria $10^4$ cells/mL, D: bacteria $10^5$ cells/mL, and E: bacteria $10^6$ cells/mL.

### Table 6. Percentage of neutrophils in each treatment during the study.

| Treatment | H+7 (Normal) | H+11 (Vaccination) | H+25 (Booster) | H+33 (Challenge test) |
|-----------|--------------|-------------------|----------------|----------------------|
| A (K-)    | 6.00 ±0.81   | 4.75 ±0.95        | 5.25 ±0.95    | 7.25 ±0.95          |

Note: Notations shown with different superscript letters in the same column indicate significantly different (p <0.05). A (K-): fish not vaccinated and challenged, B (K+): fish vaccinated and not challenged, C: bacteria $10^4$ cells/mL, D: bacteria $10^5$ cells/mL, and E: bacteria $10^6$ cells/mL.
This decrease in hemoglobin is thought to be due to the activity of Aeromonas hydrophila which has hemolysin which can lyse erythrocytes or reduce hemoglobin levels. This is also consistent with the study of [6] which states that hemoglobin levels after the challenge test decreased in accordance with a decrease in erythrocytes. The value of hemoglobin (Hb) in each treatment is still in the range of normal levels of fish Hb (6-10 g%). The result of the hemoglobin level obtained for fish is 6-10 g% [7].

Leukocytes can be used as a marker of infection in the body. The body will produce more leukocytes when foreign objects enter the body [6]. An increase in the average number of white blood cells in bacterial infection indicates that fish respond responsively to the presence of foreign material entering the body. Whereas the vaccination treatment showed a decrease in leukocytes indicating that the fish had succeeded in producing antibodies that could fight the incoming antigens. This shows that the vaccine given is able to influence the body's immune response to pathogens so that it is able to defend itself from disease attacks [8].

4. Conclusion

Based on the results of this study concluded that the administration of Aeromonas hydrophila vaccine with a density of $10^6$ cells/mm³ showed the best immune response, total erythrocytes reached $3.31 \times 10^6$ cells/mm³, hemoglobin 7.63 g%, total leukocytes were lower than normal controls, $8.3 \times 10^8$ cells/mm³, lymphocytes 85%, monocytes 8.5%, and neutrophils 6.5%.

5. References

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