Determination of LC50 and Clinical Symptoms of *Aeromonas hydrophila* Infection on the Fingerlings of Semah (*Tor soro*), the Indonesian Native Freshwater Fish

Firdausi Amalia Putri[1], Rahman[2], and *Mulya* Muhammad Arif[1]

[1]Production Technology and Management of Aquaculture Study Program, College of Vocational Studies, IPB University, Bogor
[2] Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor

Abstract. *Aeromonas hydrophila* is a bacterial pathogen in freshwater aquaculture causing the motile aeromonad septicemia (MAS) disease and inflict significant economic losses. This study aimed to determine the lethal concentration (LC50-48h) of *A. hydrophila* on *Tor soro's* fingerlings and investigated the clinical signs that appeared following the challenge test. Briefly, 150 fingerlings (3,4 ± 0,18 cm) were challenged by immersion means with 0 (control), 10^4, 10^5, 10^6, and 10^7 CFU/mL of live cells of *A. hydrophila*. Reed and Muench's method's determination of LC50-48h was calculated and all clinical signs that appeared during the test were recorded. The result showed that the LC50-48h by immersion was 1.0 x $10^{6,66}$ CFU/mL with clinical signs observed as hemorrhages, depigmentation, lesions, anorexia, loss of balance, and enlargement of the abdominal cavity. Furthermore, based on the LC50-48h and the clinical symptoms, this study reveals that even though all symptoms are consistent among the fish, the severity of the disease tends to be prevalent and intense as the concentration of pathogen is higher.

1 Introduction

Semah (*Tor soro*) is a native freshwater fish in Indonesia. This species is known as a premium freshwater commodity because of its high demand but its existence is scarce. Domestication for *Tor soro* is made to explore its potential for both aquaculture purposes and conservation efforts. The major obstacle faced in *Tor soro* aquaculture is the presence of opportunistic pathogens such as *Aeromonas hydrophila* causing the motile aeromonad septicemia (MAS) disease. MAS disease is common in freshwater-aquaculture facilities and may inflict

* Corresponding author: rahmanaqua@gmail.com

significant economic losses due to high mortality and morbidity [4].

The pathogen is a rod-shaped, motile, gram-negative bacteria with a diameter of 0.3–1 μm and a length of 1–3.5 μm, without a spore phase, usually lacking a capsule, growing optimum at 28 ºC but can also grow at extreme temperatures (4 ºC and 37 ºC). Its cosmopolite nature in the aquatic environment allows contact with fish and amphibians, and even enters the animal. Such contact can cause infection depending on its species and its virulent level [9].

Pathogenesis is the study of the course of diseases ranging from the influx of the pathogen to the onset of clinical symptoms. This study is needed to scrutinize the basic information in preventive control as well as treatment of disease. In this study, we aimed to evaluate the pathogenicity of *A. hydrophila* on *Tor soro* and observed any clinical symptoms resulting from the artificial infection [10].

2 Material and Method

2.1 Fish and acclimatization

One hundred fifty fingerlings of *Tor soro* (3,4 ± 0,18 cm) were purchased from a state-owned aquaculture facility (BPPBAT Bogor, Cijeruk Installation) and were acclimated to the Laboratory of Aquaculture, IPB Vocational School, Sukabumi (Indonesia) in tanks of 100 L of clean freshwater. Stocking density refers to the result of Priadi's *et al.* research (2021) [1]. During the rearing, fish were fed with commercial feed containing 38% crude protein for two weeks.

2.2 Pathogen preparation and Virulence Enhancement

Bacterial isolates came from the collections of the laboratory of aquatic organism health, Department of Aquaculture, Bogor Agricultural University, and was maintained in slant agar before the propagation. The liquid culture medium of trypticase soy broth (TSB, Sigma Aldrich^{TM}, USA) was prepared according to the instructions of the manufacturer by dissolving 37 g in 1 L of distilled water. The sterilization was processed by autoclaving at 121^0C for 15 min (Gea, LS50LJ). Bacterial suspensions were prepared by transferring a loop containing three to five colonies of *A. hydrophila* isolated from Petri dishes of trypticase soy agar (TSA) medium after 24 h of cultivation at 30^0C to test tubes containing 10 mL of TSB and reincubated for another 24 h in a rotary incubator at speed of 140 rpm. Virulence enhancement was done by injecting a suspension culture of *A. hydrophila* bacteria that have been washed with phosphate buffer saline (PBS) as much as 0.1 mL into intramuscular catfish (*Clarias garipienus*). After the clinical symptoms such as ulcers and hemorrhagic appeared, the bacteria was reisolated to TSA medium from the kidney of infected fish and incubated for 24 hours at a temperature of 29 °C to 30 °C. The result from reisolated bacteria was purified into four quadrants in a plate and recharacterized. Characterization of bacteria was conducted using software the API 20E KIT. The results of the characterization using the API 20E KIT showed that the bacteria were *Aeromonas hydrophila* with a similarity level of 94%.

2.3 Determination of LC50-48h

The pure cultured of *A. hydrophila* bacteria were isolated by picking a single colony from the stock culture to TSB medium as much as 50 mL and incubated in a rotary incubator at a speed of 140 rpm for 24 hours in 29 °C to 30 °C. After incubation, the logarithmic growth was measured by total plate counting. This data was used to obtain different concentrations

of the pathogen by a ten-fold serial dilution for the LC50-48h bioassay test. Bioassay was done by immersing 10 fingerlings into each different series of final concentrations 10^5, 10^6, 10^7 CFU mL^{-1} for 48 hours long. All mortalities and any clinical symptoms were recorded, while the LC50 value was generated referred to modified of Reed and Muench's (1938) method [8].

2.4 Data analysis

Data of LC50-48h were analyzed with microsoft excel software descriptively. The LC50-48h were calculated. Meanwhile, appearing clinical symptoms were analyzed descriptively.

3 Results

Mortality of *Tor soro* fingerlings did not appear after 48 hours of artificial infection with a suspension of *A. hydrophila* on 1.0 x 10^4 CFU/mL. Only when the concentration raised to 10^5, 10^6, and 10^7 CFU/ml the mortality was beginning (Table 1.) and followed the onset of the clinical symptoms. Based on the Reed and Muench modified method (1938), it was concluded that the LC50-48h was 1.0 x $10^{6,66}$ CFU/ml.

Table 1. Mortality of *Tor soro* fingerlings after 48 hours challenged by *Aeromonas hydrophila*

Pathogen concentration (CFU/ml)	Response		Accumulation		Ratio	% Mortality
	Dead	live	Dead	Live		
1.0 x 10^7	6	4	11	4	11/15	73,4
1.0 x 10^6	3	7	5	11	5/16	31,25
1.0 x 10^5	2	8	2	19	2/21	9,52
1.0 x 10^4	0	10	0	29	0/29	0

The virulence of *A. hydrophila* was able to regain after enhancement by passaging the pathogen into the body of African catfish. The clinical symptoms appearing after 24 hours on the body of catfish including inflammation, hemorrhagic, body discoloration, anorexia, and lethargy. Meanwhile, infection on *Tor soro* fingerlings exhibits moderate clinical symptoms after 48 hours where body discoloration, hyperemia, and swelling in the body cavity were prevalent (Fig 1). The fingerlings had a reduction of respiratory, loss of balance, erratic swim-movement anorexia, erosion of the caudal fin, and dropsy containing yellowish ascites fluid.

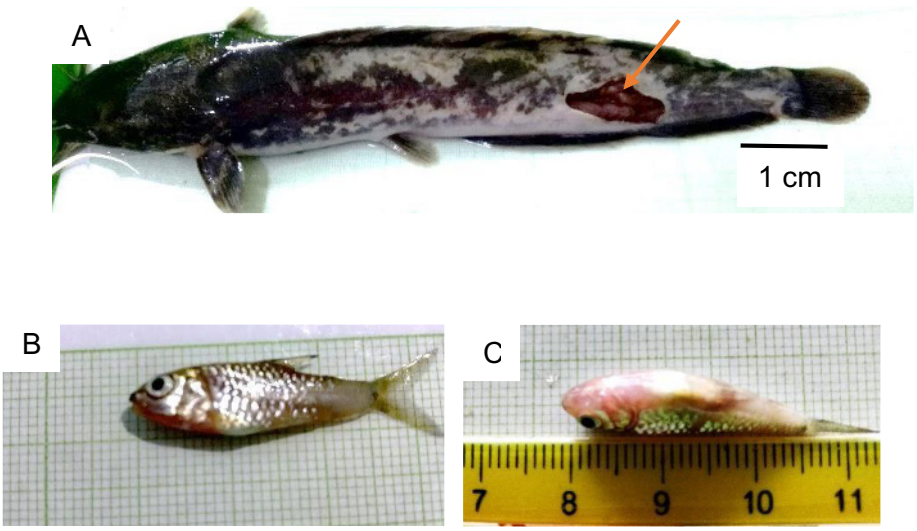

Fig. 1. The clinical symptoms of African catfish during enhancement of *A. hydropila*. (A) an open hemorrhagic (orange arrow) and body discoloration of African catfish showed after 24 hours. (B) Scales loss and hyperemia showed in the body of Tor soro fingerlings. (C) Dropsy also showed from moribund fingerlings.

4 Discussion

Motile aeromonads septicemia (MAS) is one of the major diseases that attack various species of fish [6]. The disease is caused by the bacteria *A. hydrophila* which is Gram-negative and lives with normal flora in freshwater [11]. The impact of the pathogen is hemorrhagic and often ends in death [3]. The fingerlings are the prone stage because do not have a sophisticated immune response compared to the adults that a massive disease outbreak will lead to higher mortality [7, 10]

Aeromonas hydrophila can attack the fins, tegument, and abdominal cavity, and may be able to break the blood vessels, resulting in the manifestation of hemorrhagic, hyperemia, wound, ulcer, severe scale, and blackened skin [2, 5, 12, 13]. In our bioassay with *A. hydrophila* on *Tor soro* fingerlings, the parameter of water quality was set constant and considered as suitable for most of the freshwater fish. However, the severity of the disease was increased along with the concentration of the pathogen thus influencing the mortality rate. We found that even particular clinical symptoms were consistent in all assays, the higher of pathogen concentration, the more prevalent and intense the clinical symptoms among the fish. All findings in this study provide important information not only on the pathogenesis of *A. hydrophila* but also for the development of treatment and crisis strategies for this bacteriosis. Furthermore, additional studies should be conducted in the search for improvements in the immune response against the pathogenicity of *A. hydrophila* using maternal immunization, vaccination, or stimulation by environmental enrichment.

References

1. B Priadi, D Irawan. Effect of Different Stocking Densities on the Growht of Tor Soro Fish. Aquaculture Engineering Bulletin, **19,** 85-88 (2021)

2. EH Hardi, CA Pebrianto, T Hidayanti, RT Handayani. Phatogenicity of *Aeromonas hydrophila* via some port entryin cultured Nile tilapia *Oreochromis niloticus* from Loa Kulu Kutai Kartanegara Kalimantan Timur. Journal of Veterinary Medicine **8**, 130-133 (2014)
3. GH Fu, F Liu, JH Xia, GH Yue. The *LBP* gene and its association with resistance to *Aeromonas hydrophila.* International Journal of Molecular Sciences **15**, 22028-22041 (2014)
4. J Hayes. *Aeromonas hydrophila* in Oregon State University, 24 Nov 2020, Corvallis (2000)
5. JA Plumb, A Larry, LA Hanson. Health Maintenance and Principal Microbial Diseases of Cultured Fishes in Blackwell Publishing **1**, 492 (2011)
6. JB Fernandez, AV Yambot, O Almeria. Vaccination of Nile tilapia *Oreochromis niloticus* using LPS prepared from *Aeromonas hydrophila*. International Journal of Fauna dan Biological Studies **1**, 1-3 (2014)
7. K Molnar, C Szekely, M Lang. Field Guide to Warmwater Fish Disease in Central and Eastern Europe, The Caucasus and Central Asia in Food and Agriculture Organization of The United Nations **1**, 128 (2019)
8. Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. Journal of Epidemiology **1**, 493-497 (1938)
9. RF Floyd. Aeromonas Infection in University of Florida, 27 Nov 2020, Florida (2002)
10. S Sukenda, O Carman, R Rahman, D Hidayatullah, SN Yumaidawati. Vaccination in Nile tilapia broodstock with whole-cell vaccine and disease resistance in its fry against *Aeromonas hydrophila.* Journal of Indonesia Aquaculture **2,** 268-276 (2017)
11. S. Dehghani, M Akhaghi, M Dehghani. Efficacy of formalin-killed, heat-killed and lipopolysaccharide vaccines against motile aeromonas infection in rainbow trout *Oncorhynchus mykiss,* Global Veterinaria **9**, 409-415 (2012)
12. Song, X., Zhao, J., Bo, J., Liu, Z., Wu, K., Gong, C. Aeromonas hydrophila induces intestinal inflammation in grass carp (*Ctenopharyngodon idella*): an experimental model. Aquaculture 434, 171–178 (2014)
13. T. Aoki. Motile aeromonas (*Aeromonas hydrophila*) **3**, 427-453 (1999)