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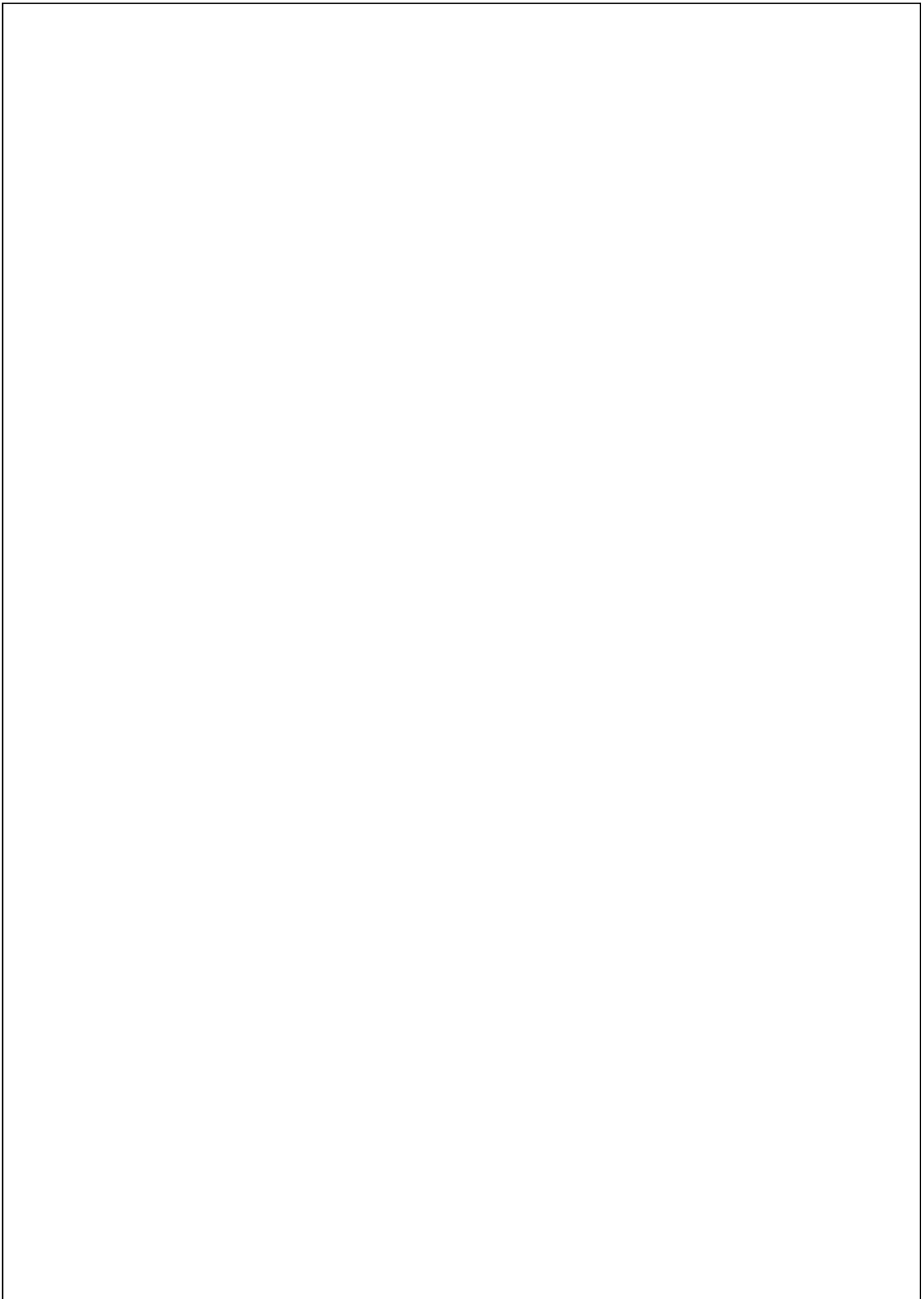
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# Bacterial Viability of *Edwardsiella tarda* from Silver Rasbora (*Rasbora argyrotaenia*) after Infection with Immersion Methods

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**Abstract.** Silver rasbora (*Rasbora argyrotaenia*) is a freshwater fishery commodity that has high economic value. However, fulfilling the demand for silver rasbora still relies on catches from nature, so cultivation is needed. The problem that occurs in the cultivation process is the *Edwardsiella tarda* infection which causes Edwardsiellosis disease. The purpose of this study was to determine the bacterial viability of *E. tarda* from silver rasbora after infection with immersion methods. The Total Plate Count (TPC) from blood, liver and kidney was taken from infected fish after 14 days immersion with bacterial suspension. The results showed that *E. tarda* infection had occurred in the blood, liver and kidneys as indicated by an increasing the density of bacteria in each organ along with the increasing of the concentration of infected bacteria. The highest density of *E. tarda* bacteria infected in silver rasbora was in the blood and the least was in the kidneys.

## 1. Introduction

Silver rasbora (*Rasbora argyrotaenia*) is a freshwater fishery commodity that has high economic value [1]. However, the fulfillment of the demand for silver rasbora still relies on catches from nature so that it can cause a decrease in fish resources [2]. Fish farming is the alternative activity to fulfill the increasing market needs. According to [3], there are several factors that influence fish farming activities, including water quality management, feed, cultured organisms, and disease control. One of the problems that occur in the cultivation process that causes economic losses is the infection of pathogenic bacteria such as *Edwardsiella* which causes Edwardsiellosis disease [4,5]. *Edwardsiella* bacteria are opportunistic bacteria that infect freshwater and marine fish [6,7].

*E. tarda* infection can cause exophthalmia, red spots on the abdomen, abdominal swelling, bleeding on the fins and skin [8,9,10]. *E. tarda* can producing hemolysin, and dermatotoxin [11,12]. Furthermore, *E. tarda* has a bacterial protein secretion system, including Type I to Type VI (T6SS) system which can penetrate, survive, and replicate in epithelial cells and phagocytes cells [13,14]. Previous study reported that *E. tarda* bacteria can infect freshwater fish species *Catla catla* from the family Cyprinidae [15]. This indicates that *E. tarda* also has the potential to infect silver rasbora, which are freshwater fish from similar family [16].

The increased viability of bacteria in the fish body due to the immune system being unable to fight infection causes *E. tarda* to spread and infect deeper tissues, reaching the circulatory and lymphatic



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systems so that it can affect the survival of fish [17]. This can affect the work of internal organs, such as the liver, kidneys, spleen, and host muscles [18,19].

However, research on the bacterial viability of *E. tarda* infected in silver rasbora (*R. argyrotaenia*) is still limited. The purpose of this study was to determine the bacterial viability of *E. tarda* from silver rasbora after infection with immersion methods. The data obtained are used as a reference for the density of bacteria in the waters capable of infecting silver rasbora to accelerate the process of handling and preventing Edwardsiellosis disease in silver rasbora cultivation.

## 2. Methods

This research was carried out from October 2020 to February 2021 at the Instrument Laboratory of the Faculty of Fisheries and Marine PSDKU Airlangga University in Banyuwangi.

### 2.1 Experimental Fish and Bacterial Preparation

Silver rasbora (*Rasbora argyrotaenia*) as many as 200 fish with a length of 5.4-7 cm and a weight of  $0.47 \pm 2.63$  grams. All fishes was immersed in NaCl solution at a dose of 30 ppm for 5 minutes to remove the ectoparasites and then acclimatized for approximately 7 days. *E. tarda* bacteria was identified biochemically using Gram staining, oxidative/fermentative test, SIM test, catalase test and oxidase test [20].

### 2.2 Bacterial infection

Bacterial infection of *E. tarda* in silver rasbora was carried out by immersion for 14 days in twenty four aquariums (40x40x30 cm, 10 liters water) with density of 10 fish/aquarium containing *E. tarda* bacteria with a density of  $10^{11}$  CFU/mL,  $10^{12}$  CFU/mL, and  $10^{13}$  CFU/mL and without bacterial immersion as a negative control.

### 2.3 Blood, Liver and Kidney Sampling

Blood, liver and kidney samples were taken from 10% of the total population of the test fish in order to represent all the test fish. The fish used as samples were fish that did not show clinical signs or fish that showed clinical symptoms of infection with *E. tarda*. Sampling organs was carried out before infection and 14 days after infection. Samples from blood, liver and kidneys that have been obtained will be diluted starting from  $10^{-2}$ ,  $10^{-4}$ ,  $10^{-6}$ , and  $10^{-8}$ , using physiological NaCl, then spread on *Xylose-Lysine Deoxycholate Agar* selective media (XLD) to be cultured and then incubated (28°C) for 24 hours. The density of the growing *E. tarda* bacterial colonies was calculated using the TPC (*Total Plate Count*) method using a hand counter.

### 2.4 Statistical Analysis

The colony of bacterial in blood, wounds, liver and kidney were analyzed statistically using analysis of Variance (ANOVA) using IBM SPSS 20 software ( $\alpha=0.05$ ). If it is significantly different, Duncan's Multiple Range Test (DMRT) (95% confidence interval) were used to analyse the significance between all treatments. Clinical symptom data were analyzed descriptively.

### 3. Results and Discussion

#### 3.1 Result

The total density of bacteria in the blood, liver and kidneys that have been infected with *E. tarda* bacteria can be seen in Table 1.

**Table 1.** Density of *E. tarda* Bacteria in Blood, Liver and Kidneys of Silver Rasbora after Immersion with *E. tarda* for 14 Days

Parameter	Bacterial Density ( $\times 10^4$ CFU/mL)			
	P0	P1	P2	P3
Blood	0 <sup>a</sup> ±0	0.245 <sup>a</sup> ±0.049	0.71 <sup>b</sup> ±0.212	0.875 <sup>b</sup> ±0.205
Liver	0 <sup>a</sup> ±0	0.185 <sup>a</sup> ±0.106	0.44 <sup>ab</sup> ±0.113	0.88 <sup>b</sup> ±0.113
Kidney	0 <sup>a</sup> ±0	0.32 <sup>a</sup> ±0.098	0.585 <sup>a</sup> ±0.374	0.45 <sup>a</sup> ±0.155

Description: P0: fish were reared without in *E. tarda* bacteria (Control), P1: fish were immersed in *E. tarda* suspension with a density of  $10^{11}$  CFU/mL, P2: fish were immersed in *E. tarda* suspension with a density of  $10^{12}$  CFU/mL, P3: fish were immersed in *E. tarda* suspension with a density of  $10^{13}$  CFU/mL. Different superscripts on the same line showed significant differences ( $P < 0.05$ ).

The results of bacterial isolation from blood showed that there was a significant difference ( $P < 0.05$ ) on the density of *E. tarda* bacteria in treatment P3 (immersion of *E. tarda*  $10^{13}$  CFU/mL), is  $0.875 \pm 0.205 \times 10^4$  CFU/mL and P2 (immersion of *E. tarda*  $10^{12}$  CFU/mL), is  $0.71 \pm 0.212 \times 10^4$  CFU/mL compared to P1 (immersion of *E. tarda*  $10^{11}$  CFU/mL), is  $0.245 \pm 0.049 \times 10^4$  CFU/mL and P0 (negative control).

The highest bacterial density was found in the blood, which was around  $0.245 \pm 0.049 \times 10^4 - 0.875 \pm 0.205 \times 10^4$  CFU/mL compared to other organs (liver and kidney). The presence of *E. tarda* in the liver was less than the total density of bacteria in the blood, which was around  $0.185 \pm 0.106 \times 10^4 - 0.88 \pm 0.113 \times 10^4$  CFU/mL in all treatments. The results of bacterial counts from the kidney organs after *E. tarda* infection also showed that there was also no significant difference ( $P > 0.05$ ) in the density of *E. tarda* bacteria from all treatments, in the range of  $0.32 \pm 0.098 \times 10^4$  CFU/mL to  $0.45 \pm 1.55 \times 10^4$  CFU/mL. The total density of bacteria in the kidneys was less than in the blood and liver, which was around  $0.32 \pm 0.098 \times 10^4 - 0.45 \pm 0.155 \times 10^4$  CFU/mL.

#### 3.2 Discussion

*E. tarda* can infect several internal fish organs, including liver, kidney, lymph and muscle [17]. The results showed that *E. tarda* infection had occurred in the blood, liver and kidneys as indicated by an increase in the density of bacteria in each organ along with the increase in the concentration of the infected bacteria. Based on the results, the highest bacterial density was found in the blood compared to other organs (liver and kidney). The components of red blood cells contain a lot of iron which is used as a source of nutrients for the growth and proliferation of bacteria [21,22]. [18, 19] stated that *E. tarda* is a septicemic bacterium, so it is commonly found in blood.

Based on the results, the presence of *E. tarda* in the liver was less than the total density of bacteria in the blood in all treatments. These phenomenon caused by the defense system that occurs in the liver, such as macrophages in the form of Kupffer cells [8,10,23]. Resident Kupffer cells play a role in the initial response to injury or damage by releasing pro-inflammatory cytokines and chemokines, such as CCL2 CCL5 [24] (Safithri., 2018), TNF- and IL-6 which function to process cell repair [25]. According to [26], Kupffer cells are a type of hepatic sinusoidal macrophage that has a function to phagocytize pathogens that enter the liver, so that when fish are infected with *E. tarda*, Kupffer cells will phagocytize these pathogens so that cell damage does not occur in the liver.

The results of bacterial counts from the kidney organs after *E. tarda* infection also showed that there was no significant difference ( $P > 0.05$ ) in the density of *E. tarda* bacteria from all treatments. Based on the results of the study also showed the presence of *E. tarda* infection in the kidneys. However, based on the results of the study, it was shown that the total density of bacteria in the kidneys was less than in the blood and liver. The density of *E. tarda* bacteria also did not cause a very significant difference between treatments ( $P < 0.05$ ). Differences in the number of bacteria in the kidneys can be caused by the body's defense system in the form of macrophages [8,10,23]. The decrease in the number of bacterial density in the kidney is thought to be caused by the defense system of *E. tarda* bacteria unable to fight macrophages in the kidney, so that some bacteria were successfully phagocytized by macrophages [27]. Based on the results of the study also showed that *E. tarda* bacterial infection did not cause external pathological changes such as bleeding and enlargement of the kidneys. According to [28] the density of *E. tarda* bacteria that can cause changes in external clinical symptoms in the kidney is  $0.3 \times 10^8$  CFU/mL. So the percentage of bacterial density  $0.32 \pm 0.098 \times 10^4 - 0.45 \pm 0.155 \times 10^4$  CFU/mL can already infect fish, but has not caused external clinical symptoms in the kidneys.

Silver rasbora after infection with *E. tarda* bacteria did not show external clinical symptoms, such as the absence of wounds and changes in behavior. It can be assumed that the total bacterial density still does not meet quorum sensing ( $0.128 - 1.024 \times 10^7$  CFU/mL) [29]. In addition, the absence of clinical symptoms, there was also no mortality or 100% survival in silvers after infection with *E. tarda*, this could be due to the level of pathogenicity of *E. tarda* bacteria determined based on the ability of these bacteria to infect non-specific immunity in fish [30] and the level of bacterial pathogenesis based on the degree of compatibility of the host (susceptible host) [31]. In an unsuitable host, even though the bacteria is pathogenic for certain types of fish, it will not produce the same effect as a suitable host [32]. Host match rates can be receptor based [33]. Based on this statement, it is suspected that bacteria that enter the body are capable of infecting, but have not affected the survival of changes in clinical symptoms and behavior.

#### 4. Conclusion

The conclusion of this study was the highest density of *E. tarda* bacteria infected in silver rasbora was in the blood and the least was in the kidneys.

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