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The 4th International Conference on Fisheries and Marine Sciences (INCOFIMS) Surabaya Indonesia, 29 September 2021

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The 4th International Conference on Fisheries and Marine Sciences (INCOFIMS) Surabaya Indonesia, 29 September 2021

International conference on fisheries and marine sciences (INCOFIMS) is an annual conference organized by Faculty of Fisheries and Marine Universitas Airlangga, Surabaya, Indonesia. The main aim is to provide a sharing platform that enables researchers, academics and practitioners from all over the world to share their most recent findings as well as to propose the best strategies to address issues and challenges which we have been currently facing in aquaculture and fisheries practices worldwide. The 1st INCOFIMS was held successfully offline in Surabaya in 2018, the second in 2019 and the third in 2020.

The 4th INCOFIMS was previously scheduled offline in Surabaya on 29th September 2021. However, due to the Covid-19 pandemic and travel restriction for foreigners come into Indonesia as well as traveling within the Indonesian islands, we had the 4th INCOFIMS in a virtual format with ZOOM on 29 September 2021, and hosted from Faculty of Fisheries and marine, Universitas Airlangga, Surabaya Indonesia. We were unable to postpone the event because INCOFIMS is our annual event and also most of the participants requested to have the conference in the virtual format (online)

The theme in the 4th INCOFIMS was "Interprofessional collaboration for enhancing the aquatic ecosystem sectors". Technically, we had the conference divided into 2 (two) sessions in general: (1) keynote speaker session and (2) guest speaker session. In the keynote session, we had 4 (four) keynote speakers delivering a speech which were Prof Felipe Polivanov Ottoni, Ph.D.; Asst. Prof. Dr. Narongrit Muangmai and Dr. TB. Haeru Rahayu, A.Pi., M.Sc. Each keynote speaker had 1.5 hours for giving a presentation using ZOOM and 30 minutes for discussion in one virtual room. After the keynote speaker session, we proceeded to the guest speaker session in which all participants were divided into 7 (seven) rooms according to our subtopics for oral and poster presentations:

Room 1: Aquaculture technology

Room 2: Fish Nutrition

Room 3: Fish Diseases

Room 4: Marine and Aquatic Sciences

Room 5: Estuarine and Coastal Ecosystems

Room 6: Fisheries Management

Room 7: Fisheries Socio-economics

In this session, every speaker had 15 minutes for presentation and 5 minutes for discussion. Total participants joined in this conference was 225 participants from at least 6 different countries (Australia, Japan, Austria, Malaysia, Taiwan and Indonesia).

The conference was in general quite successful, acknowledging the number and enthusiasms of participants during the discussion sessions in both the keynote speaker session and guest speaker's session. We thank all participants and organizing committee for their support to this conference and see you in the 5th INCOFIMS 2022.

Chairman Veryl Hasan, Ph.D

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Peer Review Statement

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Peer Review Statement

All papers published in this volume have been reviewed through processes administered by the Editors. Reviews were conducted by expert referees to the professional and scientific standards expected of a proceedings journal published by IOP Publishing.

- Type of peer review: Double Anonymous
- Conference submission management system: Morressier
- Number of submissions received: 195
- Number of submissions sent for review: 195
- Number of submissions accepted: 191
- Acceptance Rate (Submissions Accepted / Submissions Received × 100): 97.9
- Average number of reviews per paper: 2
- Total number of reviewers involved: 10
 Contact person for queries: Name: Dwi Yuli Pujiastuti, S.Pi., M.P. Email: dwiyp@fpk.unair.ac.id Affiliation: Faculty of Fisheries and Marine, Universitas Airlangga

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

N Husna¹, R Kusdarwati², M F Ulkhaq^{2*}.

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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 dermatoxin [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±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 stratistically using Analysis of Variance (ANOVA) using IBM SPSS 20 software (α =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.

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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 (x10 ⁴ CFU/mL)				
	P0	P1	P2	P3	
Blood	0ª±0	0.245 ^a ±0.049	0.71 ^b ±0.212	$0.875^{b}\pm 0.205$	
Liver	0ª±0	0.185 ^a ±0.106	0.44 ^{ab} ±0.113	0.88 ^b ±0.113	
Kidney	0ª±0	0.32ª±0.098	0.585ª±0.374	0.45 ^a ±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^{12} CFU/mL, P3: fish were immersed in *E. tarda* suspension with a density of 10^{12} 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* 10¹³ CFU/mL), is 0.875±0.205 x10⁴ CFU/mL and P2 (immersion of *E. tarda* 10¹² CFU/mL), is 0.71±0.212 x10⁴ CFU/mL compared to P1 (immersion of *E. tarda* 10¹¹ CFU/mL), is 0.245±0.049 x10⁴ CFU/mL and P0 (negative control).

The highest bacterial density was found in the blood, which was around $0.245\pm0.049 \times 10^4 - 0.875\pm0.205 \times 10^4$ 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 $0.185\pm0.106 \times 10^4 - 0.88\pm0.113 \times 10^4$ 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 $0.32\pm0.098 \times 10^4$ CFU/mL to $0.45\pm1.55 \times 10^4$ CFU/ml. The total density of bacteria in the kidneys was less than in the blood and liver, which was around $0.32\pm0.098 \times 10^4 - 0.45\pm0.155 \times 10^4$ 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.

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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. 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 0.3x10⁸ CFU/mL. So the percentage of bacterial density 0.32±0.098 x10⁴ - 0.45±0.155 x10⁴ 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 (0.128-1.024x10⁷ 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 (susceptable 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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