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Hematological alterations and bacterial abundance of *Edwardsiella tarda* in catfish (*Clarias* Sp.) cohabiting with carrier silver rasbora (*Rasbora argyrotaenia*)

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ABSTRACT

Background: Catfish farming is often performed with high stocking densities, which can result in environmental degradation, stress, and increased vulnerability to infections such as Edwardsiellosis caused by *Edwardsiella tarda*. Infection by *E. tarda* occurs through horizontal transmission during cohabitation between infected and healthy fish.

Aim: This study aimed to investigate hematological alterations and abundance of *E. tarda* in catfish following cohabitation with carrier silver rasbora.

Methods: A total of 160 silver rasboras (length: 6.02 ± 0.36 cm, weight: 2.065 ± 0.565 g) were immersed in an *E. tarda* suspension at a concentration of 10^{13} colony forming unit (CFU) /ml for 2 weeks. Subsequently, they were cohabitate with 240 catfish (length: 6.9 ± 2.35 cm, weight: 2.78 ± 1.37 g) at different density ratios: 1:1 (P1), 1:2 (P2), and 2:1 (P3), along with negative control (without *E. tarda*, NC), and with *E. tarda* at 10^{13} CFU/ml (positive control) for 5 days. The observed parameters included hematological profiles (total erythrocytes, leukocytes, hemoglobin (Hb) levels, and leukocyte differentials) and the density of *E. tarda* in various organs (liver, kidneys, and spleen).

Results: Catfish cohabiting with *E. tarda*-infected silver rasbora exhibited significant hematological alterations, including elevated percentages of neutrophils, lymphocytes, and monocytes, alongside decreased erythrocyte counts, Hb levels, and total leukocyte counts. Furthermore, the highest density of *E. tarda* was detected in the liver (1.15 ± 0.11 × 10^4 CFU/ml) compared with the other organs.

Conclusion: Cohabitation between healthy catfish and *E. tarda* carrier silver rasbora resulted in bacterial infection in the catfish.

Keywords: Catfish, Cohabitation, Edwardsiellosis, Fisheries, Silver Rasbora.

Introduction

Catfish is a widely cultivated freshwater aquaculture commodity in almost 90 countries globally, recognized for its affordability and high nutritional value, as demonstrated by a marked increase in consumer demand. This rising interest is reflected in the high demand for catfish in the community (Segaran *et al*., 2023). According to the Indonesian Ministry of Marine Affairs and Fisheries (2024), catfish production increased by 4.27% from 2018 to 2022, representing the highest average growth rate among all aquaculture commodities in Indonesia. The intrinsic characteristics of catfish, including rapid growth and resilience to environmental fluctuations, contribute to its status as a preferred species for aquaculture, supported by established cultivation techniques and technologies (Salamah and Zulpikar, 2020).

Nonetheless, the prevalence of disease is a significant challenge in catfish farming, resulting in high mortality rates and compromised harvests. The pathogen *Edwardsiella tarda*, a Gram-negative, rod-shaped, flagellated bacterium, is the causative agent of Edwardsiellosis (Goh *et al*., 2023). In addition to catfish, infections caused by *E. tarda* have been documented in various freshwater and marine fish species, including channel catfish (*Ictalurus punctatus*) (Armwood *et al*., 2022), Nile tilapia (*Oreochromis niloticus*) (Peng *et al*., 2022), iridescent shark catfish (*Pangasianodon hypophthalmus*) (Hoque *et al*., 2020), and *Labeo rohita* (Sattanathan *et al*., 2020), often resulting in elevated

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mortality rates during the grow-out phase. This phenomenon is attributed to the virulence factors of *E. tarda*, including adhesins, hemolysins, and type III and VI secretion systems, which facilitate the bacterium's invasion, survival, and replication within host epithelial and phagocytic cells (Samir *et al*., 2021).

The clinical manifestation of Edwardsiellosis in fish is characterized by symptoms such as depigmentation, exophthalmia, and red lesions on the skin and fins, as well as internal hemorrhages affecting organs such as the spleen, liver, and kidneys (Pandey *et al*., 2021). The emergence of Edwardsiellosis is exacerbated by factors such as stress, fluctuating water temperatures, and elevated organic matter levels in aquatic environments (Miniero Davies *et al*., 2018). Furthermore, Janda and Duman (2024) reported that Edwardsiellosis also infects reptiles, birds, and mammals, categorizing it as a zoonotic disease with potential implications for human health.

The horizontal transmission of *E. tarda* through water from infected fish to healthy fish has been extensively documented (Buján *et al*., 2015). Instances of cohabitation-associated *Edwardsiella* infections have been reported in species such as striped catfish (*P. hypophthalmus*), resulting in abdominal swelling and the enlargement of the liver and lymph nodes (Phuoc *et al*., 2020). Additionally, da Costa *et al.* (2021) reported horizontal transmission of *Edwardsiella* infections in *Pseudoplatystoma corruscans* originating from two invasive fish species (*O. niloticus* and *Clarias gariepinus*), leading to mortality rates of up to 50%.

The silver rasbora (*Rasbora argyrotaenia*) is a member of the family Cyprinidae and is distributed across India, China, and Southeast Asia. This species typically inhabits flowing waters, such as rivers, and calm environments, such as swamps (Anggararatri *et al*., 2023). The silver rasbora can serve as a vector for *E. tarda*. Findings reported by Husna *et al.* (2022) indicate that *E. tarda* can infect silver rasbora, with the bacteria accumulating in the blood, liver, and kidneys at densities ranging from 1.85 – 8.8 × 10^3 colony forming unit (CFU)/ml. Furthermore, fish infected with *E. tarda* exhibited decreased erythrocyte and hemoglobin (Hb) levels, along with an increase in white blood cell counts (Tirani *et al*., 2024). However, information regarding the horizontal transmission of *E. tarda* from silver rasbora carrier to healthy catfish remains undocumented. Therefore, this study aimed to analyze the hematological profile and total bacterial load of *E. tarda* in catfish following cohabitation with silver rasboras carrier. These results are anticipated to serve as a reference for preventing outbreaks of *E. tarda* in aquatic environments, particularly those caused by horizontal transmission by infected fish.

Materials and Methods

Fish and bacterial preparation

The study utilized 240 catfish (length 6.9 ± 2.35 cm, weight 2.78 ± 1.37 g) sourced from the Fish Hatchery Center Genteng in Banyuwangi, East Java, and 160 silver rasboras (length 6.02 ± 0.36 cm, weight 2.63 ± 1.5 g) obtained from the Freshwater Aquaculture Development Center Umbulan, Pasuruan, East Java. All fish were maintained separately in cylindrical fiberglass tanks with a capacity of 450 l (contains sterilized freshwater with 30 ppm chlorine and neutralized with sodium thiosulphate in the same concentration) and allowed to acclimatize for a duration of 7 days. During the acclimation period, the fish were fed a commercial pellet diet (Matahari Sakti, Indonesia; nutrient estimation: 30% protein, 6% fat, 3% carbohydrates) at 8:00 PM and 4:00 AM until satiation. Water quality parameters, including temperature, pH, and dissolved oxygen (DO), were measured bi-daily every morning (08.00 AM) and evening (04.00 PM) and maintained at optimal levels (26°C–28°C, pH 6–7, and DO at 5–6 ppm, respectively) to minimize stress in the fish.

The *E. tarda* bacteria used in this study were obtained from the Microbiology Laboratory of the Faculty of Health Sciences, Medicine, and Natural Sciences at Airlangga University and were previously identified in Husna *et al.* (2022). The bacterial isolates were recultured on selective Xylose-Lysine Deoxycholate (XLD) agar (Himedia, India) and biochemically characterized according to Indonesian Standard number 7663:2011.

Experimental design and in vivo challenge

This study was conducted using a completely randomized design with five treatments and four replications at the Instrument and Wet Laboratories, Airlangga University. The treatments consisted of: catfish immersed in an *E. tarda* suspension with a density of 10^{13} CFU/ml (positive control/PC), catfish maintained without exposure to *E. tarda* (negative control/NC), and silver rasbora carriers with *E. tarda* cohabitate with catfish in ratios of 1:1 (T1), 1:2 (T2), and 2:1 (T3). For all treatments, experiments were conducted in glass aquariums (H 40 × W 30 × L 30 cm) with an overall water volume of 160 l.

Prior to cohabitation, healthy silver rasbora were immersed in *E. tarda* at a concentration of 10^{13} CFU/ml for 2 weeks, excluding the control treatments (PC and NC), to establish them as bacterial carriers. The presence of *E. tarda* in the organs (liver, kidney, and spleen) of the silver rasbora was verified through colony counting in XLD medium using the plate count method (Madigan *et al*., 2012).

Following confirmation, *E. tarda*-carrier silver rasbora were cohabitate with healthy catfish according to established treatment ratios for a duration of 5 days. Throughout the maintenance period, the fish were fed commercial feed (Matahari Sakti, Indonesia) at satiation in the 8.00 PM and 4.00 AM. Every morning (8.00 AM), all solid waste was cleared from each aquarium. Water quality parameters, including temperature, pH, and dissolved oxygen (DO), were monitored bi-daily (8.00 AM and 4.00 PM) and were maintained within optimal ranges (26–28°C, pH 6–7, and DO 5–6

ppm). The temperature and pH were measured using a thermometer (Resun, Indonesia) and pH indicator (Merck, Germany). The DO levels were determined using a DO meter (Horiba, Poland), whereas the ammonia concentration was assessed using a SERA-ammonium/ammonia test kit (SERA, Germany).

Hematological and bacterial sampling

Blood and organ samples from catfish were collected before (0 dpi) and after (5 dpi) cohabitation with *E. tarda* carrier silver rasbora. The hematological parameters evaluated included total erythrocytes/red blood cells (RBCs), total leukocytes/white blood cells (WBCs), Hb levels, and the percentages of neutrophils, monocytes, and lymphocytes. Two catfish were randomly selected from each aquarium and transferred to an anesthetic solution containing 100-ppm eugenol for 1 minute. Blood was then drawn from the caudal vein using a syringe and transferred to a tube containing 10% ethylenediaminetetraacetic acid. Additionally, hematological profiles were analyzed according to the method described by Blaxhall and Daisley (1973).

The density of *E. tarda* was assessed by dissecting catfish to obtain liver, kidney, and spleen samples. Subsequently, 1 g of each organ was weighed, crushed using a tissue grinder (Axygen, USA), and homogenized in 9 ml of a sterile phosphate buffered saline solution. An aliquot of 0.1 ml was collected and subjected to serial dilution using the plate count method. Each dilution (0.1 ml) was inoculated onto XLD medium (Himedia, India), incubated at 37°C for 24 hours, and the colonies were counted using the following formula (Madigan *et al*., 2012):

$$PB = \frac{K}{A \times B \times C}$$

where:

PB = bacterial population (CFU/ml).
K = number of colonies.
A = inoculum volume in diluent (ml).
B = dilution factor at which the colonies are counted.
C = inoculum volume transferred to the agar medium (ml).

The survival rate of the catfish was assessed at the conclusion of the experiment using the following formula (Bera *et al*., 2020):

$$\text{Survival rate (SR)} = \frac{Nt}{No} \times 100\%$$

Nt = number of fish after the experiment.
N0 = number of fish at the start of the experiment.

Data analysis

The hematological profiles and survival rates of the catfish were analyzed descriptively and compared to normal values. In addition, the density of *E. tarda* bacteria in various organs was analyzed using a one-way analysis of variance, followed by Duncan's multiple range test when the significance level was set at $p < 0.05$.

Ethical approval

This study was conducted under the supervision and approval of the Faculty of Health, Medicine, and Natural Sciences at Airlangga University, with ethical clearance certificate number 85/UN3.1.16/2022.

Results

The hematological profile of catfish following cohabitation with *E. tarda*-carrier exhibited alterations compared to pre-challenge assessments (Fig. 1), particularly within treatment group T3 (2:1 ratio) when compared to other treatments. The total red blood cell counts (Fig. 1a), Hb concentrations (Fig. 1b), and total white blood cell counts (Fig. 1c) demonstrated a decline after cohabitation. Conversely, the percentages of neutrophils (Fig. 1d), monocytes (Fig. 1e), and lymphocytes (Fig. 1f) increased after cohabitation compared with pre-challenge values. The hematological profiles of the NC group also exhibited changes although these were more stable than those observed in the other treatment groups.

Bacterial counts in the liver, kidney, and spleen of silver rasbora showed *E. tarda* infection with bacterial densities ranging from 0.1 – 0.8 $\times 10^4$ CFU/ml across all organs examined (Table 1). In the study, the density of bacteria in the liver, kidney, and spleen of post-cohabitation catfish with silver rasbora carriers was observed, as shown in Table 1. The highest bacterial density was observed in treatment T3 (2:1), whereas the lowest bacterial density was observed in treatment T1 (1:2) for all catfish organs.

Catfish in all treatments, except the NC, exhibited 100% mortality by the 5th-day post-cohabitation (Fig. 2). Mortality began at 12 host infection (hpi) and increased exponentially starting at 72 hpi, reaching 100% at 120 hpi with *E. tarda*.

Discussion

Edwardsiella tarda is a bacterial pathogen that causes Edwardsiellosis, which results in various alterations in hematological parameters and severe fish mortality. *Edwardsiella tarda* can adapt to many habitats and infect several hosts, resulting in significant mortality rates (Goh *et al*., 2023). Edwardsiellosis in fish causes several alterations in total RBCs, WBCs, Hb levels, and differential leucocyte counts (Tirani *et al*., 2024). Catfish hematological parameters and bacterial abundance changed significantly after cohabiting with silver rasbora carrying *E. tarda*.

RBCs/erythrocytes, which are the most abundant blood cells in fish, play a vital role in the transportation of various substances (such as gases, water, minerals, nutrients, hormones, toxins, and metabolic waste products) within the body (Seibel *et al*., 2021). The decrease in the number of erythrocytes in catfish

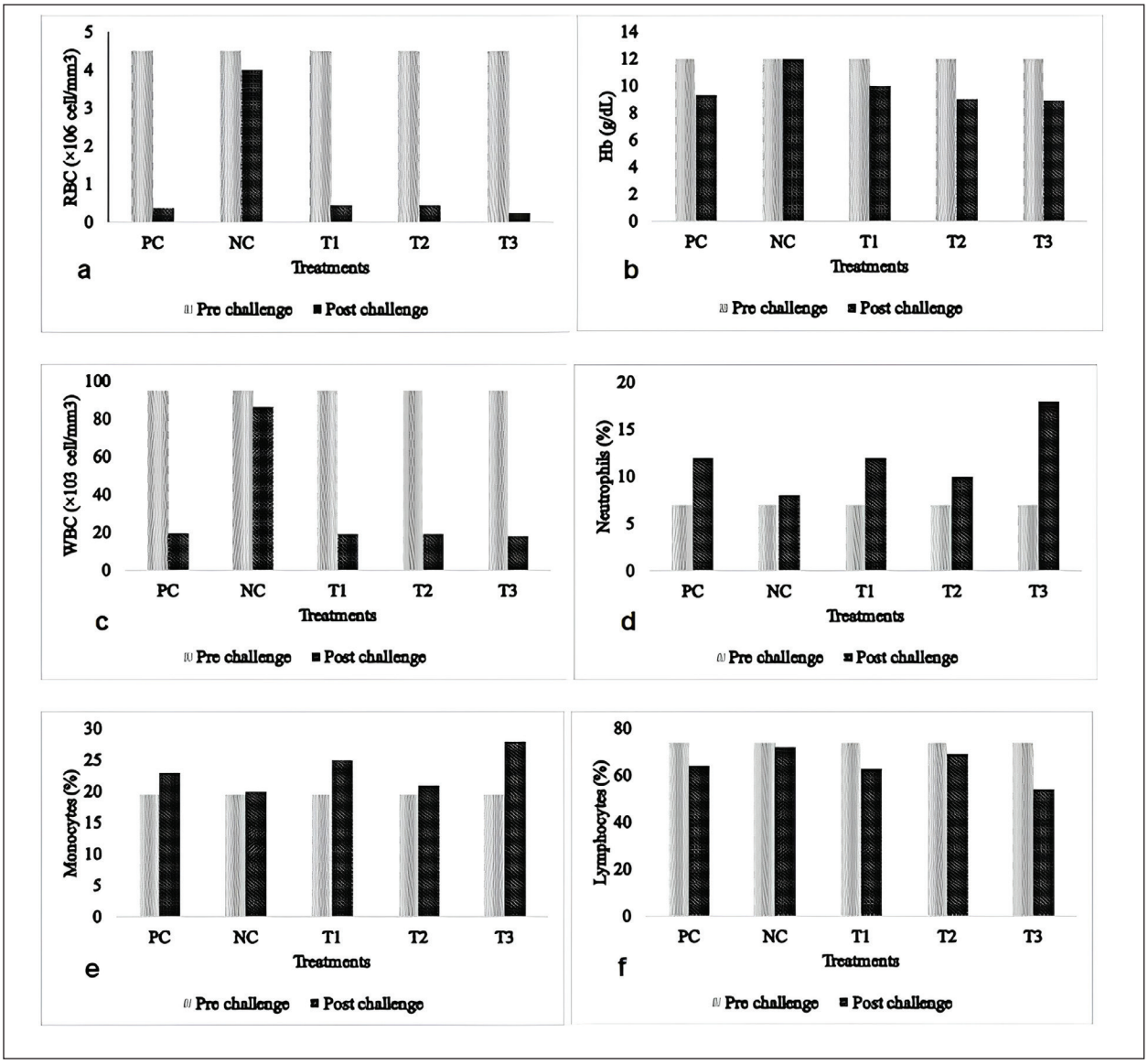

Fig. 1. Mean values for hematological parameters in catfish (*Clarias* sp.) pre- and postcohabitation with *E. tarda* carrier silver rasbora (*R. argyrotaenia*). PC: positive control; NC: negative control; *E. tarda* carrier silver rasbora cohabitate with catfish at ratios of 1:1 (T1); 1:2 (T2), and 2:1 (T3). a. RBCs, b. Hb, c. WBCs, d. Neutrophils, e. Monocytes, and f. Lymphocytes.

cohabitation with silver rasboras carrying *E. tarda*, particularly in treatment T3 (2:1), indicates that the catfish were affected by Edwardsiellosis. This finding is supported by the fact that the erythrocyte levels were significantly below the reference range of 3.02 ± 0.01 $\times 10^6$ cells/mm^3 (Fazio, 2019). Tjampakasari *et al*., (2024) reported that *E. tarda* secretes hemolysin toxins (EthA and EthB), which cause damage to the erythrocyte cell walls and result in cell lysis. Furthermore, Kalindamar *et al*. (2021) suggested that *E. tarda* hemolysin toxins facilitate adhesion and invasion within fish bodies, enabling intracellular bacterial survival. These findings are consistent with previous studies on tilapia (Sherif *et al*., 2021) and yellow catfish (*Pelteobagrus fulvidraco* (Chen *et al*., 2020) infected with *E. tarda*. The decrease in erythrocyte count corresponds to a reduction in Hb levels in catfish following cohabitation with *E. tarda* carrier silver rasbora in all treatments (except for the NC), dropping below the normal Hb level of (12.423 ± 2.21 g/dl) (Al-Deghayem *et al*., 2017). This decline can be attributed to the positive correlation between the total erythrocyte count and Hb level, with pathogen infections inducing anemia (Ahmed *et al*., 2020). *Edwardsiella tarda* is believed to encode the fur (ferric uptake regulator) gene, which enables the bacteria to exploit iron from Hb released during erythrocyte lysis

Table 1. Bacterial abundance of different organs in silver rasbora and catfish before and after cohabitation. Different superscript showed significant different ($p < 0.05$).

Organ	Bacterial abundance ($\times 10^4$ CFU/ml)				
	PC	NC	T1	T2	T3
Silver rasbora before cohabitation					
Liver	ND	ND	0.2 ± 0.04^{a}	0.6 ± 0.21^{b}	0.8 ± 0.2^{b}
Kidney	ND	ND	0.1 ± 0.01^{a}	0.4 ± 0.11^{ab}	0.4 ± 0.11^{a}
Lymph	ND	ND	0.3 ± 0.09^{a}	0.5 ± 0.32^{a}	0.6 ± 0.15^{a}
Catfish after cohabitation					
Liver	2.38 ± 0.09^{a}	ND	0.77 ± 0.17^{b}	0.86 ± 0.19^{c}	1.15 ± 0.11^{c}
Kidney	2.30 ± 0.06^{a}	ND	0.45 ± 0.14^{b}	0.54 ± 0.12^{bc}	0.68 ± 0.13^{c}
Lymph	0.95 ± 0.18^{a}	ND	0.28 ± 0.02^{b}	0.3 ± 0.03^{b}	0.43 ± 0.13^{b}

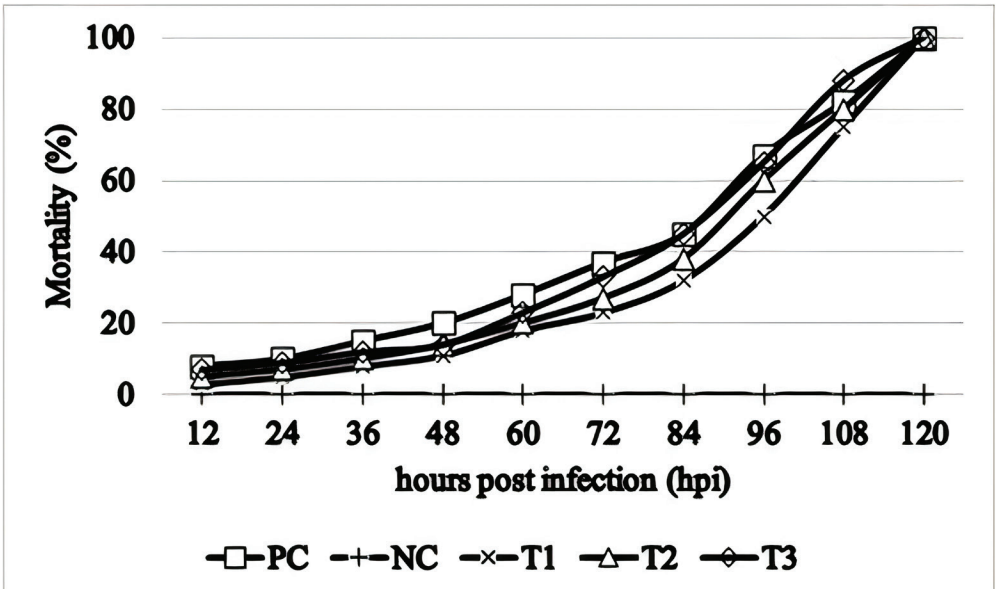

Fig. 2. Mortality graph of catfish (*Clarias* sp.) pre- and postcohabitation with *E. tarda* carrier silver rasbora (*R. argyrotaenia*) . PC: positive control; NC: negative control; *E. tarda* carrier silver rasbora cohabitate with catfish at ratios of 1:1 (T1); 1:2 (T2), and 2:1 (T3).

(Swain *et al*., 2020). Additionally, Lemos and Balado (2020) noted that *E. tarda* produces vibrioferrin, a compound similar to siderophore, which captures and extracts iron from heme-binding proteins in the host. Similar results were observed in yellow catfish (*Pelteobagrus fulvidraco*) (Chen *et al*., 2020) and Japanese flounder (*Paralichthys olivaceus*) (Sun *et al*., 2020) infected with *E. tarda*.

WBCs/leukocytes play a crucial role in fish immunity and overall health. Increased leukocyte levels are positively associated with the release of cytokines, phagocytosis activity, and inflammatory responses against pathogens (Mokhtar *et al*., 2023). In this study, we observed that leukocyte counts in catfish decreased following infection with *E. tarda* in all treatment groups, except for the NC. These leukocyte levels fell below the reference range of 20–150 $\times 10^3$ cells/mm^3 (Yanuhar *et al*., 2021), resulting in mortality by day 5 postinfection. This suggests the failure of the immune system to effectively combat *E. tarda* infection, leading to disease and death (Firdaus-Nawi and Zamri-Saad, 2016). Similar findings were reported in goldfish (*Carassius auratus*) experiencing Edwardsiellosis, where death occurred on day 5 post-challenge, accompanied by an initial leukocyte increase on day 1 post-infection (dpi), followed by a decline by day 5 (Choe *et al*., 2017). Transcriptomic analysis of Japanese flounder (*Paralichthys olivaceus*) revealed 21 genes associated with *E. tarda* infection, leading to disease and death (Sun *et al*., 2020).

Neutrophils, the first leukocytes to be activated at sites of infection, are responsible for pathogen neutralization through mechanisms such as the production of reactive oxygen species, neutrophil extracellular traps (NETs) (Buchmann, 2022), antimicrobial peptides, and proteolytic enzymes (Mokhtar *et al*., 2023). The percentage of neutrophils in catfish increased following cohabitation with *E. tarda* carrier silver rasbora in all treatment groups, especially in T3 (2:1). However, these values remained below the reference range for neutrophil levels in catfish, which is 26.2 ± 4.9% (Adamu and Solomon, 2015). This indicates that neutrophils were unable to effectively control *E. tarda* infection in catfish. This condition is suspected to be caused by premature activation of neutrophils, compromising their ability to engage in phagocytosis, NETs formation, and intracellular killing (Leliefeld *et al*., 2016). Boucontet *et al.* (2018) also suggested that a phenomenon known as neutrophil paralysis could further weaken the ability of neutrophils to neutralize invading pathogens in fish.

Monocytes play a critical role in the immune system of fish by acting as phagocytes and serving various functions during infection, inflammation, and tissue repair (Yang *et al*., 2021). They are found in bone marrow, blood, and spleen and can differentiate into macrophages upon entering tissues. Observations of monocyte percentages in catfish after cohabitation with *E. tarda* carrier silver rasbora showed an increase in all treatments, particularly in T3 (2:1), compared with pre-challenge values. However, this increase did not provide benefits to the catfish because *E. tarda* can survive within monocytes and reduce their phagocytic capacity. Tjampakasari *et al*. (2024) reported that the type VI secretion system (T6SS) of *E. tarda* plays a crucial role in adhesion, penetration, and replication within fish phagocytes. Ma *et al.* (2024) also reported that the qseB and qseC genes contribute to the intracellular replication of *E. tarda*, leading to systemic infection in fish.

Fish lymphocytes are generally similar to those in mammals, consisting of T and B cells that can express immunoglobulins (IgM, IgT, and IgD) (Sakai *et al*., 2021). Morphologically, fish lymphocytes are small, round cells with a thin, narrow rim of homogeneous basophilic cytoplasm surrounding a large, round, oval, or irregularly shaped nucleus (Witeska *et al*., 2022). The percentage of lymphocytes in catfish increased after cohabitation with *the E. tarda* carrier silver rasboras. However, this increase was still lower than the reference range for catfish lymphocytes (68%–76%) (Adamu and Solomon, 2015). The low lymphocyte percentage suggests that the catfish immune system failed to provide adequate protection against *E. tarda* infection. This condition is thought to result from immune suppression caused by the pathogen, leading to reduced cytokine production and decreased cytotoxic activity, particularly in T cells (Davoodzadeh Gholami *et al*., 2017) . Additionally, impaired B-cell maturation due to toxin activity from the pathogen hinders the production of immunoglobulins essential for defense against infections (Oakes *et al*., 2016).

Bacterial examination demonstrated the presence of *E. tarda* in silver rasbora without causing mortality, indicating a robust immune system in these fish, preventing *E. tarda* from establishing and enhancing its virulence (Ben Hamed *et al*., 2018) . This is supported by the fact that silver rasbora are not susceptible hosts for *E. tarda*. Buján *et al*. (2018) noted that *E. tarda* infections are more prevalent in catfish, eels, flounders, and turbots.

The presence of *E. tarda* in catfish organs suggests that the immune system, particularly phagocytic cells, was unable to eradicate the pathogen. Kupffer cells also known as macrophages, are found in the liver and play a crucial role in the early immune response to liver infection. They release proinflammatory cytokines and chemokines (Slevin *et al*., 2020; Hussein *et al*., 2023). Macrophages in the kidneys and spleens are organized into melanomacrophage centers (MMCs) that act as primary phagocytic cells (Bjørgen and Koppang 2024). MMCs can also serve as biomarkers for environmental contamination (Marteja and Modina 2021).

Edwardsiella tarda is an intracellular pathogen that can survive and coexist within macrophages. It enters cells through mechanisms such as macropinocytosis and endocytosis pathways involving cholesterol and dynamin compounds in the cell membrane (Hu *et al*., 2019). In phagocytic cells, *E. tarda* penetrates cells through similar pathways mediated by clathrin and caveolin compounds (Sui *et al*., 2017). Additionally, *E. tarda* regulates pro-apoptotic and anti-apoptotic genes by enhancing its survival and replication within host cells, preventing apoptosis, which is an immune mechanism for eliminating pathogens (Zhou and Sun 2016).

Horizontal transmission occurs from infected fish to healthy fish that cohabit in aquatic medium (Zaheen *et al*., 2022). Infected fish will excrete the bacteria through the skin, bile, feces, and urine and can infect other fish by ingestion or through the skin and gills. Furthermore, pathogens can also penetrate the physical defenses of the skin, gastrointestinal tract, and gills (Quintanilla *et al*., 2021). Horizontal transmission of *E. tarda* has been documented to exist in both the intraspecies (Phuoc *et al*., 2020) and different species of fish (da Costa *et al*., 2021). This condition is due to *E. tarda* is an opportunistic pathogen in water that can infect stressed and immunocompromised fish (Manzoor *et al*., 2023). Moreover, catfish are also susceptible to infection by *E. tarda* (Janda and Duman 2024).

The mortality of catfish on the 5th day post-cohabitation indicates that the catfish were infected with edwardsiellosis and are susceptible hosts for *E. tarda*. These findings are consistent with Butar Butar *et al*. (2020), who reported *E. tarda* infections in catfish

in North Sumatra, Indonesia. Additionally, Algammal *et al*. (2022) documented *E. tarda* infections in *O. niloticus* and *C. gariepinus* in Dakhlia Governore, Egypt, with clinical symptoms including excessive mucus production, abdominal swelling, operculum reddening, and fin and tail rot.

Conclusion

Catfish cohabitating with silver rasbora infected with *E tarda* experienced 100% mortality by the fifth day postinfection (dpi). Moribund fish exhibited altered hematological profiles, including increased percentages of neutrophils, lymphocytes, and monocytes, as well as decreased total erythrocyte, Hb levels, and total leukocyte counts. Furthermore, the liver was detected to have the highest concentration of *E. tarda* compared with other organs.

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Conflict of interest

The author(s) declare(s) that they have no conflict of interest.

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Authors' contributions

MFU, DK, EF: Supervision, Conceptualization, Formal analysis, Investigation, Methodology, Writing-original draft, writing review, and editing. WT: validation, investigation, and formal analysis. MAP: Formal analysis, investigation, writing original draft, writing review, and editing. JYL: writing-review and editing. All authors have read, reviewed, and approved the final manuscript.

Data availability

All data supporting the findings of this study are available in the manuscript.

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