RESEARCH ARTICLE

EFFICACY OF AUTOGENOUS FORMALIN KILLED WHOLE CELLS VACCINE AGAINST EDWARDSIELLOSIS IN STRIPED CATFISH (*Pangasionodon hypophthalmus*)

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ABSTRACT

This research aims to make a vaccine from *Edwardsiella tarda* isolated from local isolate. It was carried out at Aquaculture Laboratory of Aquaculture Department, Sriwijaya University. Bacterial isolate was a stock from Fish Quarantine and Inspection Agency, South Sumatera, Indonesia. Prior to vaccination, fish had already adapted to pellets and aquaria conditions. After 14 days post-vaccination (dpv) catfish was infected by *E. tarda* 10^7 CFU.ml^{-1} administrated by injection. The result revealed that vaccination can protect fish from edwardsiellosis better than non-vaccinated fish with Relative Percent Survival (RPS) value was 63.64 % and antibody titer increased from 7 dpv and greater at 14 dpv. In fact, the RPS value was less than 70% but there were no hematological disorders after vaccination. It means the whole cells vaccine can be used as a vaccine candidate to protect striped catfish from edwardsiellosis. Furthermore, some experiments need to be developed to increase RPS value in the future.

Keywords: Autogenous vaccine, *Edwardsiella tarda*, *Pangasianodon hypophthalmus*, whole cell vaccine

INTRODUCTION

South Sumatera is the largest province that produce striped catfish (*Pangasionodon hyphophthalmus*) in Indonesia. In 2019, South Sumatera striped catfish production almost 50% from total production of Indonesia (https://statistik.kkp.go.id). However, infectious diseases such as edwardsiellosis are a major problem in striped catfish culture because its infection can cause more than 50% mortality depends on virulency, environment and fish health status. Edwardsiellosis caused by bacteria *Edwardsiella tarda* and *E. ictaluri*. Moreover, both

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bacteria were reported to attack striped catfish in Indonesia which caused extensive mortalities in striped catfish cultured on cage, in Cirata Lake (Mawardi et al. 2018; Susanti et al. 2016). Thus, fish health management including prevention and treatment needs to be developed to avoid disease outbreaks in striped catfish.

Fish vaccination is already well known as prevention action in aquaculture to minimize risk of failure due to disease outbreaks (Ma et al. 2019). Some commercial vaccines have been released to fish farmers with various types of vaccine and their efficacy for some fish spesies. Vaccine efficacy is strongly influenced by several variables including: (i) antigen dose, exposure and absorption, (ii) improving vaccination strategies, (iii) adjuvant, (iv) water temperature, (v) fish size and life stage and (vi) type of vaccine, virulence and administration (Evensen 2020; Ma et al. 2019; Miccoli et al. 2021). The simplest type of vaccine or traditional vaccine is a killed whole cells vaccine. The advantages of this type are no risk of inducing diseases as they do not contain live component, provide sources of potential antigen and cheaper to produce than any type of vaccines (Adams, 2019; Ma et al. 2019). Autogenous vaccine is custom made that are produced based on local pathogen or isolated from farm which they are to be used in small to medium scale (Bwalya et al. 2020).

This is preliminary research to produce autogenous vaccine that expected able to protect local striped catfish from *E. tarda* infection. As hypothesis, the vaccinated fish has higher immunity than non-vaccinated fish. Furthermore, this research can be used as basic information to enhance vaccine efficacy in order to protect striped catfish better by boosting cellular immune response from edwardsiellosis.

MATERIAL AND METHODS

Time and Place

This research was carried out in June – August 2021 at Aquaculture Laboratory, Department of Aquaculture and Genetics and Biotechnology Laboratory, Department of Biology, Sriwijaya University.

Research Materials

Juvenile striped catfish were bought from local fish farmers with 10-11 cm in length for vaccination and 17-18 cm for collecting virulence type of bacteria. Fish were fed with commercial pellet (HI Pro-Vite 781-2, CP Prima). *E. tarda* isolate were collection from Fish Quarantine and Inspection Agency, South Sumatera. Medium culture was Brain Hearth Infusion Agar (BHIA) and Brain Hearth Infusion Broth (BHIB) (Oxoid, CM1136B, CM1135, ThermoFisher Scientific, UK). Neutral Buffer Formalin 10% (v/v) was made from 37% formaldehyde (Sigma Aldrich, Merck, Germany). Fish IgM Elisa kits (E0025FI, Bioassay Technology Laboratory, Sanghai Korain Biotech Co, Ltd). The equipment was nanospectophotometer (NanoDrop™ Lite Spectrophotometer, ThermoFisher, UK), thermal centrifuge (microfuge 20R- Beckman Coulter Life Science), Laminar air flow (Robust Laminar air flow), microhematocrit (Marinefeld Micro Hematocrit) and aquarium 50 x 50 x 50 cm^3.

Research Design

The research design was experimental study with two treatments which was vaccinated and non-vaccinated fish. As control of rearing, there was negative control which was no infection and no vaccination. Each treatment has 3 replications and 2 repetitions (Figure 1).

Work Procedure

The research divided into 4 parts including bacterial and growth conditions, vaccine preparation, fish vaccination and bacterial challenge. All procedures are described below.

Bacterial and growth conditions

The *Edwardsiella tarda* were used for antigen preparation and infection respectively. One colony from the stock isolate cultured on BHIA were transferred into 20 ml BHIB. It was grown in BHIB with hand shaking regularly for 24 h at room

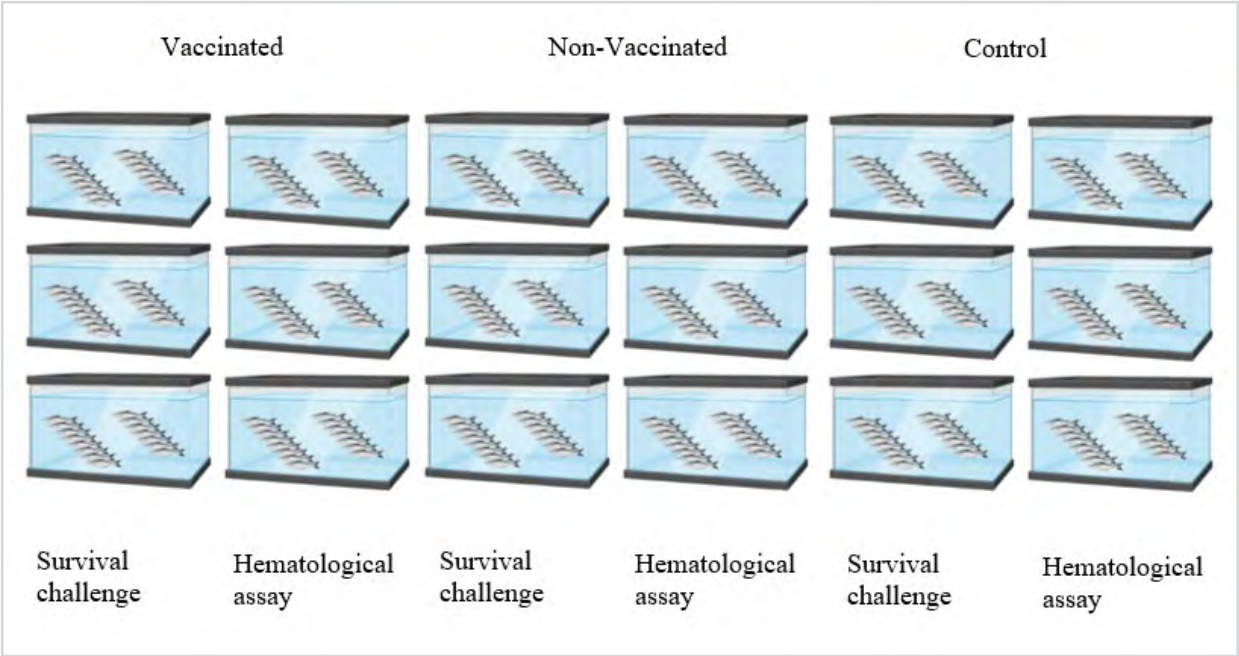

Figure 1 Research design of experiment created by BioRender.com (n=15 fish/aquarium)

temperature (25-26°C). 10 ml of pre-cultured cells were inoculated into 90 ml BHIB with the same condition. Prior vaccine production, the isolate had been tested to striped catfish for virulence factor. Re-isolation of bacteria from infected fish has been done with postulate Koch approach on BHIA. The total bacteria were counted with Total Plate Count (TPC) method.

Vaccine preparation

Whole cells vaccine with formalin-inactivated or killed methods was made as previously described by Cao et al. (2015) and Dwinanti and Fitrani (2016) with modifications as follows. Inoculant were diluted into final concentration 10^{11} CFU.ml^{-1}. Bacterial cells were inactivated by addition of 10% (v/v) neutral buffer formalin and incubated at room temperature for 24 h. The suspension was washed twice with phosphate buffer saline. Supernatant was separated by centrifugation at 10.000 rpm for 5 minutes 4°C. Sterility of vaccine was confirmed by viability test which was no growth of bacteria on BHIA plate. Absence of toxicity was confirmed by injecting the vaccine candidate into 10 striped catfish and if no death the vaccine ready to used.

Fish vaccination

The healthy fish were reared in aquarium with water volume was 15 L and fish density was 1 individu.L^{-1}. The vaccine was injected intramuscular 0.1 mL.Individu^{-1} while negative and positive were injected by phosphate buffer saline with same volume. Hematological tests (hematocrit, total leukocytes, titer antibody) were measured at day 7 and 14 post-vaccination (dpv).

Bacterial challenge

Striped catfish fingerling reared for 14 days post-vaccination (dpv) was injected with *E. tarda* (10^4 CFU.mL^{-1}) 0.1 ml per individual. The percentage of mortality, relative percent survival (RPS) and prevalence of infection on fingerlings were calculated 7 days after the challenge test.

Parameters

Hematological (hematocrit, total leukocytes and antibody titer)

Antibody titer was measured by using fish serum and ELISA kits by Bioassay Technology Laboratory. The curve standard was made before

measuring the sample. All protocols followed the manual procedure from the kit. Total leukocytes and hematocrit were measured according to Blaxhall and Daisley (1973).

Efficacy of vaccine (Relative Percent Survival, Prevalence Rate, Survival Rate)

Relative percent survival, prevalence rate and survival rate were calculated in the end of experiment. Relative percent survival (RPS), prevalence rate and survival rate were calculated using the following formula:

$$\text{RPS (\%)} = \frac{\text{1-\%mortality of vaccinated fish}}{\text{\%mortality of non vaccinated fish}} \text{ x } 100$$

$$\text{Prevalence rate (\%)} = \frac{\text{total of infected fish}}{\text{total of fish}} \text{ x } 100$$

$$\text{Survival rate (\%)} = \frac{\text{number of survived fish}}{\text{total initial of fish}} \text{ x } 100$$

Water quality

The water quality during research was well maintained such as pH, water temperature, ammonia and dissolved oxygen.

Data Analysis

This study used a completely randomized design method. Data obtained was collected using Microsoft Office Excel 2010 and analyzed using one way ANOVA method.

RESULTS

The efficacy of the vaccine can be represented by the relative percent survivor value (RPS) which is calculated from mortality in control group and vaccinated group after infection. Based on experimental, survival rate and prevalence rate are presented in Table 1 while Relative Percent Survivor (RPS) value of vaccine is presented in Table 2.

Vaccinated fish have better protection than unvaccinated fish. Based on the results of statistical tests, it was seen that the vaccinated fish produced significantly different survival rates and prevalence rate than the unvaccinated fish. During the study, fish that were not infected with the bacteria also showed high survival rates. This explains that the catfish rearing technique has been implemented properly.

Table 1 Percentage fish both survival and prevalence rate infected with *E. tarda* after 7 days post infection (dpi)

Treatments	Survival rate (%) $LSD_{0.05}$	Prevalence rate (%) $LSD_{0.05}$
Control -	$93.33^{c} \pm 6.67$	-
Control +	$51.11^{a} \pm 3.85$	$48.89^{b} \pm 5.44$
Vaccinated	$82.22^{b} \pm 3.85$	$22.22^{a} \pm 3.44$

Note: different manuscripts in the same column indicate significantly different (at least $p<0.05$)

Infected fish by *E. tarda* clearly show clinical sign like whiteish necrotic abscesses which is showed in Figure 2.

The antibody level after vaccination was increase after 14 days post vaccination (Figure 4). The experiment revealed that vaccinated fish produce antibody since 7 dpv and almost doubled increase in 14 dpv. The first 7 dpv, showed that antibody titer not significantly different ($p<0.05$) which means during the period the antibody not produced optimum yet. However, in 14 dpv titer antibody significantly different which vaccinated fish had higher 1.2-fold antibody titer than non-vaccinated. By using regression on standard curve (Figure 3), the amount of antibody for 7 dpv and 14 dpv were 237.7 $mg.ml^{-1}$ and 589 $mg.ml^{-1}$ respectively.

Table 2 Relative Percent Survivor (RPS) value of vaccine

Treatment	Mortality rate (%)	RPS (%)
Control -	6.77	
Control +	48.89	**63.64**
Vaccinated	17.78	

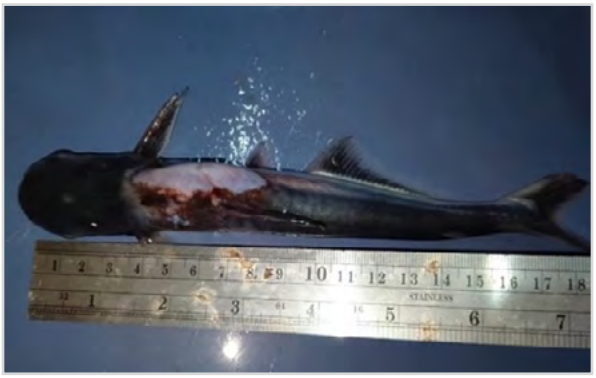

Figure 2 Striped catfish infected by *E. tarda* (→: whiteish necrotic abscesses)

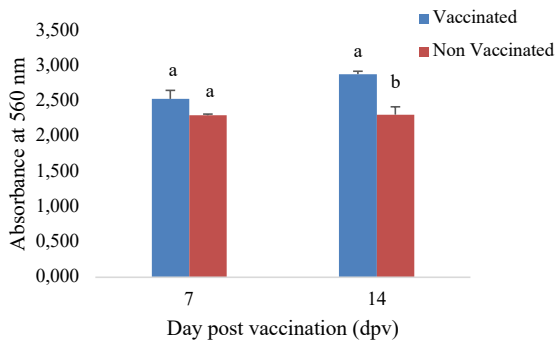

Figure 4 Antibody titer of striped catfish after vaccination

DISCUSSION AND CONCLUSION

Fish vaccination is revealed for some commercial fishes as a tool to prevent diseases outbreaks and avoid mass mortality due to pathogen infection (Gudding 2014). Vaccine is made from pathogen (antigen) or its any derivatives that able to stimulate fish immunity to produce antibody in order to protect fish from specific infection (pathogen is same with material vaccine). Various types of vaccine has been researched, developed, commercialized, successfully applicated and reviewed (Collins et al. 2019, Dwinanti et al. 2014; Ma et al. 2019, Nor et al. 2020).

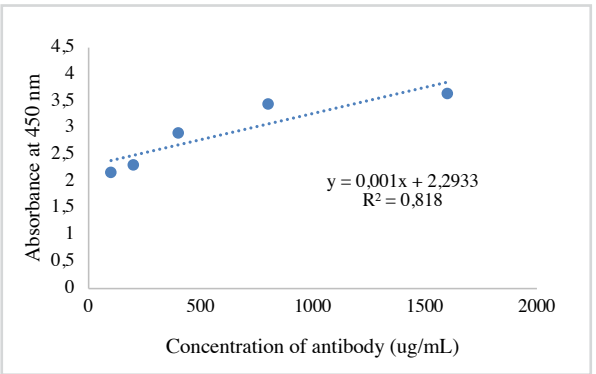

Figure 3 Curve standard of antibody titer

Edwardsiellosis or Edwardsiella septicaemia in fish has clinical sign such as necrotic abscesses in the muscle that can be whiteish or show petechia and mortality pattern may be acute or chronic depending on environment, virulency factor and fish immunity. *Edwardseilla tarda* that were used in this experiment was virulence type and clinical sign were observed after striped catfish injected (Figure 2). Transmission of *E. tarda* in catfish might be through the water from an infected source (carrier animal feces, water or mud) to susceptible fish since this bacterium is common in the aquatic environment (Evans et al. 2011).

Nowadays, fish farmers emphasizing on diagnosis and prevention of edwardsiellosis infections and promote health and production efficiency by environmental manipulation, proper nutrition and immunological protection such as vaccination (Hoque et al. 2020).

Vaccination in fish can trigger the fish immune response to produce antibody to protect fish from specific pathogen.

In general, the specific system of fish defense requires the presence of an antigen (vaccine). The antigen will initiate reactions and culminate in the increase of circulation of specific antibodies, besides promoting immune memory. Moreover, antigens that enter the body will be recognized and processed by the innate system by antigen presenting cells (APC- macrophages, dendritic cells and B lymphocytes), to process microorganisms in molecular units, and at first trigger immune response of proliferation, and in a second moment, the response of memory (Biller-Takahashi and Urbinati 2014). In line with antibody titer, the relative percent survival (RPS) revealed that the autogenous formalin killed whole cell vaccine able to protect striped catfish from edwardsiellosis (Table 2). RPS value in this experiment reached about 63.64% which indicates that the vaccine can be used for striped catfish farmer or developed to increase the protection value in the next future research. The similar result about whole cells vaccine also gives the RPS value of around 50-75% in different fish but the same type of vaccine (Sughra et al. 2021; Taukhid et al. 2018).

During experiment, water quality was maintained into certain condition which were temperature 28-30 ^{o}C, pH 6-7, dissolved oxygen 4-5 mg.L^{-1} and ammonia 0.07-0.17 mg.L^{-1}. The behavior of striped catfish was also monitored as control to environment disorder. Based on Nasional Standard of Indonesia, the conditions of fish rearing are 27-30 ^{o}C, 6.5-8.5 and >5 mg/l for temperature, pH and dissolved oxygen respectively (Standar Nasional Indonesia 2000).

To sum up, the formalin killed cells vaccine from autogenous bacteria had a good performance with efficacy of vaccine was 63.64%. It means that the vaccine can protect striped catfish from edwardsiellosis caused by *Edwardsiella tarda* and might cause mortality around 36.36 % when the outbreak occurs. The efficacy value needs to be increased in order give more protection to striped catfish. Thus, some researches are needed to be developed such as application of adjuvant into vaccine or modification of material for vaccine.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – SHD, T; Design – SHD, T, NAU; Resources – SP, SHD, MAR; Materials – SHD, T, NAU; Data collection and/or processing – NAU, MAR, RCM; Literature research - NAU, SP, SHD; Writing Manuscript – SHD, T, NAU; Critical Review - SHD, T, RCM

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EFIKASNOST AUTOGENOG CJELOSTANIČNOG CJEPIVA TRETIRANOG FORMALINOM PROTIV EDWARDSIELLOZE KOD PRUGASTOG SOMA (Pangasionodon hypophthalmus)

SAŽETAK

Cilj istraživanja je napraviti cjepivo korištenjem Edwardsielle tarda iz lokalnog izolata. Istraživanje je izvedeno u Laboratoriji za akvakulturu Katedre za akvakulturu Univerziteta Sriwijaya. Bakterijski izolat potječe iz zaliha Agencije za karanten ribe i inspekcijski nadzor iz Južne Sumatere u Indoneziji. Prije cijepljenja, ribe su već bile prilagođene na pelete i akvarijske uvjete. 14 dana nakon cijepljenja (dpv), somovi su inficirani sa E. tarda 107 CFU/mL-1 po injekciji. Rezultati su pokazali da je riba cijepljena protiv edvardsieloze bolje zaštićena od necijepljene ribe, pri čemu je vrijednost relativnog procenta preživljenja (RPS) iznosila 63,64%, a titar antitijela povećan sa 7 dpv na više od 14 dpv. Ustvari, RPS vrijednost je iznosila manje od 70%, ali nisu zabilježeni hematološki poremećaji nakon cijepljenja. Možemo zaključiti da su cjelostanična cjepiva potencijalni kandidati za zaštitu prugastog soma od edvardsieloze. Nadalje, u budućnosti je potrebno provesti određene eksperimente u svrhu povećanja RPS vrijednosti.

Ključne riječi: Autogeno cjepivo, cjelostanično cjepivo, Edwardsiella tarda, Pangasionodon hypophthalmus