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Detection of *Edwardsiella tarda* Infection of Catfish (*Clarias gariepinus*) in Central Tapanuli Regency, North Sumatra, Indonesia

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Abstract:*Edwardsiella tarda is one of zoonotic bacteria being able to colonize and infect across biological organisms. The species is regarded as important pathogenic bacteria in the field of aquaculture which is needed to be monitored and prevented for disease incidence. Recent investigation in Central Tapanuli as aquaculture site for catfish (Clarias gariepnius) revealed that there was a high incidence (25-30%) of detected E. tarda based on culture-based method following the methodology of Indonesian National Standard. The study sites namely Site I, II, III, and IV showed a differences in proportion of E. tarda while mud pond showed a higher incidence than tarp pond. Infected C. gariepinus showed a morphological sign of lesions on body surface indicating the infection of E. tarda. Based on target organ infection, the highest detection (%) was obtained from kidney followed with muscle, lymph, and liver with the percentage of 47.62, 44.76, 5.71, and 1.90%, respectively. Confirmation of E. tarda was further performed by testing co-agglutination method on catfish samples. By comparing the proportion of detection with serological test, there was no significant differences between culture-based technique and immunological test (P>0.05).*

Keywords: *Clarias gariepinus, Edwardsiella tarda, North Sumatra*

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I. Introduction

Indonesian aquaculture relies heavily on extensive and semi-intensive fishpond through integrated or non-integrated (conventional) concepts (FAO, 2005). Catfish (*Clarias gariepinus*) is an important species as a freshwater commodity in Indonesia. Its production is raised annually. However, disease occurring within aquaculture of this species may hinder the fulfill of mass production target leading to low selling price and low fish consumption by the community (Tran et al., 2017). Indonesian Ministry of Fisheries has a target to increase catfish production by 35% in the consecutive year, starting from 670,000 tons in 2013 by applying improvement in production, marketing and disease management (DJPB KKP, 2013). Multifactor interaction or abiotic-biotic relationship in aquaculture environment may trigger disease incidences in freshwater fishpond (Matthew et al., 2014). Among significant micoorganisms, pathogenic bacteria are regarded as primary infectious agents in aquafarming practices which cause serious decline in global fish production (Camus et al., 1998; Mohanty and Sahoo, 2007).

Edwardsiella spp. is a group of important pathogenic bacteria in aquaculture from freshwater to saline with interspecific infection (Subasinghe, 2005). The infected fishes may produce clinical signs such as abnormal swimming behavior, physiological disorders (hematological) and organ disruption regarding the disease severity and infection level (Buller, 2014). There are currently four species *E. tarda, E. hoshinae, E. piscicida,* and *E. ictaluri* (Park et al., 2012; Reichley and Ware, 2015). Biochemical characteristics of these species are facultative anaerobic organisms, gram negative, negative oxidase, positive catalase, and poor carbohydrate utilizers (Wang et al., 2011).

Edwardsiella tarda is categorized as one of zoonotic pathogenic bacterial species which is able to infect humans (Gormaz et al., 2014). *Edwardsiella tarda* is an elusive opportunistic pathogen with wide ecological niche and broad host specificity starting from fishes, reptiles and humans (Alcaide et al., 2006; Waoo and Bruno, 2011). In monitoring practice, there is an urge to survey for any possibilities in disease outbreak or epidemic caused by *E. tarda*, especially for a country with significant production of freshwater commodities.

Detection and epidemiological studies on *E. tarda* infection in various fish species has been documented in Indonesia. *Pangasius pangasius* or catfish was reported to be infected by *E. tarda* with target

organs of colonization in cloacal, abdominal, and intestinal region by immunochemistry technique (Andriyanto et al., 2009). Four Indonesian strains of *E. tarda* were detected from tilapia which possessed similar genetic characteristics with human pathogenic strain (Narwiyani and Kurniasih, 2011). In addition, *E. tarda* heavily infected *lele dumbo* or *C. gariepinus* in Palembang, city of South Sumatra (Yuliantoro et al., 2017). According to previous informations, infection prevalence of *E. tarda* from other fish species may also be documented through field observations and laboratory investigations.

Aquaculture practices in North Sumatra still apply conventional management run by local communities. Disease transmission is theoretically frequent in conventional fishpond, supported by unstable environmental condition which later become stressors to fish commodity. Stresses play a significant role in opportunistic bacterial infection, in specific to *E. tarda* (Waoo and Bruno, 2011). There is a possible finding on high prevalence of *E. tarda* infection, one of which occurring in Central Tapanuli, known as a massive fishpond region adjacent to freshwater region of Lake Toba. Surveys are then conducted to detect any presence of *E. tarda* in study region through conventional and serological test.

II. Material And Methods

Study site and sampling methods

Central Tapanuli is a regency in North Sumatra, known for its conventional aquaculture practice of catfish (*Clarias gariepinus*) along with other common fish commodities in four sites, assigned as Site I, II, III, and IV as representative study sites. Fishponds are managed by local communities, characterized with two types of substrates, i.e mud and tarp pond.A cross-sectional study was started by exploring equal number of fishponds managed in different sites within each site. Sampling size was calculated based on Naing et al (2006) with 50% possibility of infected catfishes in which resulted into 387 samples, fulfilled into 400 individu and with confidence interval of 95%. For each sites, 100 individu of catfishes were randomly caught from each 50 mud and 50 tarp ponds origin. Catfishes were preserved in cold condition prior laboratory investigation. Fish samples were slaughtered by pithing (Medgela et al., 2006), while organ dissection was performed to observe any clinical signs of Edwardsiellosis from kidneys, livers, muscles and lymphs. All dissected organs were preserved in 4^{o}C in Nutrient broth (NB) medium prior isolation.

Isolation of *Edwardsiella tarda*

Isolation technique in recovering *E. tarda* from organ samples was based on *Standard Nasional Indonesia* (SNI 7663:2011, ICS 65.150). Briefly, *Clarias gariepinus* was visually observed for any clinical signs of Edwardsiellosis. Target organs were crushed using pistilles and aseptically streaked by using loop into sterile Tryptone Soy Agar (TSA) and Brain Heart Infusion Agar (BHIA). Plates were incubated at 26^{o}C for 24-48 hr. Single colony of *E. tarda* was tested for its morphological, biochemical and gram staining characteristics. Confirmation of presumptive colony of *E. tarda* by transferring a loopful of colony into *Edwardsiella ichtaluri* Medium (EIM). The presence of green colony with dark center indicate a positive result of *E. tarda* occurrence in samples.

Serological test of *Edwardsiella tarda* isolates

The serological test to confirm the presence of *E. tarda* was based on co-agglutination test by Yoshimizu and Kimura (1985). Target organs were homogenized with physiological saline solution (1:1, w/v) and heated in 100^{o}C for 30 min. The homogenates were centrifuged at 4000 rpm for 20 min. Supernatants were collected and spotted on object glass. The co-agglutination kit was spotted on top of supernatants. Positive control used the antigen of *E. tarda* ATCC 15947 while negative control used *E. ictaluri* type strain NCIMB 13272.

Data analysis

Numerical data obtained in this study were analyzed descriptively and displayed in proportion and percentage (%). Chi-square test of independence was performed using Minitab ver. 16.0 in comparing occurrence of *E. tarda* in respective to fishpond types, and target organs. A confidence interval of 95% was used to interpret statistical association and significance was considered when *P*-value <0.05. Secondary data or environmental parameters i.e temperature (*T*), pH, and dissolved oxygen (DO) were also documented as supporting data (Table 1).

Table 1. Physicochemical characteristics of water quality among different sites and substrate types in Central Tapanuli regency

No	Parameter	Site							
		Site I		Site II		Site III		Site IV	
		Mud	Tarp	Mud	Tarp	Mud	Tarp	Mud	Tarp
1	*T* (°C)	26.0	26.6	26.9	26.9	25.3	27.1	26.6	26.5
2	pH	6.2	6.6	5.8	6.7	6.0	6.5	6.2	6.7
3	*DO* (mg/L)	4.1	4.8	3.9	4.8	4.3	4.6	4.0	5.0

III. Results and Discussion

Total of 400 catfish samples were surveyed in Central Tapanuli regency revealing a slight differences in detected proportion of E. tarda (Figure 1). The higher proportion of E. tarda was observed from site III with the percentage of 30%, followed by site II, IV, and I with the percentage of 25, 24, and 24%, respectively.

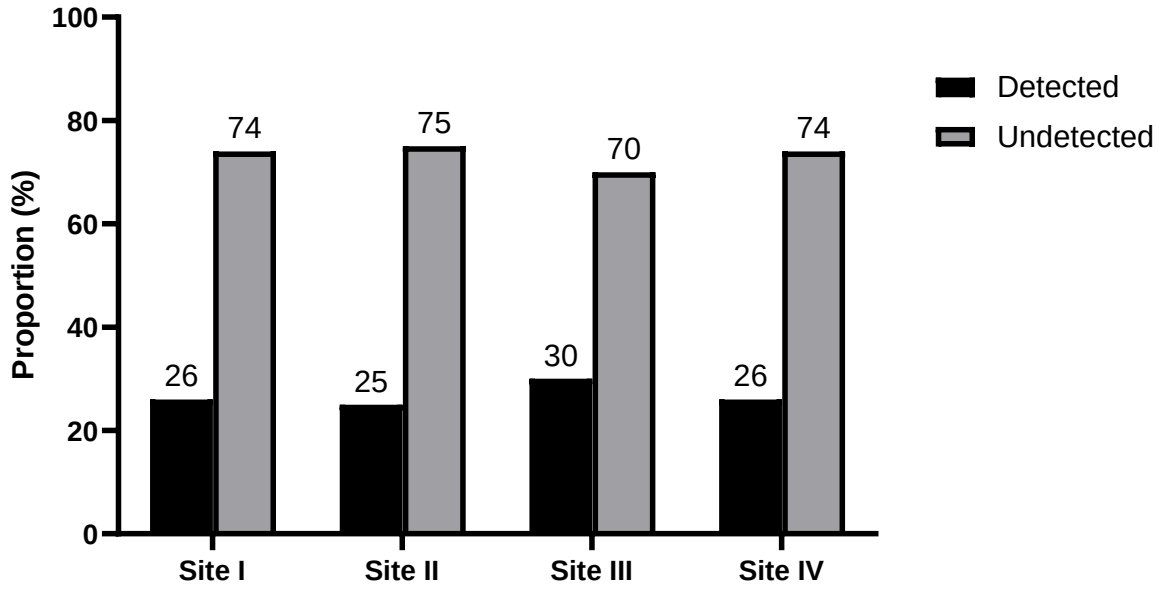

Figure 1. Proportion (%) of E. tarda in catfish (C. gariepinus) in respective to different sites in Central Tapanuli regency, North Sumatra, Indonesia

The high percentage of E. tarda presence in site III may be due to unstable or fluctuative environmental condition in aquaculture site, such as higher water temperature, poor water quality, and high organic content in fishpond (Park et al., 2012). However, our data on environmental condition did not imply any significant value to the transmission of E. tarda regarding its survivability in fishpond (Table 1). Therefore, the differences in infection prevalence may also depend on dimension of fishpond and catfish population in aquaculture pond. According to previous survey in Bihar and West Bengal, India, it revealed a percentage of 14.41% of E. tarda infection among 118 commercial fishes, with higher prevalence in semi-natural pond than in fishpond (Kumar et al., 2016). Infection prevalence of E. tarda was also documented from natural habitat in C. gariepinus by reaching the percentage of 23.52% from Lake Hawassa, Ethiopia (Nemo et al., 2017).

Our finding on high proporiton of *E. tarda* in fish commodity should be considered as serious threat for any future possible bacteremia or Edwardsiellosis. However, clinical case or any reported disease severity caused by this pathogen may be regarded as rare case in human with health importance (Hirai et al., 2015). Hence, this pathogen is also reported to colonize the human intestinal tract with the percentage of 80% population in fecal samples (Janada dan Sharon, 1993). Based on fishpond types, infection prevalence of *E. tarda* in all sites showed a higher percentage in mud pond with the range of 24 to 28%. In other case, the prevalence was higher in tarp pond at site III with the percentage of 32% (Figure 2).

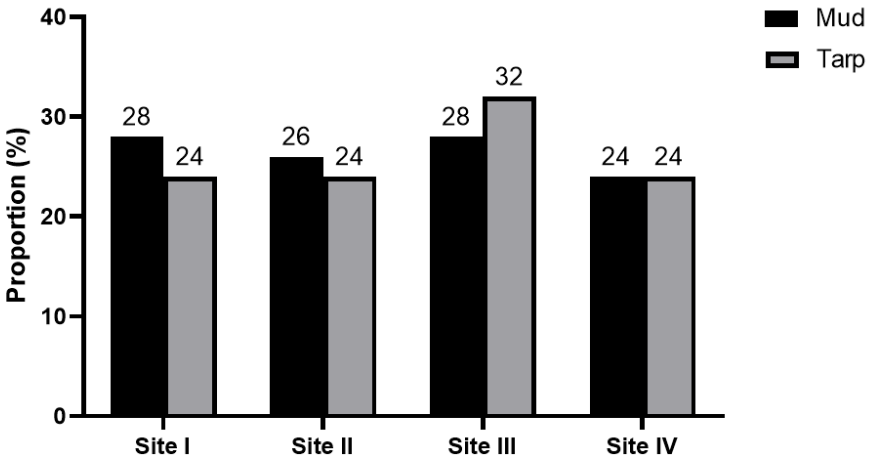

Figure 2. Proportion (%) of *E. tarda* presence in different sites based on fishpond type (*N* = 50 individu/pond)

The niche flexibility of *E. tarda* thoroughly support its growth and persistence across host specificity and different habitats. Previous study reported that 75% of pond water was detected being colonized by *E. tarda* while 64% among other bacterial species was also recovered from mud as the substrate type for aquaculture site of catfish in Texas, USA (Wyatt et al., 1979). The ecological study of *E. tarda* distribution in Fukuyama region, reported that 86% of *E. tarda* population was recovered from pond water, 44% from sediment or mud, while 14% from the fish commodities (Rashid et al., 1994). Our results may show that the pond water used four different sites, may be heavily polluted by *E. tarda* whichindicate poor water resources for catfish aquaculture in Central Tapanuli regency.

Based on target organ colonization, we obtained two heavily-colonized organs in *C. gariepinus* which were kidneys and muscles with the percentage of 47.62 and 44.76%, followed by lymphs with 5.71% and livers with 1.90% (Figure 3). However, no significant differences was observed from two pond types in organ colonization although the tarp pond revealed a higher percentage than mud in all organs (*P*>0.05) (Figure 4, Table 2).

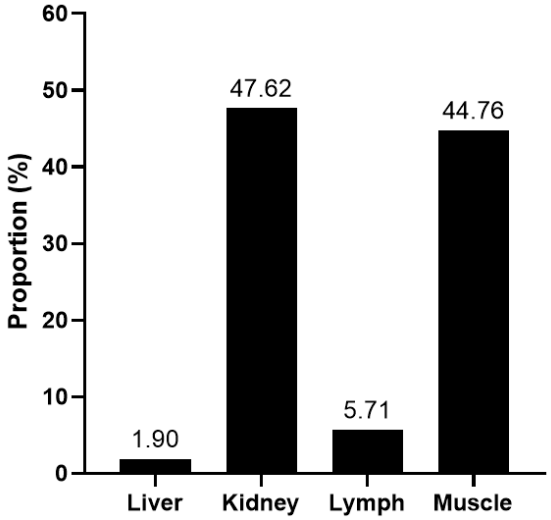

Figure 3. Proportion (%) of *E. tarda* presence in different target organ for colonization in *C. gariepinus*

Table 2. Chi-square (χ^2) analysis of differences in *E. tarda* presence between pond type

	Total *N* = 105		Mud *n* = 53		Tarp *n* = 52			
	N	%	*N*	%	*n*	%	χ^2	*P*-value
Liver	2.00	1.90	2.00	3.77	0.00	0.00	2.012	0.571
Kidney	50.00	47.62	25.00	47.17	25.00	48.08		
Lymph	6.00	5.71	3.00	5.66	3.00	5.77		
Muscle	47.00	44.76	23.00	43.40	24.00	46.15		

Kidney was the massively colonized organ by *E. tarda* in *C. gariepinus* in this study. The high incidence may be due to high organic content or pollutant and poor water quality in pond water which increase the physiological activity of kidney higher than usual. Nemo et al (2017) argued that the presence of *E. tarda* and other strains in kidney and liver of aquatic organisms may be result of accumulative damages with subclinical signs supported by poor environmental condition exposed in aquaculture which later suppress the immunity of aquatic organisms. However, many clinical signs may be imposed from different host as evidenced from previous reports.

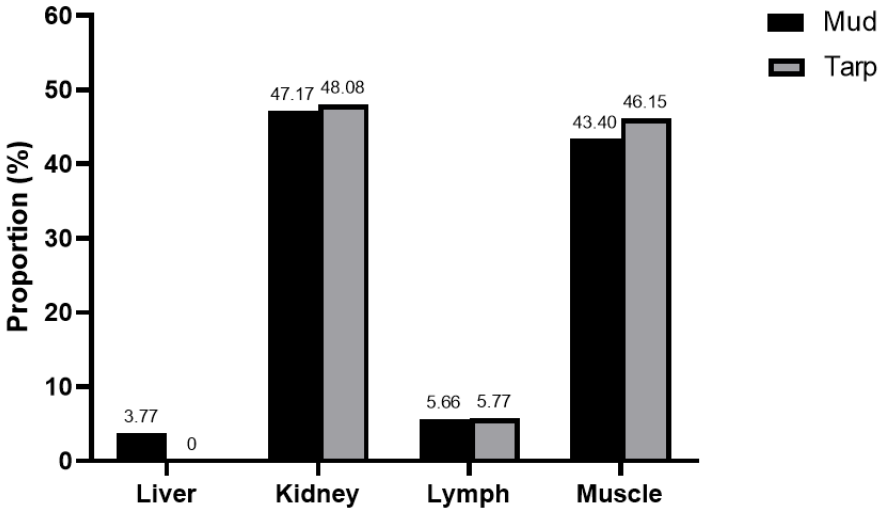

Figure 4. Proportion (%) of *E. tarda* presence in different organs based on fishpond type (*N* = 105 ind.)

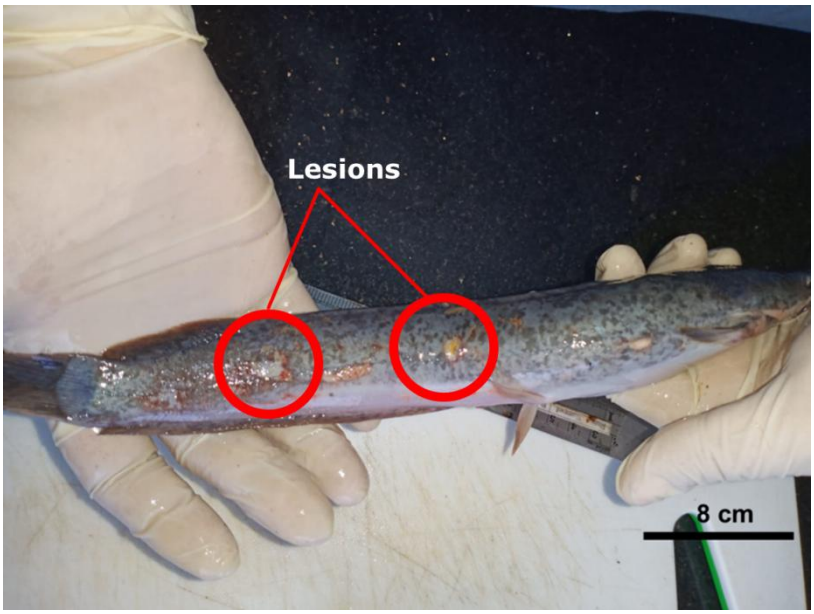

Figure 5. Presence of lesions on the surface of the skin of *C. gariepinus* from Central Tapanuli regency

Majority of *E. tarda* infections in catfish (*Pangasius pangasius*) originating from kidney organs showed pathophysiological features in the form of loss of skin pigmentation due to lesions, abdominal swelling, hemorrhage in the fins, and necrosis in the fin area (Murwantoko et al., 2019). In addition, previous studies of

African *C. gariepinus* in Lake Hawassa, Ethiopia showed that the percentage of *E. tarda* colonization in succession to kidney, liver and intestine organs was 0.9, 1.9, and 5%, respectively. Based on comparison of the results of previous studies, it shows that *E. tarda* colonization of target organ of aquatic biota may differ between natural and artificial habitats.

Liver was detected as the target organ with the minimum percentage of colonization by *E. tarda* in catfish in this study. *Edwardsiella tarda* was able to infect the liver of *C. gariepinus* by showing pathophysiological features in the form of swelling of the liver and pale hepatic (liver) (Arimbi et al., 2017). The most common feature for infected catfish in Central Tapanuli regency is only lesions on the surface of the skin (Figure 5). Meanwhile, the pathophysiological characteristics of each catfish's internal organs cannot be observed as a sign of *E. tarda* infection.

Confirmation of *E. tarda* presences in our samples were tested by biochemical or co-agglutination kit. The presence of granular or clumping suspension in samples indicate a positive result of test based on visual inspection by the complex reaction between the agglutinin kit and dissolved *E. tarda* antigens in samples (Figure 6). There is no significant differences ($P>0.05$) between culture-based and serological method in confirming *E. tarda* isolates from *C. gariepinus* in Central Tapanuli. Here, we may conclude that isolation-based identification of *E. tarda* or the conventional method following the Indonesian national standard (SNI) is still reliable in producing good results of detection. However, specificity of detection may be further improved through the molecular detection for differentiating *E. tarda* out of *E. tarda* –like bacteria.

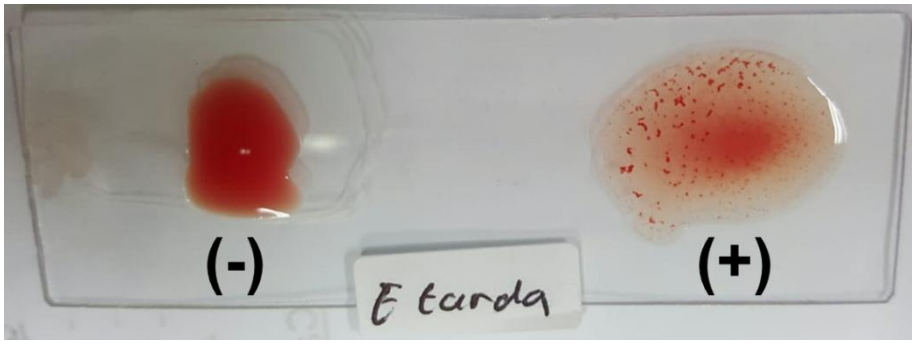

Figure 6. Detection of *E. tarda* using the serological co-agglutination method. The presence of granular suspension in sample indicate a positive result (+) or the presence of *E. tarda* in sample

Table 3. Chi-square (χ^2) analysis of differences in *E. tarda* presence between detection test

Mud						
	Conventional $n = 53$		Serology $n = 50$			
	n	%	*n*	%	χ^2	*P*
Liver	2.00	3.77	2.00	4.00	0.196	0.978
Kidney	25.00	47.17	23.00	46.00		
Lymph	3.00	5.66	2.00	4.00		
Muscle	23.00	43.40	23.00	46.00		
Tarp						
	Conventional $n = 52$		Serology $n = 51$			
	n	%	*n*	%	χ^2	*P*
Liver	0.00	0.00	0.00	0.00	0.097	0.953
Kidney	25.00	48.08	26.00	50.98		
Lymph	3.00	5.77	3.00	5.88		
Muscle	24.00	46.15	22.00	43.14		

IV. Conclusion

Edwardsiella tarda is detected in the proportion of 25-30% of total catfish (*Clarias gariepinus*) sampled from Central Tapanuli, North Sumatra, Indonesia. Based on fishpond types, the incidence is detected higher in mud pond than tarp pond. The highest percentage of colonized target organ in catfish is kidney (47.62%), followed with muscle (44.76%), lymph (5.71%), and liver (1.90%). The clinical sign of majority infection by *E. tarda* is shown by the presence of lesions on the catfish body. The detection based on serological test by testing similar samples shows that there is no difference between culture-based or conventional detection and immunological detection ($P>0.05$).

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