MOLECULAR STUDY OF ISOLATES *Edwardsiella tarda* FROM SEVERAL ISLANDS IN INDONESIA

SITI NARWIYANI*, KURNIASIH**, ASMARANI K.**

*Balai Besar Karantina Ikan Hasanuddin Makassar

**Fakultas Kedokteran Hewan -UGM,Yogyakarta

ABSTRACT

Edwardsiella tarda is an enteric pathogen that causes diarrhea, wound infection, and death due to septicemia. This species is capable of invading fish and human epithelial cell lines, and *E. tarda* escapes from the endocytic vacuole within minutes of entry and then replicates within the cytoplasm moving directly from cell to cell. *Edwardsiella tarda* was much more frequently isolated from lake fish. The aim of research was to find out the strain variation of several isolates of *E. tarda* from different islands in Indonesia. Four isolates of *E.tarda* from catfish (Java and Sumatra), *Cyprinus carpio* (Kalimantan), Brazilian turtle (imported), and ATCC from Singapura were identified by morphology. All isolates were DNA extracted and DNA amplified by PCR. Products of amplification were digested with *Alu*I and *Hae*III enzymes (RFLP) for 1 hour. They were visualized with electrophoresis in 8% polyacrylamid gel and stained with silver. The restriction endonuclease result showed that 3 isolates from fish had the same bands. Two isolates from imported turtle had the same band as ATCC isolates. Digestion with *Hae*III enzyme gave more different bands than with *Alu*I enzyme. It was thought that isolates of *E. tarda* from turtle and from ATCC were originated from human.

Keyword : *Edwardsiella tarda*, PCR, RFLP

INTRODUCTION

Edwardsiella tarda is an enteric pathogen that causes diarrhea, wound infection, and death due to septicemia. This species is capable of invading fish and human epithelial cell lines, and *E. tarda* escapes from the endocytic vacuole within minutes of entry and then replicates within the cytoplasm moving directly from cell to cell (Strauss *et al.,* 1997). *Edwardsiella tarda* was much more frequently isolated from lake fish (Van Damme & Vandepitte., 1980). Eye tumefaction, inflammation, haemorrhages, ascites and the presence of a purulent fluid were the main macroscopic lesions observed (Padros *et al.,* 2006). Internal organs were congested, and livers showed patchy discoloration and petechiae. Histologically, the liver, kidney, and spleen had severe multifocal necrotizing inflammation. No bacteria were isolated from infected fish after 6 d Post infection (Darwish & Plumb, 1989; Sahoo *et al,* 2000). Clinical signs and internal gross lesions declined by 8 days post infection and were absent thereafter.

MATERIALS AND METHODS

Five isolates of *Edwardsiella tarda* were identified conventionally in the Microbiology laboratory, Faculty of Veterinary medicine, Gajah Mada University. Each isolate ($2mlx10^8$) of *Edwardsiella tarda* was DNA exctracted with Qiagen kit, Germany. They were amplified with specific primers of *E. tarda* from fish. Eta 2-351 F (5'-TAG GGA GGA AGG TGT GAA-3'). Edwsp-780 R (5'-CTC TAG CTT GCC AGT CTT-3'). And from human Eta 1-363 F(5' – GTG TCC GTG TTA ATA GCA - 3').

The PCR programme was denaturation in 94^0C for 2 minutes, following by 26 cycles of programme in 94^0C for 30 seconds, 50^0C for for 30 seconds, 72^0C for 1 minute, and final extension in 72^0C for 5 minutes.

The PCR products were then digested with *Alu*I and *Hae*III enzymes for one hour. All digestion products were visualized with electrophoresis in 8% polyacrylamide gel and stained with silver.

RESULT AND DISCUSSIONS

The isolation and identification of 5 different isolates showed the same result (Table 1)

Table 1. The result of biochemistry characteristic of *Edwardsiella tarda*

Media	ATCC	Kalimantan	Sumatra	Java	Brazilia
Gram test	-	-	-	-	-
Oxidase	+	+	+	+	+
Catalase	+	+	+	+	+
Motility	+	+	+	+	+
Indole	+	+	+	+	+
H_2S in TSIA	+	+	+	+	+
Voges-proskover	+	+	+	+	+
Urea hydrolysis	-	-	-	-	-
Lysine decarboxylase	+/-	+/-	+/-	+/-	+/-
Ornithine decarboxylase	-	-	-	-	-
Gelatine hydrolysis	+	+	+	+	+
Gas of glucose	+	+	+	+	+
Acid of:					
Glucose	+	+	+	+	+
Lactose	+/-	+/-	+/-	+/-	+/-
Sucrose	+	+	+	+	+

The result of DNA extraction had the different contents of DNA, and the DNA amplification product showedt he bright band in 216 bp.(Figure 1). It is the same as reported by Baird *et al.* (2003). Isolate of *Edwardsiella tarda* from imported turtle could only be amplified with human primer.

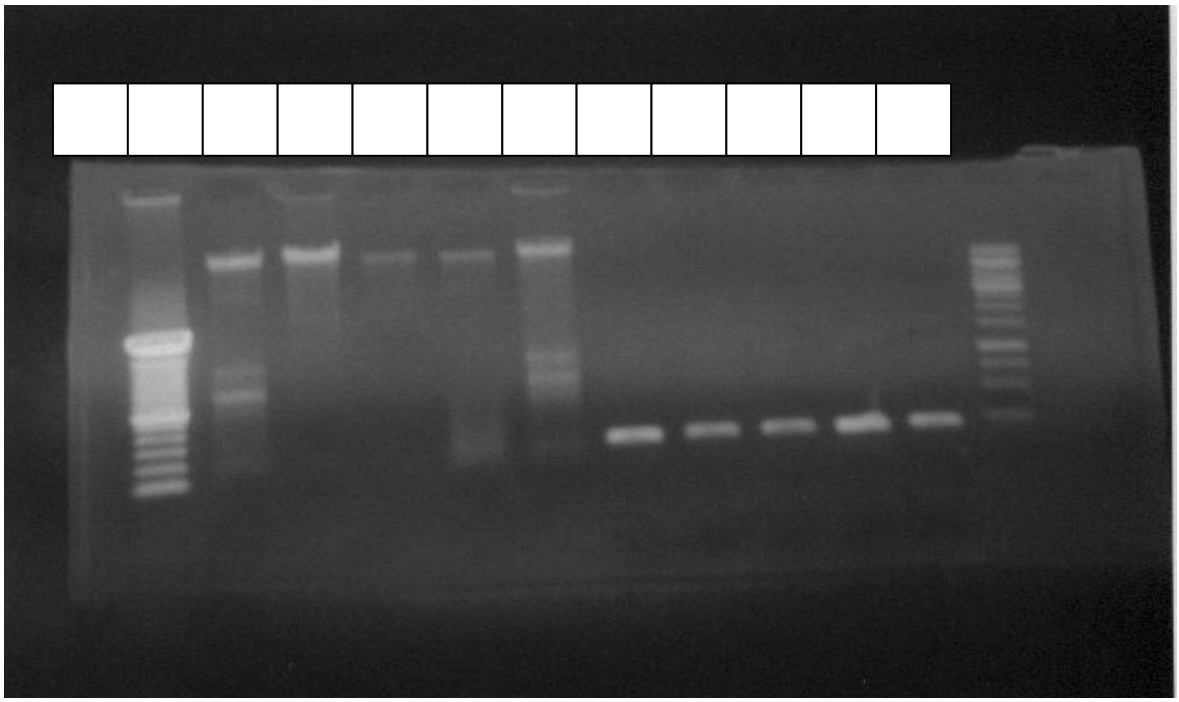

Figure 1. The result of DNA extraction of *E. tarda* (lane 1-5), and the PCR product of *E. tarda* (lane 6-10). Lane 1, 2, 3, 4, and 5 were isolate from ATCC, Kalimantan, Sumatra,Brazil, and Java. The sequence of lane 6-10 was the same as lane 1-5.

The endonuclease restriction using *Alu*I enzyme had no different among 5 isolates of *Edwardsiella tarda* (Figure 2). However, using *Hae*III enzyme showed 2 different groups of endonuclease restriction sites. Isolate of *E. Tarda* from fish in Kalimantan, Java and Sumatra had the same bands restriction. Isolate of *E. Tarda* from imported turtle showed the same band as isolate of *E. Tarda* from ATCC (Figure 3).

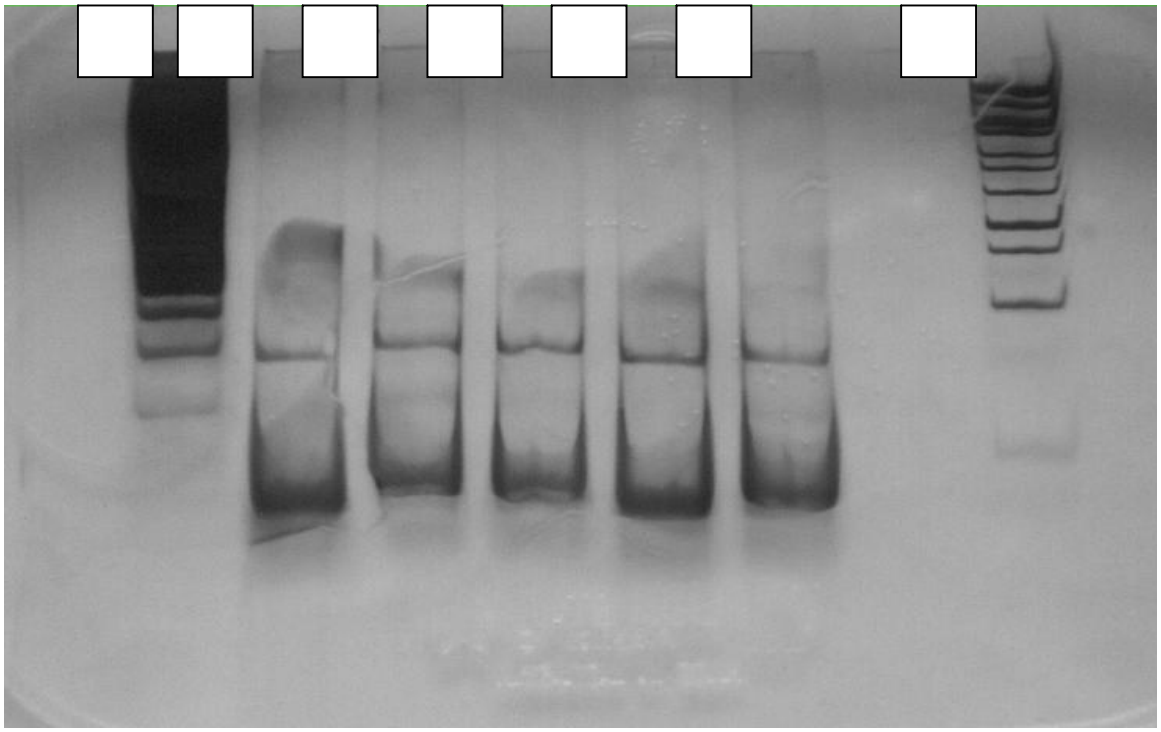

Figure 2. The RFLP result of *E. tarda* (lane 1-5) used *Alu*I enzyme. The sequens isolate in lane 1, 2, 3, 4, and 5 were isolate from ATCC, Kalimantan, Sumatra,Brazil and Java.

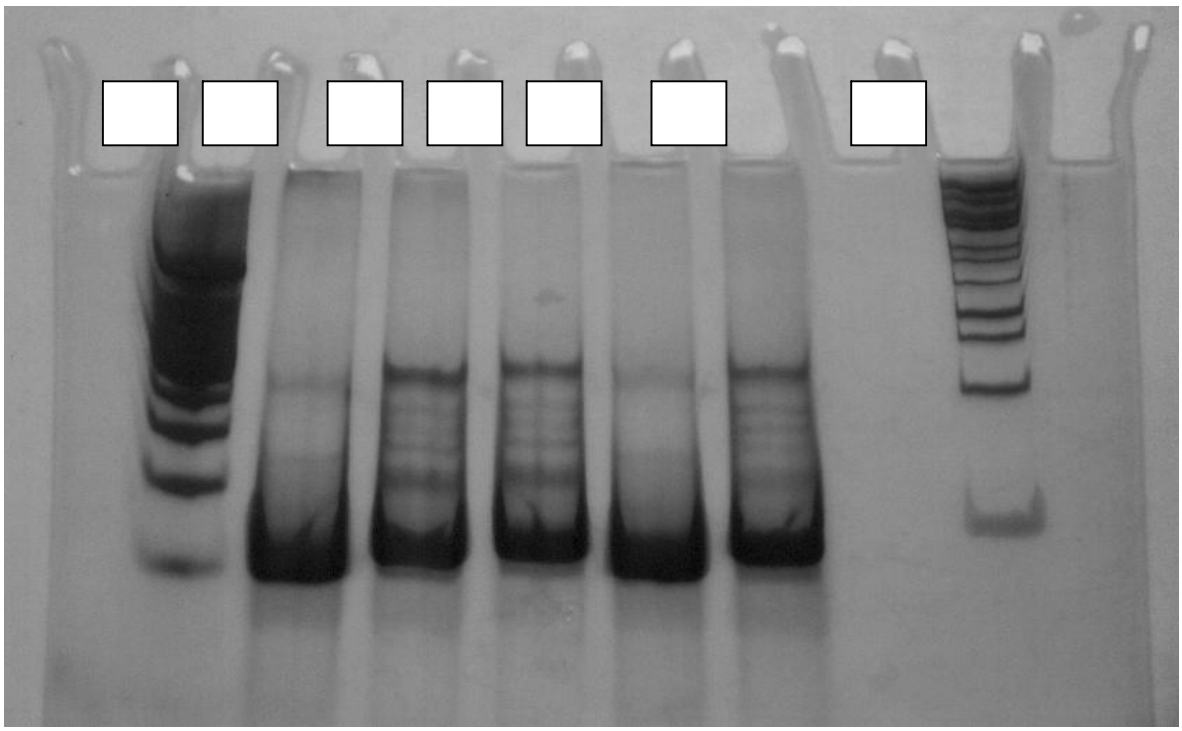

Figure 3. The RFLP result of *E. tarda* (lane 1-5) used *Hae*III enzyme. The sequence Isolates in lane 1, 2, 3, 4, and 5 were isolate from ATCC, Kalimantan,Sumatra,Brazil, and Java.

The phenotype characterization and numerical analysis of *E. Tarda* in wild Asian Swamp eel in Malaysia were share similar numerical taxonomy and the same strain of *E. Tarda* (Najiah & Lee, 2006). The prevalence of site-specific genotypes with PCR-RFLP of 16s rDNA was found to be specific to detect habitat specific isolates, or all the fish isolates were found only in fish, not in water or sediment (Acharya *et al.*, 2007).

CONCLUSION

Four isolate of *E. Tarda* from Indonesia had 2 strain variation compared with ATCC. Isolate of *E. Tarda* from fish in Kalimantan, Java and Sumatra were the same strain/ group. Isolate of *E. Tarda* from imported turtle was the same as ATCC isolate, or it was originated from human.

REFERENCES

Acharya, M., Maiti, N.K., Mohanty, S., Mishra, P., And Samanta, M. Genotyping Of *Edwarsiella Tarda* Isolated From Freshwater Fish Culture System. J. Comparative Immunology,.Microbiology & Infections. 30 :33-40. 2007.

Baird, K.D., Chikarmane, H.M., Smolowitz, R., And Uhlinger, K.R. J. Biological Laboratory. 205 : 235-236. 2003.

Damme, I.R.V., And Vandepitte, J. Frequent Isolation Of *Edwarsiella Tarda* And *Plesiomonas Shigelloides* From Healthy Zairese Freshwater Fish: A Possible Source Of Sporadic Diarrhea In The Tropics. J. Applied And Environmental Microbiology. 476-479. 1990.

Darwish, A. Histopathology And Pathogenesis Of Experimental Infection With *Edwardsiella Tarda* In Channel Catfish In Textbook Of Fish Health. Dr. George Post.

Najiah, M., Lee, S.W., And Lee, K.L. Phenotypic Characterization And Numerical Analysis Of *Edwarsiella Tarda* In Wild Asian Swamp Eel, *Monopterus Albus* N Trengganu. J. Of Sustainability Scienceand Management. 1 : 85-91. 2006.

Padros,F., Zarza, C., Dopazo, L., Cuadrado, M., And Crespo, S. Pathology Of *Edwardsiella Tarda* Infection In Turbot, *Scophthalmus Maximus* (L.). J. Of Fish Diseases. 29 : 79-86. 2006

Sahoo, P.K., Swain, P., Sahoo, S.K., Mukherjee And Sahu, A.K Pathology Caused By The Bacterium *Edwarsiella Tarda* In *Anabas Testudineus (Bloch).* J. Asian Fisheries. 13 : 357-362. 2000.

Strauss, E>J., Ghori, N., And Falkow, S. An *Edwardsiella Tarda* Strain Containing A Mutation In A Gene With Homology To *Shlb* And *Hpm**b*** Is Defective For Entry Into Epithelial Cells In Culture. J. Infection And Immunity. 415 : 723-2671. 1997.