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Full Length Research Paper

Identification of *Edwardsiella Tarda* in Indonesia

Surya Amanu[1], Kurniasih Imanudin[1*], Siti Narwiyani[2]

[1]Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta
[2]BKIPM Hasanudin, Makasar

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Edwardsiellosis **is a bacterial disease that can infect fish and humans. The aim of this study was to determine a possibility subspecies of *Edwardsiella tarda* based on molecular studies isolated from several hosts in Indonesia. *E. tarda* isolates were taken from Tilapia (Yogyakarta), catfish (Semarang and Jambi), imported Tortoise (Brazil) and Goldfish (Pontianak). Atypical isolates of *E. tarda* (ATCC) were imported from Singapore used in comparison with 4 isolates of *E. tarda* from Indonesia. All isolates of *E. tarda* would be extracted, amplified with the SSU rRNA-16S and sequenced. Multiple sequence alignment used CLUSTAL W version 1.8. Neighbour-Joining (NJ) method and maximum parsimony method were used to analyze the phylogenetic tree. Identification was supported by Agar Gel Precipitation (AGP) method. The result showed that three isolates of *E. tarda* from Pontianak, Jambi, Yogyakarta was same strain originating from fish, whereas the isolates of *E. tarda* from turtle was same strain with ATCC isolates from human origin.**

Keywords: Phylogenetic tree, *Edwarsiella tarda*, Fish, Turtle, Agar gel precipitation.

INTRODUCTION

Edwardsiellosis/Emphysematous Putrefactive disease of Catfish (EPDC) or *Edwardsiella Septicaemia* (ES) was caused by *Edwarsiella tarda. Edwardsiellosis has occurred in* Europe, Japan, Taiwan, Thailand, United States, Singapore, Malaysia, and Indonesia. *Edwardsiella tarda is* an enteric bacteria, transmitted horizontally among fish, carrier fish, via water and mud (Wakabayasi and Egusa, 1973). *Edwardsiella tarda* can be alive in fresh water or marine and transmitted by reptile (turtle), frog, lobster, swine, and humans (Wyatt, 1979). *Edwarsiella tarda* can infect humans via oral-faecal through faeces and water. Strain *E. tarda* in fish is usually transmitted among fish (Nucci et al., 2002).

Edwarsiella tarda was isolated 80% from domestic catfish (Wyatt et al., 1979) and 30% from imported fish. *Edwardsiella tarda* was isolated 75% from catfish in the ponds, 64% from catfish-muddy ponds and 100% from frog, turtle, and crayfish. This evidence that *E. tarda* is included as microflora inside the catfish ponds, so that it was difficult to eradicate (Inglis et al., 1993).

*Corresponding Author Email: kurniasih_1951@yahoo.co.id

Edwardsiellosis could be diagnosed with several *factors* such as: Morphological identification of *E. tarda* (Inglish et al., *1993*), ELISA (Swaim et al., 2001), Immunohistochemistry on the infected organ (Pirarat *et al.*, 2008), Molecular test (Chen and Lai, 1998), and Precipitation agar test.

MATERIALS AND METHODS

Edwardsiella tarda has been isolated and identified morphologically from Gold fish (Pontianak), Tilapia (Yogyakarta), Catfish (Semarang and Jambi), Imported turtle (Brazil), and ATCC isolate (Singapore). All isolates were extracted with Qiagen kit, amplified with PCR method in 16S rRNA region. Primer used was: Forward Eta 2-351(5'-TAG GGA GGA AGG TGT GAA-3'). Reverse Edwsp-780r (5'CTC TAG CTT GCC AGT CTT-3') and from human Eta 1-363 F (5'-GTG TCC GTG TTA ATA GCA-3') (Baird et al., 2003). Amplification products were then purified and sequenced. Multiple sequences were aligned with CLUSTAL W version 1.8, and analysed with *neighbour-joining* and *maximum parsimony* methods to produce phylogenetic tree (Saitou and Nei, 1987).

Agar solution was poured on the petri-dish, several

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#ETD                GTA TAT --A TTT ATC TTT TGT TTT CTC TTT  [ 30]
#KK                 ACT TAC CGA AAA AAC ACC GGG CTA CTC CGT  [ 30]
#LL                 AAT AAT AAA AAA AAA AAA GAA CGC AAG AGG  [ 30]
#E2                 ACC TAC AGA AGA AGC ACC GGC TAA CTC CGT  [ 30]
#ETP                ACC TAC AGA AGA AGC ACC GGC TAA CTC CGT  [ 30]
#Edwardsiella_tarda ACC TAC AGA AGA AGC ACC GGC TAA CTC CGT  [ 30]
#ETD                TTT TTC TCC GAT CTT CCC TCT GGA TGC GTC  [ 60]
#KK                 GCC AGC AGC CGC GGT AAT ACG GAG GGT GCA  [ 60]
#LL                 CAG AGG AGG GGA TAA AAT AAG AAG AAC GTA  [ 60]
#E2                 GCC AGC AGC CGC GGT AAT ACG GAG GGT GCA  [ 60]
#ETP                GCC AGC AGC CGC GGT AAT ACG GAG GGT GCA  [ 60]
#Edwardsiella_tarda GCC AGC AGC CGC GGT AAT ACG GAG GGT GCA  [ 60]
#ETD                CCA GTT AA- GCT GGT GTT CTT T-- --C AAT  [ 90]
#KK                 AGC GTT A-- ATC TGA ATT ACT GGG CGT AAA  [ 90]
#LL                 TAA GAA AAT AAC CAC AGC AAA CAA AAG AAA  [ 90]
#E2                 AGC GTT AT- ATC GGA ATT ACT GGG CGT AAA  [ 90]
#ETP                AGC GTT A-- ATC GGA ATT ACT GGG CGT AAA  [ 90]
#Edwardsiella_tarda AGC GTT A-- ATC GGA ATT ACT GGG CGT AAA  [ 90]
#ETD                TTA CCA ATC GCT GCT GTC TTA CTC CCT TAA  [120]
#KK                 GCG CAC GCA GGC GGT TTG TTA ATA ATT GGA  [120]
#LL                 ATT TTT ATT TCT TTC CCT TTA CGC CCC CAA  [120]
#E2                 GCG CAC GCA GGC GGT TTG TTA A-- GTT GGA  [120]
#ETP                GCG CAC GCA GGC GGT TTG TTA A-- GTT GGA  [120]
#Edwardsiella_tarda GCG CAC GCA GGC GGT TTG TTA A-- GTT GGA  [120]
#ETD                TTC GTA AAT --G CTG CTC CTC CTA TGG CCT  [150]
#KK                 TGT GAA ATC --C CCG GGC TTA ACC TGG GAA  [150]
#LL                 ACA ACA ACT TAC CCA AAA CTC ATT TTA TTA  [150]
#E2                 TGT GAA ATC --C CCG GGC TTA ACC TGG GAA  [150]
#ETP                TGT GAA ATC --C CCG GGC TTA ACC TGG GAA  [150]
#Edwardsiella_tarda TGT GAA ATC --C CCG GGC TTA ACC TGG GAA  [150]
#ETD                GGT GTT GCT AGT ATT TGC AGG TGT TAC  [177]
#KK                 CTG GAT CCA AGA CTG G-C AAG CTA CAG  [177]
#LL                 AAA TTT ACG AAA TCG CAC AAC AGT ACA  [177]
#E2                 CTG CAT CCA AGA CTG GGC AAG CTA CAG  [177]
#ETP                CTG CAT CCA AGA CTG GGC AAG CTA CAG  [177]
#Edwardsiella_tarda CTG CAT CCA AGA CTG G-C AAG CTA GAG  [177]
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Figure 1. *Sequencing* results of five rRNA isolates of *E. tarda* in 16S region.

wheel for field antigen was made surrounding the antibody wheel (*Edwardsiella tarda*). The antibody was made from the serum of rabbit that was injected periodically intraperitoneally with increasing dose of *E. tarda* during four weeks. Serum containing antibody of *E. tarda* was collected at week fifth.

RESULTS

Isolation and identification results showed that all isolates were *Edwardsiella tarda* compared with ATCC isolate. Sequence results in *small sub unit* (SSU) 16S region had genotype variation (Figure 1).

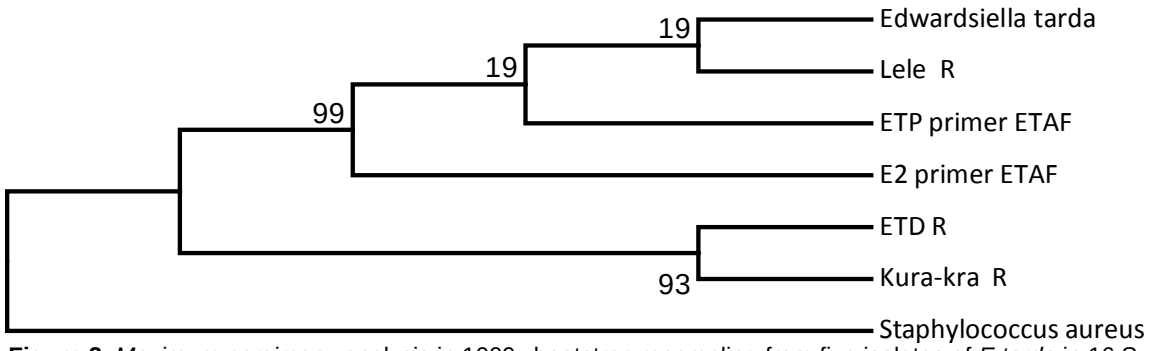

Figure 2. *Maximum parsimony* analysis in 1000x bootstrap resampling from five isolates of *E.tarda* in 16 S region

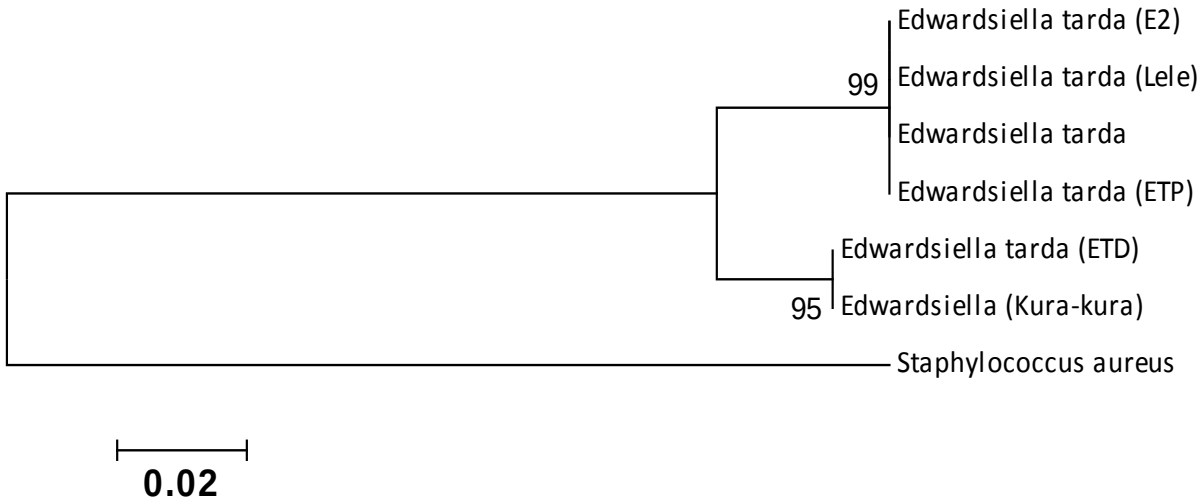

Figure 3. *Maximum parsimony* analysis in 1000x bootstrap resampling from five isolates of *E.tarda* in 16 S region

Maximum parsimony analysis showed that three isolates of *E. tarda* from Pontianak, Yogyakarta and Jambi were clustered with *E. tarda* from *Genbank* (99% of validity). *E. tarda* isolates from Brazil turtle was clustered with human ATCC isolate (93% of validity) see Figure 2.

Neighbour joining analysis showed that three isolates of *E. tarda* from Pontianak, Yogyakarta and Jambi were clustered with *E.tarda* from *Genbank* (99% of validity). *E. tarda* isolates from Brazil turtle was clustered with human ATCC isolate (95% of validity) see Figure 3.

Agar gel precipitation test showed that isolates from infected fish were only precipitated with the antibody of *E. tarda* (in the middle sites). Antibody of *E. tarda* was produced by rabbit serum (Figure 4).

However, precipitation band did not appear between antibody of *Aeromonas salmonicida* (Ab A.salm) in the middle wheel and antigen of *E. tarda* from infected fish (B).

DISCUSSION

This study shows that isolates from turtle were originated from human faeces. *Edwardsiellosis* can infect human causing haemorrhagic enteritis. Human transmission can be from pregnant mother to the baby, or doing their activity near the polluted water or river (Mowbray et al., 2003). *Edwardsiellosis* in human usually caused a serious symptom such as haemorrhagic enteritis and nephritis compared with *Edwardsiellosis* in fish (Nucci et al., 2002). The result of *Phylogenetic tree* supported the conjecture that isolates from turtle was clustered with human isolate (ATCC).

Analysis of *Edwardsiella tarda* with *maximum parsimony* and *neighbor-joining* showed that three isolates from fish clustered together are in comparison with isolate from turtle that is clustered with human isolate. Precipitation gel agar test would only produce precipitation between antigen *E. tarda* and antibody of *E. tarda.*

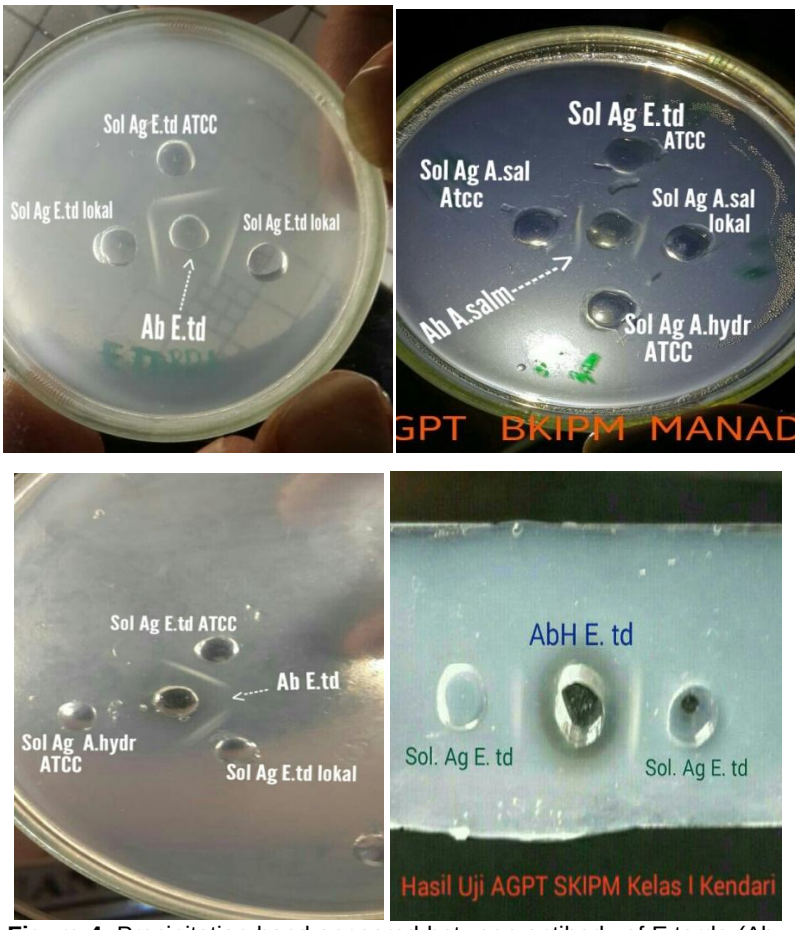

Figure 4. Precipitation band appeared between antibody of E.tarda (Ab E td) in the middle wheel and antigen of *E. tarda* from infected fish (A, C, D).

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