# Edwardsiellosis in Sea Bream *Sparus Aurata* from Suez-Bay

**Eissa, I.A.M, Maather El-Lamie, M., Amina Eldesoky, Abd-Elrehim, A.A.M***

*Dept. of Fish Diseases and Management, Faculty of Vet. Medicine, Suez Canal Univ. *Royal International Inspection Lab. (RIIL), Ain-Elsokhna, Suez governorate*

**Abstract**

The present study was carried out to investigate presence of edwardsiellosis in Sea bream *Sparus aurata* fished from Attaka fishing port, Suez-bay, Suez Governorate. 40 Fish *S. aurata* were collected randomly and freshly from Attaka fishing port during spring 2015. Naturally infected fish showed hemorrhages on different parts of body surface including fins and Gills cover, swollen abdomen with scales detachment. Isolation of the causative agent was performed from spleen, kidneys, liver and gills. Biochemical identification was performed using Microbact 24E which revealed 2 *Edwardsiella tarda* isolates (A and B). Experimental infection by I/P route in Koi fish using (A), (B) isolates revealed 70 and 80% mortalities respectively. The LD50 was $2X10^6$ CFU/ml for (A) and (B) isolates. Histopathological examination on infected *S. aurata* showed necrosis and degeneration in gills, liver, kidneys and spleen.

Key words: Edwardsiellosis**,** *Edwardsiella tarda, Sparus aurata*, Experemintal infection, Histopathologial alterations.

## Introduction

It has been shown that different strains, serotypes, genotypes and biotypes of bacterial pathogens vary in their ability to cause disease within aquaculture, so accurate identification and characterization of a pathogen is of paramount importance for both control and epidemiological investigations ***(El-Seedy et al. 2015)***.

Edwardsiellosis caused by *E. tarda* has been known as a disease of primary importance and required a great attention for successful aqua farming accordingly diagnosis of a particular infection depends on detection and identification of its causative agent ***(Das et al., 2014).***

The present study was planned for investigating Edwardsiellosis in Sea bream *Sparus aurata* fished from Attaka fishing port, Suez-bay, Suez Governorate through clinical picture, bacterial isolation and identification, determination of LD50, experimental infection on Koi fish and histopathological alterations.

## Material and Methods

**Fish:**

Forty *Sparus aurata* samples were randomly and freshly collected from Attaka fishing port, Suez-bay, Suez Governorate with an average body weight 274.4 ± 10g. The collected fish specimens were kept in ice-box to keep them in fresh state and transferred to Royal International Inspection Laboratory (RIIL), Ain El-Sokhna, Egypt to be examined without delay.

**Fish for pathogenecity test and LD50:**

A total number of 150 of Koi fish *C. carpio* were randomly collected alive from fish market in Suez governorate. The average body weight was 60± 10g. divided into 30 fish for pathogenicity test and 60 fishes for each isolate for LD50.

**Clinical examination:**

According to ***Austin and Austin (1989)*** fish were examined clinically by naked eyes for any clinical abnormalities on the external body surface such as scale detachment, necrotic blisters filled with gas, paleness, swollen abdomen, skin hemorrhages, eyes opaqueness and protruded anus. Internal organs were examined for any abnormalities as foul odor emitted from abdomen, paleness of liver and hemorrhagic enteritis.

**Bacterial isolation and identification:**

Isolation of bacteria was done from spleen, kidneys, liver and gills according to ***Wei and Musa (2008).*** Isolation was done on XLD agar plate with NaCl 4% by spread plate method followed by 48 hr incubation at 28±1$^{o}$C.

Biochemical identification was carried-out with Microbact 24E to detect the different isolates.

**Determination of (LD50):**

The method was done according to ***Li et al. (2011)***. For each 2 isolates of *E. tarda*, 60 fish were divided into six groups (10 fish/ group). Overnight cultures of each isolate were adjusted with the tenfolds dilutions to $2X10^{3-7}$ conc. Then 0.3ml of each dilution was injected I/P in each fish group while the 6$^{th}$ group was kept as control and injected with 0.3 ml sterile Maximum Recovery Diluent MRD only. Mortalities were recorded for 15 days for each isolate.

**Pathogenicity of *E. tarda* in Koi fish:**

The detected two isolates (A and B) were inoculated separately onto TSA at 30$^{o}$C for 18 hours. The pure culture from each one was suspended into MRD and adjusted at $2X10^{7}$ CFU/ml according to ***Austin and Austin (2007)***. A total of 30 apparently healthy Koi fish with an average body weight (60 ±10 g) were divided into three equal groups (10 fish/ group). Fish in first and second groups were injected Intra peritoneal (I/P) with 0.3 ml of $2X10^{7}$ CFU/ml of *E. tarda* isolates A and B respectively. Fish in group three was kept as control and injected with 0.3 ml of sterile MRD. All fish groups were kept under daily close observation for three weeks with recording mortalities

and clinical signs. All dead fish were submitted to bacteriological isolation and identification of *E. tarda* to verify the specificity of mortality.

**Histopathological examination:**

Fresh tissue specimens from the liver, kidneys, spleen and gills were collected from affected fish. Specimens were fixed in 10 % neutral buffer formalin ,processed by conventional method , embedded in paraffin, sectioned and stained with Haematoxyline and Eosin stain. The protocol was carried out according to ***Bancroft and Gamble (2008).***

**Results**

**Clinical picture findings:**

The clinical examination of the affected fish showed eye swelling starting necrosis, swelling of the abdomen with anal protrusion, hemorrhage on fins and scale detachment, gills were nearly brown coloration with sloughed parts in some cases. Internally, foul odor emitted from abdomen, liver was enlarged and congested with blood and kidneys were enlarged with hemorrhage surround it**.**

**Isolation and Identification:**

Clear colonies with black center and pink peripheral ring in diameter 1 to 2 mm grow on X.L.D. medium.

According to the morphological and biochemical reaction, the isolation and identification revealed isolation of two isolates of *E. tarda* (A) and (B). They were differentiated according to biochemical characters.

**Results of LD50 determination for each isolate for edwardsiellosis:**

Koi fish mortalities were observed from the first day for each isolate for 15 days, within 10 days all fishes were died .

LD50 for first isolate(A) was $2x10^6$ CFU/ml conc. and LD50 for second isolate(B) was $2x10^6$ CFU/ml conc.

**Results of experimental infection:**

The pathogenicity of the three isolates were tested by I/P injection of 0.3 ml of $2X10^7$ CFU/ml for each isolate in Koi fish *C. carpio*. The external and internal findings were the same for the two isolates. They were hemoarhages on different parts of fish bodies, after 72 hours the swelling of the abdomen and hemorhages all over the body with eye redness have been appeared and scales detachment with anal protrusion. plate (2), The internal findings after 72 h post infection were foul odour after opening the fish with sever internal hemorhage with presence of sereous exudates in the abdomenal cavity, pale gills with enlarged liver and spleen.plate (3). Mortality rates for the two isolatesA and B experimentally infected fish concentration $2X10^7$ CFU/ml were 70 and 80% respectively and control group mortality rate was 0%.

**Histopathological findings:**

Histopathological examination of the diseased seabream (*S. aurata*) revealed that liver infected with

Edwardsiellosis showed activation of melano-macrophages and necrosis of hepatocytes and hepatopancrease, kidneys showed activity of melanomacrophage centers (MMC) and diffuse degeneration to necrosis of renal tubular epithelium, spleen showed activity of melanomacrophage centers ( MMC ) along with depletion and necrosis of lymphocytes and gills showed congestion of blood vessels and degeneration and necrosis of secondary lamellae

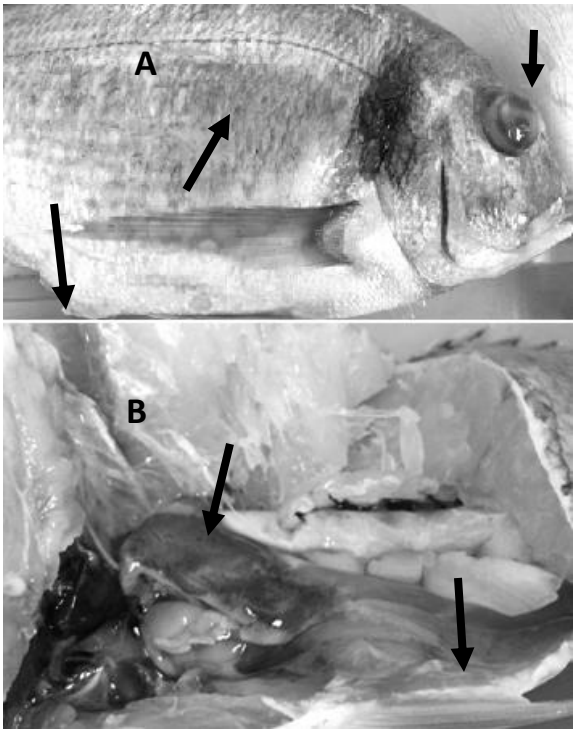


Plate. (1): **A**. *Sparus aurata* externally showing hemorrhage on the body and fin, eye protrusion and anal protrusion (Arrows), **B**. *S. aurata* internally showing hemorrhagic swollen liver and intestines (Arrow).

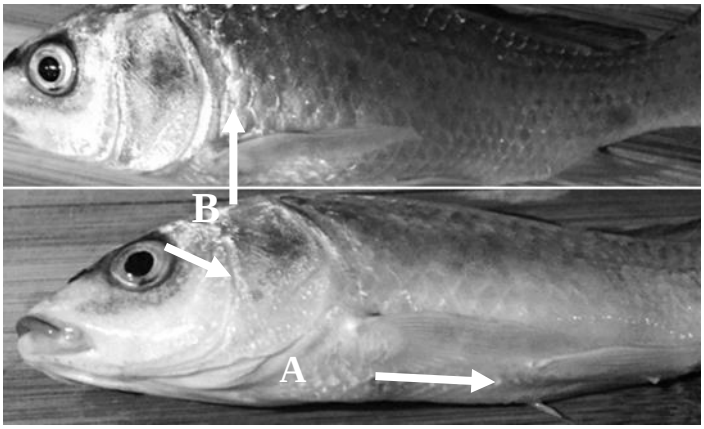


**Plate (2)**: Showing (**A**) hemoarhages on different parts of Koi fish body (**B**) eye redness.

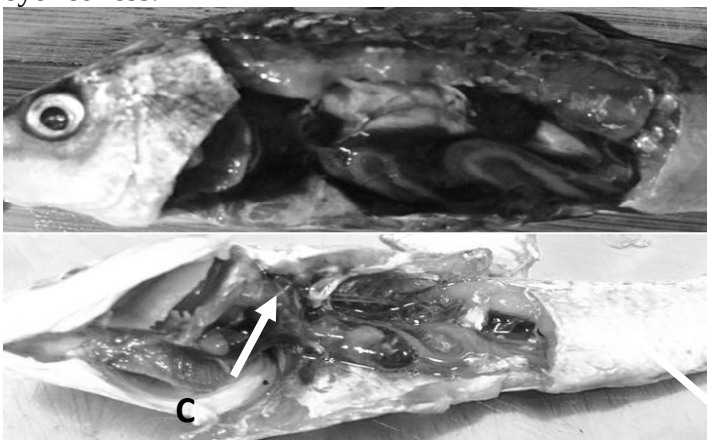


**Plate (3)**: Showing (**A**) Koi fish with sever internal hemorrhage(**B**) with sereous exudates in abdomenal cavity, (**C**) pale gills.

**Table (1):** Showing mortality rate and pathogenicty of two isolates of *E. tarda*.

| FISH GROUPS | *E. tarda* ISOLATES | Dose/fish 0.3ml | No. Of fish | No. Of dead fish/ day | | | | | Total no. Of dead fish | Mortality % |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5-21 days | | |
| 1 | A | $2X10^7$ | 10 | 4 | 2 | 0 | 1 | 0 | 7 | 70 |
| 2 | B | $2X10^7$ | 10 | 4 | 3 | 1 | 0 | 0 | 8 | 80 |
| 3 | Control | MRD | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

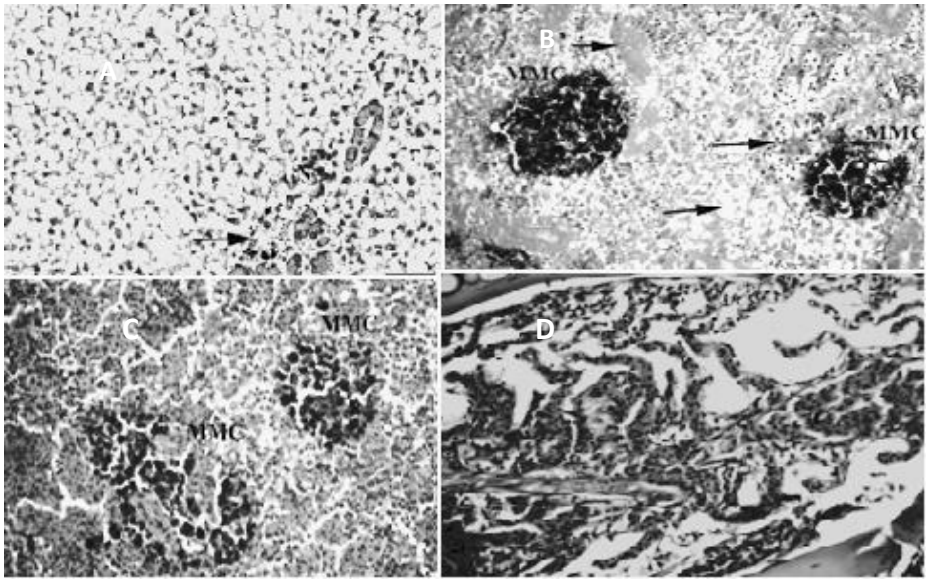


**Plate (4)**: Naturally infected S. aurata **A**. Liver showing activation of melanomacrophages ( arrow ) and necrosis of hepatocytes and hepatopancrease ( N ), **B**. kidneys showing activity of melanomacrophage centers ( MMC ) and diffuse degeneration to necrosis of renal tubular epithelium (arrows ), **C**. Spleen infected with Edwardsiellosis showing activity of melanomacrophage centers ( MMC ) along with depletion and necrosis of lymphocytes, **D.** Gills infected with Edwardsiellosis showing congestion of blood vessels ( C ), degeneration and necrosis of secondary lamellae. H&E. X 400.

**Discussion**

In naturally infected Seabream with edwardsiellosis, the present work showed eye swelling starting necrosis, swelling of the abdomen with anal protrusion, hemorrhage on fins and scale detachment. Gills were nearly brown coloration with sloughed parts in some cases. Internally foul odor emitted from abdomen, liver was enlarged and congested with blood and kidneys were enlarged with hemorrhage surrounding it**.** These results agree with ***Lan et al. (2008) and Park et al. (2012)*** who isolated *E. tarda* from cultured turbot. However, disagree with that recorded by ***Ibrahem et al. (2011)*** as they recorded presence of large blisters and abscesses in catfishes and tilapias and ***El-seedy et al. (2015)*** who mentioned presence of white nodules on liver and kidney. This disagreement may be attributed to different fish species, site of study and environmental conditions.

Regarding the LD50, it was $2X10^6$ CFU/ml conc. for isolate (A) and (B) which goes with ***Li et al. (2011), Fatma Korni (2012)*** for her citrate negative isolates of *E. Tarda*

and ***Mo et al. (2015)*** who mentioned that LD50 was $10^6$ CFU/ml conc.. Such results were lower than that obtained by ***Maiti et al. (2011)*** who recorded that LD50 was $2.7X10^9$ CFU/ml conc. Also higher than results mentioned by ***Ibrahim et al. (2011), Hashiem and Abd El-Galil (2012)*** *and* ***Pridegeon et al. (2014)***-on catfish- as LD50 ranged between $10^4$ and $10^5$ CFU/ml conc. This difference may be attributed to environmental condition, difference in fish species and different sites of the study.

Concerning the pathogenicity test on Koi fish for the two isolates A and B the mortality rates were 70 and 80% respectively. This result nearly goes with ***Ibrahem et al. (2011)*** who found mortality rate from *E. tarda* ranged 70 to 60% in African catfish and Nile tilapia, ***Hashiem and Abd El-Galil (2012)*** who reported mortality rate caused by *E. tarda* as 60% and ***Pridgeon et al. (2014)*** who said that mortality rate for their *E. tarda* isolate was ranged from 70-80%.

These results were higher than that mentioned by ***Fatma Korni (2012)*** who mentioned that $1.5X10^8$ CFU/ml of citrate positive *E. tarda* isolate showed a mortality rate as 20%. This may be attributed to the different locations of study or fish species or environmental conditions.

Regarding to the histopathological findings, in this study, the infected liver showed moderate to severe congestion of central veins. The hepatocytes showed diffuse hydropic degeneration. These findings go with the findings of ***Ibrahem et al. (2011)*** .Others showed fatty changes. Some hepatic cells revealed signs of coagulative necrosis. These findings go with ***Zhou et al. (2014) and Mo et al. (2015)*** as they found massive necrosis in the hepatocytes and also there was an increase in the melano-macrophage cells. In this study, the kidneys showed hyperactivity of melano-macrophage centers. The renal tubules showed different degenerative changes and necrosis, these findings go with ***Ibrahem et al. (2011)*** *and* ***Mo et al. (2015).*** Spleen of naturally infected fish showed mild to moderate necrosis of lymphocytes. Mild to moderate activity of melan-omacrophage centers and these results go with ***Ibrahem et al. (2011), Zhou et al. (2014)*** and ***Mo et al. (2015).*** Regarding gills, they revealed severe congestion of blood vessels in gill arch and gill lamellae and necrosis of lamellar epithelium. These results are nearly similar to that found by ***Garcia et al. (2012)*** *and* ***Zhou et al. (2014).*** The histopathological findings in this study may slightly disagree with what mentioned by ***Woo and Bruno (2011)*** who found as presence of granulomatous inflammation and abscesses in internal organs which progress to granulomas in different kinds of fishes. This disagreement may be attributed to different sites

of study and different species of fish.

**References**

**Austin B. and Austin D.A (1989):** "Methods for the microbiological examination of fish and shellfish"**;** Ellis Haward limited halsted press, Newyork, Brisban, Toranto, 59-68.

**Austin B. and Austin D.A (2007):** "Bacterial fish pathogen: Diseases of farmed and wild fish" 4th edition Springer Dordrecht Berlin Heidelberg New York.

**Bancroft J.D and Gamble M. (2008): "**Theory and Practice of Histological Techniques", 6th ed. Elsevier Health Sciences. China.

**Das B.K., Sahu I., Kumari S., Sadique M. and Nayak K.K. (2014):** "Phenotyping and Whole Cell Protein Profiling of *Edwardsiella tarda* strainisolated from infected Freshwater Fishes" Int.J.Curr.Microbiol.App.Sci, 3(1): 235-247**.**

**El-Seedy F. R., Radwan I. A., Abd El-Galil M. A. and Sayed H. H. (2015): "** Phenotypic and Genotypic characterization of *Edwardsiell tarda* isolated from *Oreochromis niloticus* and *Clarias gariepinus* at Sohag Governorate" Journal of American Science;11(11) 68-75.

**Fatma Korni (2012);** "Edwardsiellosis in some fresh water fishes" Thesis for PhD to Dept. of Fish Diseases and Management, Beni- Suef University.

**Garcia V. N., Iregui – Castro C. A.** and **Hirono I.; (2012)** " Edwardsiellosis, common and novel manifestations of the disease: A review" Revista Colombiana de Ciencia Animal, 5 (1) ,82-90.

**Hashiem M. and Abd El-Galil M. A. A.** (**2012**): "Studies on Edwardsiellosis in *Clarias Gariepinus* Fish at Sohag Governorate", Journal of American Science, 8(4): 438-444.

**Ibrahem M. D., Shaheed I. B., Abo El-Yazeed, H. and Korani H.; (2011):** "Assessment of the susceptibility of polyculture reared African Catfish and Nile tilapia to *Edwardsiella Tarda"* Journal of American Science,;7 (3) 779-786.

**Lan J., Zhang X.-H., Wang Y., Chen J. and Han Y. (2008)** "Isolation of an unusual strain of *Edwardsiella tarda* from turbot and establish a PCR detection technique with the *gyrB* gene", Journal of Applied Microbiology 105:644–651.

**Li G.Y., Li J., Xiao P., Guo Y.H. and Mo Z.L.(2011):** " Detection of type III secretion gene as an indicator for pathogenic *Edwardsiella tarda*", Letters in Applied Microbiology, 52: 213–219.

**Maiti B., Shetty M., Shekar M. and Karunasagar I. (2011):** "Recombinant outer membrane protein A (OmpA) of *Edwardsiella tarda*, a potential vaccine candidate for fish, common carp" Microbiological Research 167: 1–7.

**Mo Z.-Q. , Zhou L. , Zhang X. , Gan L. , Liu L. and Dan X.-M. (2015):"** Outbreak of *Edwardsiella tarda* infection in farm-cultured giant mottled eel *Anguilla marmorata* in China**",** Fisheries Science, 81(5):899-905.

**Park S. B., Aoki T., and Jung T. S. (2012):** "Pathogenesis of and strategies for preventing *Edwardsiella tarda* infection in fish", Veterinary Research, 43(67):1-11**.**

**Pridgeon J. W., Klesius P. H., Lewbart G. A., Daniels and Jacob H. V. M.;(2014):***"Edwardsiella tarda* and *Aeromonas hydrophila* isolated from diseased Southern flounder (*Paralichthys lethostigma*) are virulent to channel catfish and Nile tilapia", Journal of Coastal Life Medicine; 2(5): 337-34 .

**Wei L. S. and Musa N. (2008):** "Phenotyping, Genotyping and Whole Cell Protein Profiling of *Edwardsiella tarda* Isolated from Cultured and Natural Habitat Freshwater Fish" American-Eurasian J. Agric. & Environ. Sci., 3 (5): 681-691.

**Woo P.T.K. and Bruno D.W. (2011):** "Fish diseases and disorders. Volume 3: viral, bacterial and fungal infections. In Edwardsiella septicaemias". 2nd edition Edited by Evans J.J., Klesius P.H., Plumb J.A. and Shoemaker C.A. Wallingford: CABI International:512–534.

**Zhou Y. , Geng Y., Wang K., Huang X., Chen D., Peng X., Zhong Z.**
**and Chen Z. (2014):** "*Edwardsiella tarda* infection in cultured Ya-fish,
*Schizothorax prenanti*, in China" John Wiley & Sons Ltd, Aquaculture Research: 1–6.