

## FIELD AND LABORATORY INVESTIGATION ON THE *E. COLI* AS A CAUSATIVE AGENT OF DEATH IN CAPTIVE DOLPHIN *TURISOPS TRUNCATUS* AT MAGIC LAND , EGYPT .

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### SUMMARY

Characterization of pathogenic *E. coli* isolated from sudden death of captive dolphin *Turisops truncatus* through bacteriological identification and electrophoretic pattern as well as its histopathological effect on the infected dolphin has been investigated . The proteins of *E. coli* isolate was pronounced 2 heterogenous polypeptide bands with molecular weight 51 and 59.5 KD respectively . The estimated protein value of *E. coli* in free cell buffer was calculated 0.193 % through UV spectrophotometer at wave length 280 nm. The histopathological effect revealed a significant interstitial pneumonia, granular degeneration in the hepatocyte and sloughing of intestinal villi with necrosis and few lymphocytic infiltration in the epithelial lining .

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### INTRODUCTION

Pathogenic strain of *E. coli* has been reported as a most common cause of death in captive dolphin and small cetaceans . The respiratory tract diseases especially bacterial infection of lungs were pronounced as the most frequent systemic diseases of small cetaceans especially dolphin (Sweetney and Ridgway , 1975 ) . Bronchopneumonia in captive oceanic mammals has presented problems which is very rare in their native state *E. coli* has been proved to be a causative agent of hepatic failure and bronchopneumonia in adult *Turisops truncatus* ( Medway and Schryver, 1973) . The purified pili of *E. coli* gave only one band in the SDS-PAGE with a molecular weight 17 KD , while a minor band of 18 KD became visible when the gel overloaded with pilus *E. coli* preparations( Korhonen et al. , 1980 ) . In 1999 a captive dolphin *Turisops truncatus* has suddenly died at Magic Land , Egypt with purulent bacterial pneu-

monia, the causative agent of which was proved to be *Pseudomonas fluorescens*. Moreover, the histopathological examination revealed tissue alterations in kidney, liver and intestines (Mohamed Ali, 1999). The present study was aimed to characterize the causative agent of pathogenic *E. coli* isolated from captive dolphin for the first recorded in Egypt.

## MATERIALS AND METHODS

### A- Materials :

An entertainment captive female dolphin *Turisops truncatus* was recently died in Magic Land, Egypt.

### Postmortem examination :

The carcass was examined for the cause of death and recorded the macroscopic criteria.

### Samples :

Special selective samples were collected from the different organs, lung, heart, liver, spleen, kidney, gastrointestinal tract (GIT), ovaries and uterus for laboratory investigation.

### B- Methods :

#### 1- Bacteriological examination :

The bacteriological identification was done according to Quinn et al. (1994). Swabs were taken under aseptic precautions from lungs, spleen and intestine of recently dead dolphin *Turisops*

*truncatus* and cultured on specific MacConkey agar plates (Oxoid), Eosin Methylene Blue (E.M.B) plates and further purified on sheep blood agar and E.M.B. plates (Britania). The isolated purified pathogenic *E. coli* colonies were identified biochemically according to the specific scheme (Table 1) according to Quinn et al., (1994).

#### 2- Electrophoretic diagnosis :

The pure culture of *E. coli* in saline was subjected to sonication then purification through ultracooling centrifuge at 1400 rpm/15 minutes after which the supernatant was filtered through 0.8  $\mu$ m filter. The protein value of the filtrate was evaluated through UV spectrophotometer at WL 280 nm. The protein sample of the isolated *E. coli* was run in SDS-PAGE (1 dimension vertical slab 10 X 10 cm<sup>2</sup>, 1.5 mm gel thickness) compared with the standard molecular weight of high range (Sigma) in (Hoefer Mini vertical slab Model 250) for 2 hours. The gel was fixed, stained with coomassie brilliant blue stain (R 250 Sigma) and decolourised (Hudson and Hay, 1989).

#### 3- Histopathological examination :

The histopathological changes were investigated in tissue specimens that fixed in 10% formal saline, dehydrated by ethyl alcohol, embedded in paraffin wax, sectioned at 4 - 5 $\mu$  thickness and stained with H & E according to Dury and Wallington (1980).

## RESULTS

### A- Postmortem examination :

The post mortem examination of the recently dead dolphin revealed subcutaneous petechial haemorrhages . The heart chambers were full with clotted blood and the heart muscles were flabby .The lungs and trachea were congested , hepatised with dark necrotic patches and full filled with frothy fluid . Gastrointestinal tract was characterized by severe gastritis and enteritis with engorged blood vessels free from blood inside . Bloody discharges all over body cavities, and inflammatory purulent areas of genitalia have been also detected.

### B- Bacteriological identification :

The isolated pathogenic *E. coli* colonies on MacConkey agar plates ( Oxoid ) were lactose fermentable pink in colour , on E.M.B plate were green metallic ( Fig. 1 ) , on blood agar were B-haemolytic . The isolated bacteria were green negative short bacilli , motile and characterized by the following profile of biochemical analysis in Table ( 1 ) .

### 2- Electrophoretic diagnosis :

The electrophoretic pattern of protein component of the isolated *E. coli* was proved in two heterogenous polypeptide bands of 51 and 59.5 KD respectively in relation to standard molecular weight of high range ( Sigma ) Table ( 2 ) and Fig. ( 2 ) .

**Table ( 1 ) :** The biochemical characters of isolated pathogenic *E. coli*.

No.	Reagent	Result	No.	Reagent	Result
1	Oxidase	-ve	8	Case in glucose	+ve
2	Phenyl alanine diaminas	-ve	9	Acid in mannitol	+ve
3	Gelatin lcoquifaction	-ve	10	Lactose	+ve
4	H <sub>2</sub> S production	-ve	11	Salicin	+ve
5	Urea utilization	-ve	12	Indol	+ve
6	Citrate uti;ization	-ve	13	Methyl red	+ve
7	Voges Proskauler	-ve	14	Decarboxylase lysinc	+ve

**Table ( 2 ) :** The electrophoretic characterization of pathogenic *E. coli* proteins at molecular level .

Protein species	Lane No.	Heterogenous polypeptide bands	Relative molecular weight (Mr./Kda)* (Quality control marker)								
			200	116	97	66	59.5	51	42	31	21
Ecoli	2	2	-	-	-	-	1	1	-	-	-
			-	-	-	-	1	1	-	-	-

\* Mr./Kda: molecula weight/ Kilo dalton.

**Table (3):** Sodium dodecylpolyacrylamide gel electrophoresis ( SDS-PAGE ) techniques .

Protein species	Lane No.	Protein concentration (µg/ml)	Sample buffer µl	Total sample µl
E. coli	2	1.9	10	20

**Technical specification :**

Instrument : Hoefer Mini vertical Model 250 .

Electric power : 20 mA 120 volt.

Time : 2 hrs.

SDS-PAGE : 10 X 10 cm<sup>2</sup> 1.5 mm thickness 7.5 % SDS.

**3- Histopathological examination :**

The histopathological examination was carried out according to Drury and Wallington ( 1980 ) in tissue specimens taken from lungs , liver and intestines of the recently dead dolphin. The most common detected tissue alterations could be summarized as follows:

**1- Lung :**

The lung showed interstitial pneumonia with emphysema and red hepatization in some alveoli (Fig. 3 ) .

**2- Liver :**

The liver specimens showed vascular and granular degeneration in the hepatocytes, while the central vein and blood sinusoids were congested (Fig. 4 ) .



**Fig. ( 1 ) :** The isolated pathogenic E. coli on Eosin Methylene Blue (EMB) media showing green metallic colonies.

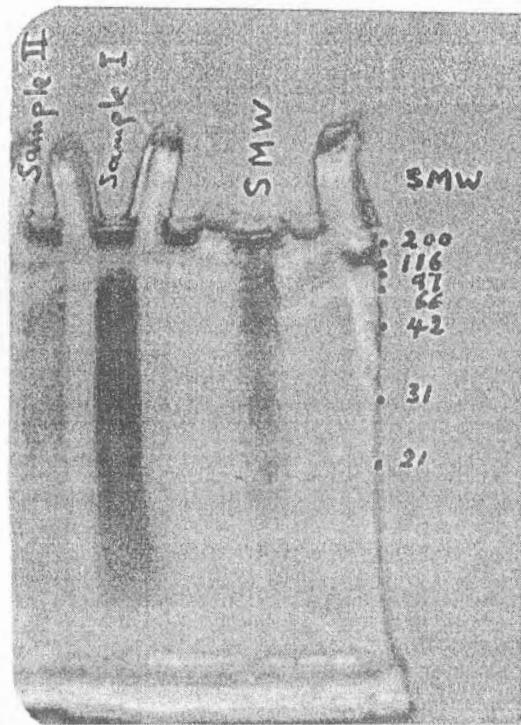


Fig. (2): The protein sample of the isolated *E.coli* in SDS-PAGE showing two heterogenous polypeptide bands at 51 and 59.5 Kda respectively in relation to standard molecular weight.

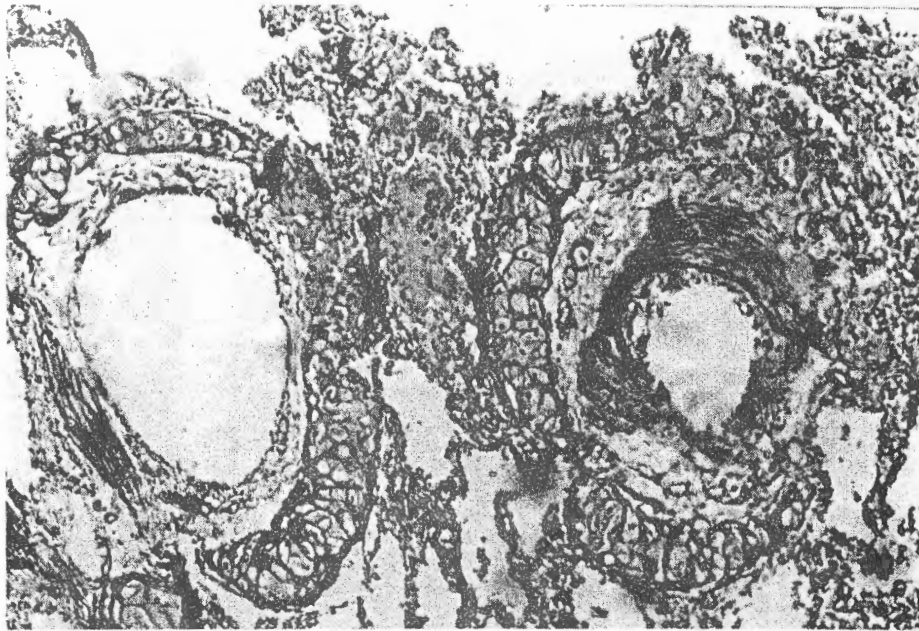
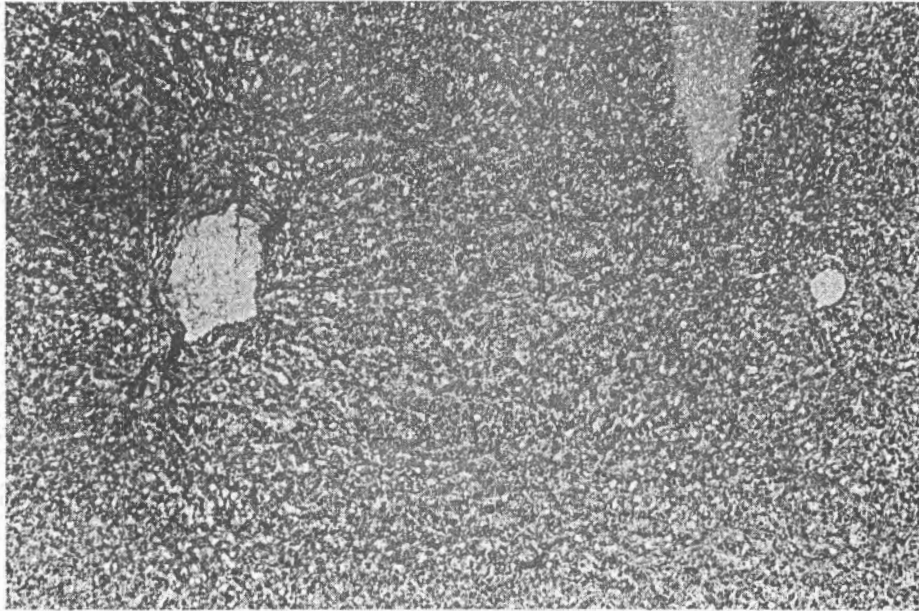
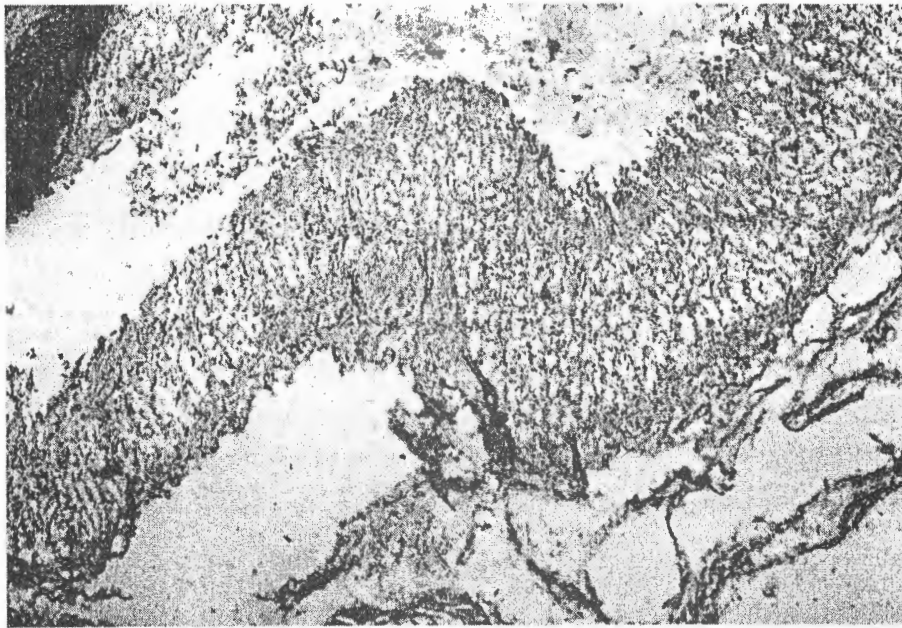


Fig.(3): The lung of recently dead dolphin showing interstitial pneumonia with emphysema and red hepatization in some alveoli.



**Fig. (4) :** The liver of recently dead dolphin showing vascular and granular degeneration in the hepatocytes while central veins and sinusoids were congested.



**Fig. (5) :** The intestinal tissues of recently dead dolphin showing sloughing of surface epithelial villi, proliferation of C.T. in lamina propria with few lymphocytic infiltration.

### 3- Intestines :

The histopathological changes in intestinal tissue specimens showed sloughing of surface epithelial villi , proliferation of connective tissue in lamina propria with few lymphocytic infiltrations . The epithelial tissues and the intestinal glands showed necrotic changes ( Fig. 5 ) .

The histopathological alterations proved that captive dolphin *Turisops truncatus* died due to severe pneumonia , hepatitis and septicaemic enteritis referring to pathogenic *E. coli* as a causative agent .

### DISCUSSION

The present study is proved the role of haemolytic *E. coli* as one of the main causative agents of death in captive entertainment dolphin in agreement with Sweeney and Ridgway ( 1975 ) . The main pathogenic strain of haemolytic *E. coli* was isolated and identified in the bacteriological lab . , Fish Diseases Department , Animal Health Research Institute , with bacteriological identification techniques according to Quinn et al., (1994) (Fig.1),Table (1) . The postmortem findings reported in this study revealed that the captive dolphin was died from severe pulmonary and gastroenteric infection with acute heart failure which was in agreement with what proved by Medway and Schryver ( 1973) The bronchopneumonia in captive oceanic mammals has presented prob-

lems where *E. coli* has been proved to be one of the causative agents. The electrophoretic analysis was carried out on the proteins of haemolytic *E. coli* isolate according to the methods of Laemeli (1970) ; Korhonen et al., (198 ) and Hudson and Hay (1989 ) Korhonen et al., ( 1980 ) detected two molecular weights in SDS-PAGE related to the purified pili of *E.coli* at 17and 18 Kda . While in the present study two heterogenous polypeptide bands at 51 and 59.5 relative molecular weights for the whole proteins were detected ( Fig. 2 and Table 2 & 3 ) .

The histopathological examination in the present study was confirmed the postmortem findings revealed that dolphin was died from severe pulmonary and gastrointestinal infection. The lungs showed interstitial pneumonia with emphysema and red hepatization in some alveoli ( Fig. 3 ) . Moreover , the intestinal tissue specimens showed sloughing of surface epithelial villi with C.T. proliferation and few lymphatic infiltrations in lamina propria (Fig. 5). The present study confirmed the haemolytic *E. coli* as a new bacterial agent causing death of the captive entertainment dolphin at Magic Land , Giza , Egypt . The reviewing literature was regarding to what identified by Mohamed Ali ( 1999 ) in morbid and dead captive dolphins at Magic Land where *Pseudomonas fluorescense* was proved as a causative agent . Moreover , the mimic criteria in postmortem and

histopathological examination with bacterial causative agents are pointing to more interest in diagnosis and control of dolphin diseases in Egypt .

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## REFERENCES

- Drury , R. A. and Wallington , E. A. 1980 : Carlton's histological techniques . 5th Ed. Oxford Univ. Press , Oxford, England .
- Hudson , L. and Hay , F. 1989 : Practical immunology . 3rd Ed. , Blackwell Scientific Publications . Oxford , London , England :
- Korhonen , T. K. ; Nurmiäho , E. ; Ranta , H. and Svanborg , C. 1980 : New method for isolation of immunologically pure pili from *E. coli* . *Infect. Immun.* , 27 ( 2 ) : 569 - 575 .
- Laemmli , U. K. 1970 : Cleavage of structural proteins during the assembly of head of bacteriophage T4 . *Nature* , London , 227 : 680 - 685 .
- Medway , W. and Schryver , H. F. 1973 : Respiratory problems in captive small cetaceans . *JAVMA* , 163 ( 6 ) : 571 - 572 .
- Mohamed Ali , M. N. 1999 : Laboratory investigations in dolphins *Tursiops truncatus* and sea cat *Odobceivus rosmarus* affected with haemorrhagic septicaemia in the Media production City in Egypt . *Egypt. Vet. Med. Ass. J.* , 59 ( 2 & 3 ) : 315 - 335 .
- Quinn , P. J. ; Carter , M. E. ; Markey , B. K. and Carter , G. R. 1994 : Clinical veterinary microbiology . Mosby Yearbook , Europea Limited .
- Sweeney , J. C. and Ridgway , S. H. 1975 : Common diseases of small cetaceans . *JAVMA* , 167 ( 7 ) : 533-534 .