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# A CONTRIBUTION AND PATHOLOGY OF E.COLI AND PSEUDOMONAS AERUGINOSA BACTERIAL INFECTION ON SOFT SHELL TURTLE RAFETUS EUPHRATICUS (GRAY, 1864) EAST HAMMAR MARSHES, IRAQ

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**Abstract**
*E.coli* and *P. aeruginosa* bacterial infection on Soft shell turtle *Rafetus euphraticus*. Isolation and identification diagnosis which that on these species affected, biochemical test and clinical signs. Samples of the Soft shell turtle *Rafetus euphraticus* Gray, 1864 were collected from East Hammar marshes during summer season of the year 2016 .The infection isolated with a percent of 40%. Clinical Finding obvious that the incidence of *E.coli* and *P. aeruginosa* infection. Some of Soft shell turtle show clinical abnormalities with *E.coli* and *P. aeruginosa*. The most common clinical signs were external haemorrhage, Histopathological changes revealed degeneration and necrosis in all organs associated with Chronic inflammatory cell infiltration and melanomacrophage cells were detected in all turtle tissues. This study showed that *P.aeruginosa* infection is common in the Soft shell turtle *Rafetus euphraticus*. So, this study was designed to make a survey of bacterial infestation of Soft shell turtle *Rafetus euphraticus* Gray, 1864 East Hammar marshes during the summer of 2016.

**Key words:** *E.coli*, *Pseudomonas aeruginosa*, bacterial infection, Soft shell turtle *Rafetus euphraticus* Gray, 1864, East Hammar marshes, Iraq

# БАКТЕРИАЛЬНАЯ ИНФЕКЦИЯ ВЫЗВАННАЯ ВОЗБУДИТЕЛЯМИ E.COLI И PSEUDOMONAS AERUGINOSA И ОБУСЛОВЛЕННАЯ ЕЮ ПАТОЛОГИЯ РАЗВИТИЯ МЯГКОТЕЛЫХ ЧЕРЕПАХ RAFETUS EUPHRATICUS (GRAY, 1864), ОБИТАЮЩИХ В БОЛОТАХ (МАРШАХ) НА ВОСТОКЕ ХАММАРА (ИРАК)

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**Реферат**

В данной работе описана бактериальная инфекция мягкотелых черепах Rafetus euphraticus, вызванная E.coli и P. aeruginosa. Мы определили и идентифицировали возбудитель инфекции, поражающий черепах данного вида, сделали биохимический анализ, выявили клинические признаки заражения. Экземпляры мягкотелых черепах Rafetus euphraticus Gray, 1864 были собраны летом 2016 года на болотах (маршах) в восточной части Хаммара. 40% общего количества собранных черепах было инфицировано. Клинические исследования наглядно показали наличие инфекции E.coli и P. aeruginosa. У некоторых экземплярах мягкотелых черепах были обнаружены клинические проявления аномалий, вызванных возбудителями E.coli и P. aeruginosa. Наиболее общим клиническим признаком явилось наружное кровотечение. Гистопатологические изменения проявлялись в виде дегенерации и некроза всех органов. Хронический воспалительный клеточный инфильтрат и мелано-макрофагальные клетки были обнаружены во всех тканях черепахи. Данное исследование показало, что инфекция, вызванная возбудителем P.aeruginosa, является обычной для мягкотелых черепах Rafetus euphraticus. В данной работе представлен обзор случаев бактериального заражения мягкотелых черепах Rafetus euphraticus Gray, 1864, собранных летом 2016 года на болотах (маршах) в восточной части Хаммара.

Ключевые слова: E.coli, Pseudomonas aeruginosa, бактериальная инфекция, мягкотелые черепахи Rafetus euphraticus Gray, 1864, восточный Хаммар.

## Introduction

The Euphrates soft shell turtle *Rafetus euphraticus* is classified as Endangered on the IUCN Red List and is thought to have undergone large, recent population declines, Species information in Iraq is limited to a few rapid surveys with little detailed information on breeding and distribution (Nadheer *et al*., 2015). Some of literature mentioned Global range and of distribution occurs in the Euphrates and Tigris rivers, from south-eastern Turkey, through Syria and Iraq to the Arabian Gulf extending into southwestern Iran. It occurs from elevations of 1000 m to sea level (Baran & Atathur, 1998). The range in Iran is limited to central and western Khuzestan province and related to the main rivers. The border areas of Iran and Iraq contain many important rivers considered as the “habitats” of *R. euphraticus* including Karkheh, Dez, Karoon, Djarrahi, Shahoor and Bahmanshir. Other important habitats are the wetland sites listed as “international wetlands (Ramsar Site) like Shadegan and Hour-Al Azim (Asghar and Mola, 2011).

All populations of organisms, including aquatic animals is limited partly or wholly in the ecosystem (Real, 1996). The prevalence of the disease in the ecosystem is affected by many environmental factors, including infectious and stressors (Nils Kautsky *et al*., 2000). Bacteria are everywhere, and occur in most freshwater environments, can be found in the water column and in the sediment (Hazen, 1979). A bacterial are adapted to environments that have a wide range of conductivity,

turbidity, pH, salinity and temperature (Hazen *et al.,* 1978). Optimum temperature may depend on the particular strain is under investigation, but generally range from 25℃ to 35 degrees Celsius (Meyer, 1970).

*Pseudomonas* spp. are widely spread in natural sources of water and associated with septicemia in aquatic animals (Roberts, 2001). These bacteria are considered opportunistic pathogens, causing disease when the host is subjected to stress. A number of aquatic animals including fish, frogs and soft-shelled turtles are reported to be susceptible to *Pseudomonas spp*. with moderate to high losses (Somsiri and Soontornvit, 2002). The etiological agents commonly found are *P. diminuta, P. fluorescens, P. putida* and *P. aeruginosa* with different degrees of virulence. The characteristic symptom of the disease produced by *Pseudomonas* bacteria is a remarkable septicemia hemorrhage in the skin of the mouth region, opercula and ventral side of the body (Wakabayashi and Egusa, 1972).

Conventional microbiological methods needed to identify bacteria from fish are often limited by the length of time required to complete the assays. In recent years, PCR have overcome problems associated with culture- based techniques, enabling the detection of bacteria directly in clinical samples without the need for previous culturing (Gonzalez *et al*., 2004). *Escherichia coli* is a commensal bacterium lives harmless in intestinal microflora in variety of animals including man, however sometimes they cause fatal diseases in humans, mammals and birds (Belanger *et al.,* 2011) Based on pathogenicity and site of infection *E. coli* strains are classified into three distinct groups such as commensal strains, intestinal pathogenic strains, and Extra intestinal pathogenic *E. coli* (ExPEC) strains (Lyhs *et al*., 2012)

So, this study was designed to make a survey of bacterial infestation of Soft shell turtle *Rafetus euphraticus* in the marshes

**Materials and Methods**

A total of 15 Soft shell turtle *R. euphraticus* were collected from East Hammar marshes during the summer of 2016; the infected turtle were acclimatized in fresh water of glass aquarium in the laboratory conditions approximately at 25°C. The turtle were allowed to feed on commercial fish-food daily and were regularly monitored whether any death or ulcerative symptoms occurred. The bacteria isolates and culture from affected organs by a sterile loop and streaked on the pre-prepared sterilized nutrient agar. Media were subjected to various tests beginning from the study of their growth morphology on different agar media to different microbiological identification tests such as gram staining and biochemical identification tests such as oxidase, methyl red, Vogesproskeur, urase, $H_2S$, Triple sugar iron, indol. A sterile wire loop was used to collect a loop full of each undiluted and inoculated on the surface of nutrient agar, MacConkey agar and Pseudomonas base agar to obtain pure cultures as well as study their morphological characteristics. The agar plates were incubated at 37°C for 24 h for appropriate colony formation.

The predominant bacterial colonies from the media were isolated, purified and characterized following standard methods (Sneath, 1986; Lacey, 1997; Pelczar, 1957).

Results

Isolation and identification diagnosis of *E.coli and Pseudomonas aeruginosa* bacterial infection on Soft shell turtle Rafetus euphraticus can be based on these species are affected, biochemical test and clinical signs of the disease. The biochemical test of *E.colai* and *Pseudomonas aeruginosa* were negative to test urease, coagulase and Voges–proskauer. Morphology of colonies of *Pseudomonas aeruginosa* are sticky ,shiny, slanted to green yellow color with thick growth of spiky

do not contain whereas the *E.coli* are convex spherical colonies that are depended on the color of the colony media MacConkey agar where rosy color and is non – composed of the spores (table 1). The infection isolated with a percent of 40%. Clinical Finding study found that the incidence of *E.coli* and Pseudomonas aeruginosa infection. Some of Soft shell turtle show clinical abnormalities of *E.coli* and Pseudomonas aeruginosa were isolated and identified, and all of the collected Soft shell turtle showed one or more from the following signs according to the stage of disease; darkness in the color of the skin, Skin showed vacuolar degeneration and necrosis in the epidermal cells with mononuclear inflammatory cells infiltration in between the epidermal cells, detachment of the scales, large irregular hemorrhages on the body surface, Ulcerative on the skin varied from shallow to deep necrotizing ulcers, inflamed vent, exophthalmia, abdominal distension with sero-hemorrhagic fluids exuded from the vent as shown in Figure 2. The most common sign of disease, occurring in 2 of turtles examined. The dorsum of the neck flippers and tail, Eye lesions took several forms: a yellow deposit on the cornea adjacent to the eyelids indicated keratocon- junctivitis. Diseases of the skeletal system were uncommon. Ulcerative shell disease was characterized by the appearance of dark, focal discolorations on the carapace of farmed turtles. These progressed into pits or ulcers.

Fig (1) culture of the *Pseudomonas aeruginosa* and *E.colai* of bacterial infection

Table (1) Biochemical tests of contribution of *E.colai* and *Pseudomonas aeruginosa* bacterial infection on Soft shell turtle *Rafetus euphraticus*

| Biochemical test | Cramstin | Oxidase | Mir | Vp | Urase | H2s | Citrate | Triple suger iron | Indol | Motality | Coagulase |
|---|---|---|---|---|---|---|---|---|---|---|---|
| E.coli | - | - | - | - | - | - | - | A/A | + | + | - |
| *Pseudomonas aeruginosa* | - | + | + | - | - | - | + | A/K | - | + | - |

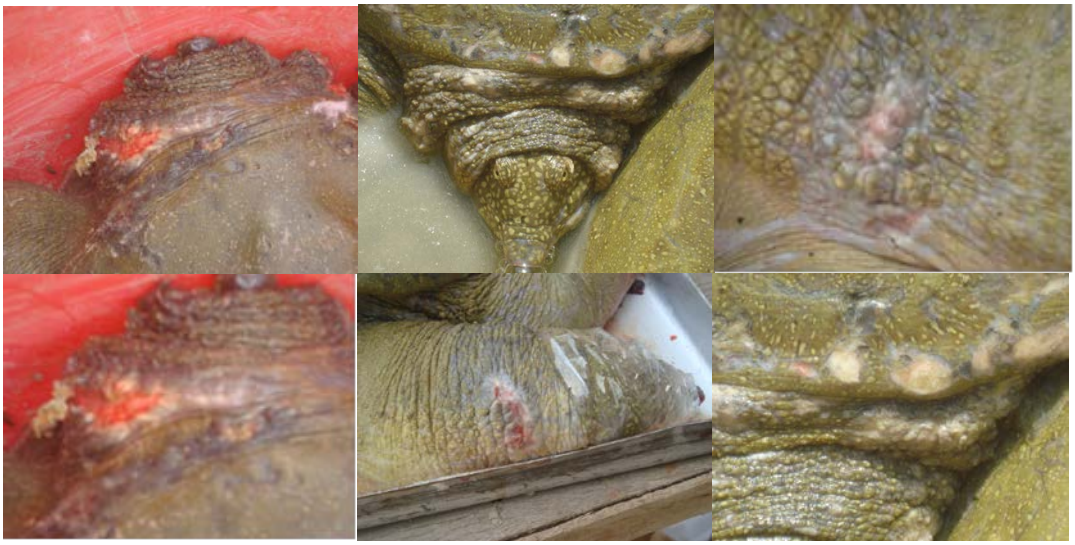

Fig (2) Soft shell turtle *Rafetus euphraticus* Gray, 1864 site of bacterial infection

**DISSCUTION**

Bacterial infections are a common cause of disease in turtles and tortoises. Proper nutrition, housing, and sanitation are the best methods of prevention ,however, even in the well-cared for turtles or tortoises, infections can still occur. The genus *Pseudomonas* is one of the most diverse bacterial genera, and its taxonomy has undergone many changes. During 2004, *Pseudomonas* spp. was isolated from Nile tilapia and African catfish (*Clarias gariepinus*), silver carp (*Hypophthalmichthys molitrix*) and grey mullet (*Mugil cephalus*) that were being reared in seventeen commercial fish farms in Kafr EI-Sheikh Governorate (Mesalhy, 2013). Bacteriological examinations of private fish farms in Kafr EI-Sheikh Governorate suffered from high mortalities, ranging from 17.6 to 22.9%. Revealed that 38 fish (36.9%) were infected with *Pseudomonas fluorescens*, 30 (29.1%) with *Pseudomonas aureginosa*, 19 (18.5%) with *Pseudomonas anguilliseptica* and 16 (15.5%) with *Pseudomonas pseudoalkaligene* (Masbouba, 2004). *Pseudomonas* infection has been incriminated as the most common bacterial infection among fish and appears to be stress related disease of freshwater fish especially under culture conditions (Kitao *et al*., 1993). *Pseudomonas* aerugenosa was isolated from skin, gills and stomach content of cultured *Clarias gariepinus* fingerlings in Nigeria (Oni *et al.*, 2013). Data clearly indicates that infection is responsible for huge losses to aquaculture. *Pseudomonas* spp. Caused septicemia in aquatic animals (Roberts, 1978) and a number of aquatic animals including fish, frogs and soft-shelled turtles are reported to be susceptible to *Pseudomonas* spp. with moderate to high losses (Somsiri and Soontornvit, 2002). *Pseudomonas* spp. bacteria are considered as opportunistic indoor pathogens as their infection initiates an inflammatory response (Hirvonen *et al.*, 2005; Huttunen *et al.*, 2003).

Eissa *et al.* (2010) isolated different strains of *Pseudomonas* species namely *Pseudomonas putida, P. aeruginosa, P. fluorescens* and *P. anguilliseptica* from *Oreochromis niloticus* in Qaroun and Wadi-El-Rayan Lakes, Egypt, They reported that infected fishes showed irregular hemorrhages on body surface, especially at the ventral part of abdomen, eyes cloudiness; scales detachment and congested gills were observed. Internally, there were sanguineous fluids in the abdominal cavity of some fish. Bacteria invade the host tissue and cause infection and bacteremia in immune compromised hosts (HIV/AIDS, cystic fibrosis, bronchiectasis, severe chronic

obstructive pulmonary disease, burns, malignancy or diabetes mellitus) (Feldman *et al*., 1998; Liu and Mercer, 1963). Furthermore extra intestinal pathogenic *E. coli* live as commensal in the intestines but sometimes they infect extraintestinal sites (Smith *et al*., 2007). It can cause various infections including gastroenteritis, urinary tract infection, meningitis and septicemia (Von BH and Marre R. 2005). *E. coli* is often considered an opportunistic pathogen (Hussain *et al*., 2014). Many strains of *E. coli* have specific virulence factors that provide them the inherent capability to cause disease (Hartl DL and Dykhuizen DE. 1984*). E. coli* is also a common contaminant of different food sources and water (Newell DG et *al*., 2011). And is frequently used as indicator organism to estimate fecal contamination in water.

The histopathological changes: *Pseudomonas sp.* could be considered as an opportunistic pathogen, which can survive on the surface of water or in the gut and may cause disease when unfavorable conditions developed (Kumaran *et al*., 2010).

Also, these results are in agreement with those of Hossain *et al*.(2006) and Musa *et al*.(2009). Similar lesions have been reported to occur in *P.aeruginosa* infected crabs gills where haemocytes are accumulated in the haemocoelic space. Necrosis is seen in most of the gill rachis. Epithelial lifting and Disrupted Pillar cells were also observed (Devakumar *et al.*, 2013).

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