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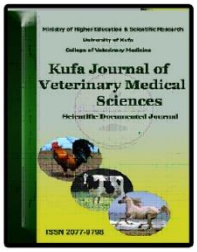

Isolation and Identification *Vibrio parahaemolyticus* Which Causing Black Skin Lesion From Golden Fish

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Abstract

The aim of this research was aimed to isolate and diagnose the *Vibrio. Parahaemolyticus* that cause black skin lesion from golden fish and study the histopathological changes in the skin and fins. Seventy-two samples of gold fish were taken from Ornamental Fish markets in Al- Najaf city, The bacteria was diagnosed through culture on normal and special media then conducted by biochemical tests as well as the examination was conducted by API20. The samples of fins and skin were examined for histopathological examination,

Histopathologically indecated the presence of degeneration and necrosis of the muscles fibers as well odema were seen in interstitial tissue, , congestion and hemorrhage were recorded in the same area with the presence of infiltration of inflammatory cells among the skeletal muscle. This study concluded that *V. parahaemolyticus* are responsible for the severe histopathological changes in all organs of infected fishes

Key words: *Vibrio Parahaemolyticus* , Golden Fish,skin**.**

عزل وتشخيص جراثيم *Vibrio parahaemolyticus* المسببة لأفة اسوداد الجلد في الاسماك الذهبية

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الخلاصة:

هدفت الدراسة الحالية الى عزل وتشخيص جراثيم *V. parahaemolyticus* المسببة لمرض اسوداد الجلد من الاسماك الذهبية ودراسة التغيرات المرضية النسجية في الزعانف والجلد. تم اخذ اثنان وسبعون عينة من الاسماك الذهبية من الاسواق المحلية في مدينة النجف، شخصت الجراثيم من خلال زرعها على الاوساط الزرعية الاعتيادية والخاصة وبعد ذلك اجريت عليها الفحوصات الكيموحيوية وكذلك اجري فحص API20 لتاكيد التشخيص ،اخذت عينات من الزعانف والجلد للفحص المرضي النسيجي حيث بينت نتائج الفحص

المرضي النسيجي وجود تهتك وتنخر للعضلات وكذلك تجمع السوائل ، احتقان ونزف الاوعية الدموية ووجود ارتشاح للخلايا الالتهابية. نستنتج من الدراسة وجود تاثيرات وتغيرات مرضية نسجية في الاعضاء المصابة بجراثيم *V. parahaemolyticus* .

Introduction:

Vibrio parahaemolyticus is an emerging enteric pathogen that was first discovered in Japan in 1950 as the cause of a food poisoning outbreak , This Gram-negative marine bacterium is now the major cause of seafood borne gastroenteritis within the United States and the major cause of all foodborne infections in Asian countries [1].

Vibrio parahaemolyticus is a gram-negative, halophilic bacterium widely distributed in coastal waters worldwide that is associated with gastroenteritis due to the consumption of raw or improperly cooked seafood. The thermostable direct hemolysin (TDH) and TDH-related hemolysin are the major virulence factors[2].

The onset of the illness usually takes place 3 to 24 h after consumption of *V. parahaemolyticus*-contaminated food. The symptoms include diarrhea, abdominal cramps, nausea, vomiting, headache, and low-grade fever [3].

Vibrio parahaemolyticus is a marine bacterium which is also responsible for acute diarrhoeal illness in human beings. Presence of *Vibrio parahaemolyticus* infection in seafish, fish products or in fresh water fishes is of public health importance. Infection *Vibrio parahaemolyticus* is self limited infections, socioeconomic loss and rarely death are some of the problems. Immunocompromised patients may die due to consumption of contaminated raw seafish or under cooked fish products[4]. The aim of this study was to investigate the isolation and identification of *Vibrio parahaemolyticus* from the goldfish samples from Ornamental Fish market in Al-Najaf city**.**

Materials and Methods

Specimens: seventy two gold fish samples were collected from Ornamental Fish Shops Each fish sample was placed in sterile small plastic bag, labeled and sealed separately to avoid contamination. The samples were placed in sealed containers with ice cubes and transported to the laboratory.

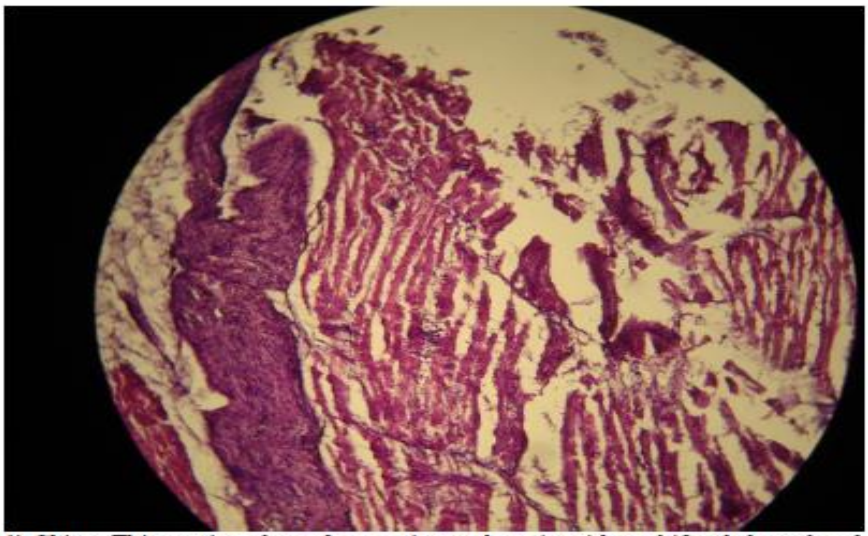
Isolation and identification of *V. parahamolyticus*: Cotton swabs were taken from the skin and fins of a golden fish and put it in alkhaline peptone water and incubated at a temperature of 37 ° C for 18 hours after that a loopful of broth was placed on Thiosulfate Citrate Bile Salts Sucrose (TCBS) at 37°C. Next day the colonies were tested for typical green color. The biochemical test were done to sure the isolate by used Api 20.

Histopathology: for histological studies ,skin and fin were dissected from the infected gold fish ,. The tissue samples were fixed in formalin(12%) fixative for 24 hrs and washed with distilled water. The samples were dehydrated in different grades of alcohol series and processed further. Sections of 5-6 µm thickness were taken using a microtome and stained using haematoxylin and eosin. [5].

Result:

Isolation and Identification: The results were showed isolation and identification of *V.parahaemolyticus* from skin and fin of golden fish. The isolate was gram negative, colones on TCBswas green color.

Histopathological examination: The results of this study reveal that there is a excessive melanosis in the dermal with multifocal dermal and subepiderml odema , there is slouphing of the outer epidermal layer.(fig1), Also in interstitial tissue, odema was seen which induced marked seperation of endomyocin with the surrounding muscle fiber , Degeneration and necrosis of muscles were also seen (fig 2), Hemorraghe and congestion were recorded(fig 3), in the same area there were dialeted blood vessele(3)., Aamong the skeletal muscle ,a generalised inflammatory infilterate of neutrophile ,mononucler cell (fig 4 ,5), Erythrocyte and macrophge are expressed abundantly among the fragmented skeletal muscle fiber. Degeneration and necrosis of the dermis and skeletal muscle fiber were seen(fig 4,5), no colonies of bacteria were detected . while, there is necrosis and hemorrhagic area were seen in the fin (fig 6).



(Fig.1) Skin : This section showed excessive melanosis with multifocal dermal and subepiderml odema , there is slouphing of the outer epidermal layer.

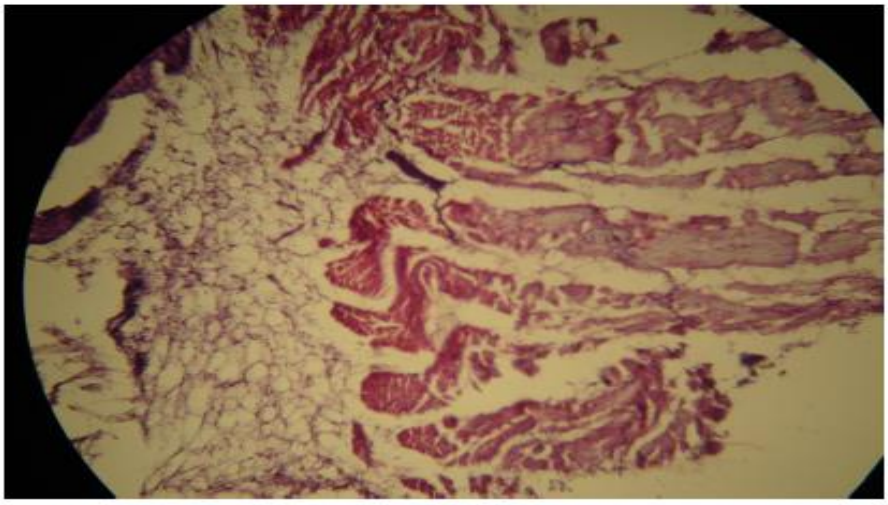


Fig.2: Skeletal muscle : This section show in interstitial tissue, odema was seen which induced marked seperation of endomyocin with the surrounding muscle fiber , Degeneration and necrosis of muscles.

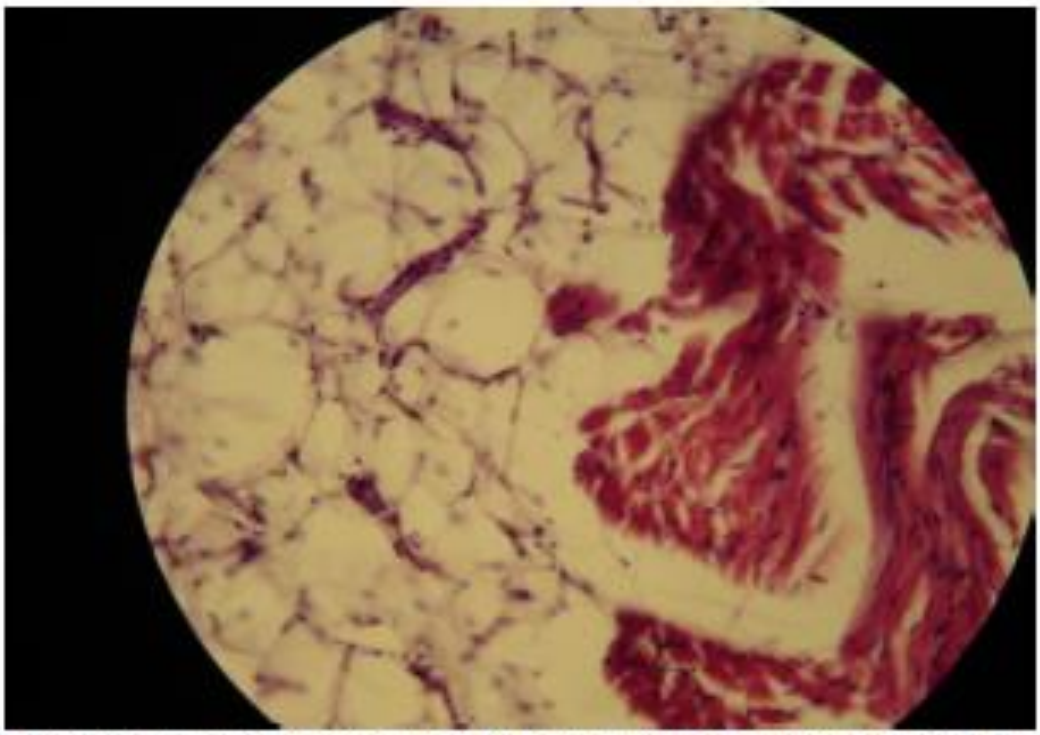


Fig.3: Skeletal muscle:This section revealed Hemorraghe and congestion, also there were dialeted blood vessel.

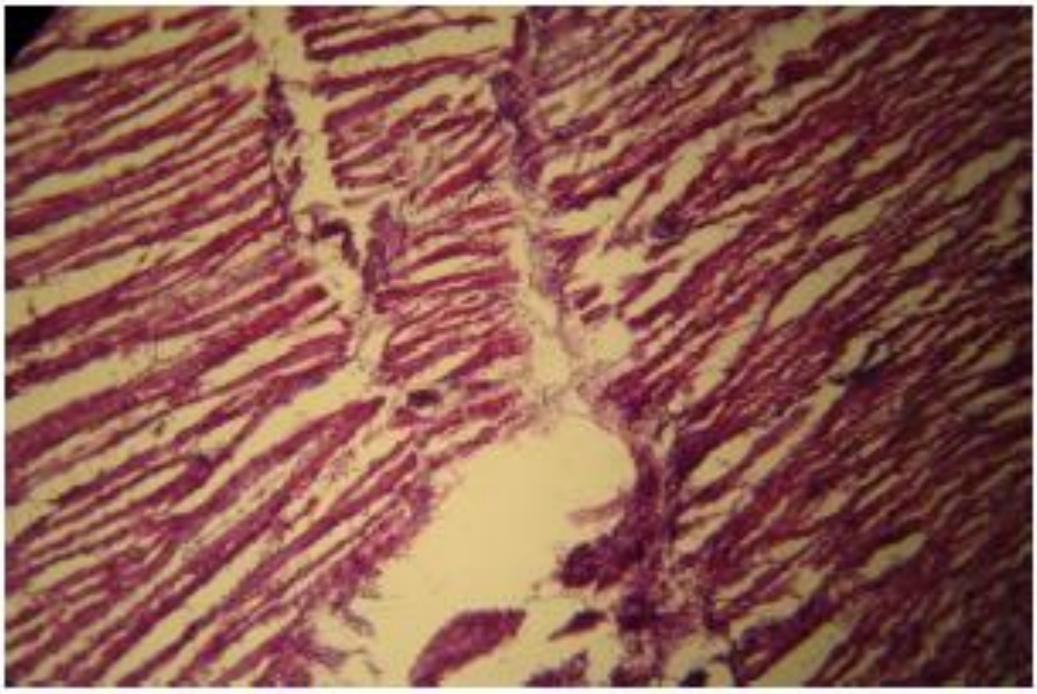


Fig.4:Skeletal muscle : This section show a generalised inflammatory infilterate of neutrophile , mononucler cell.

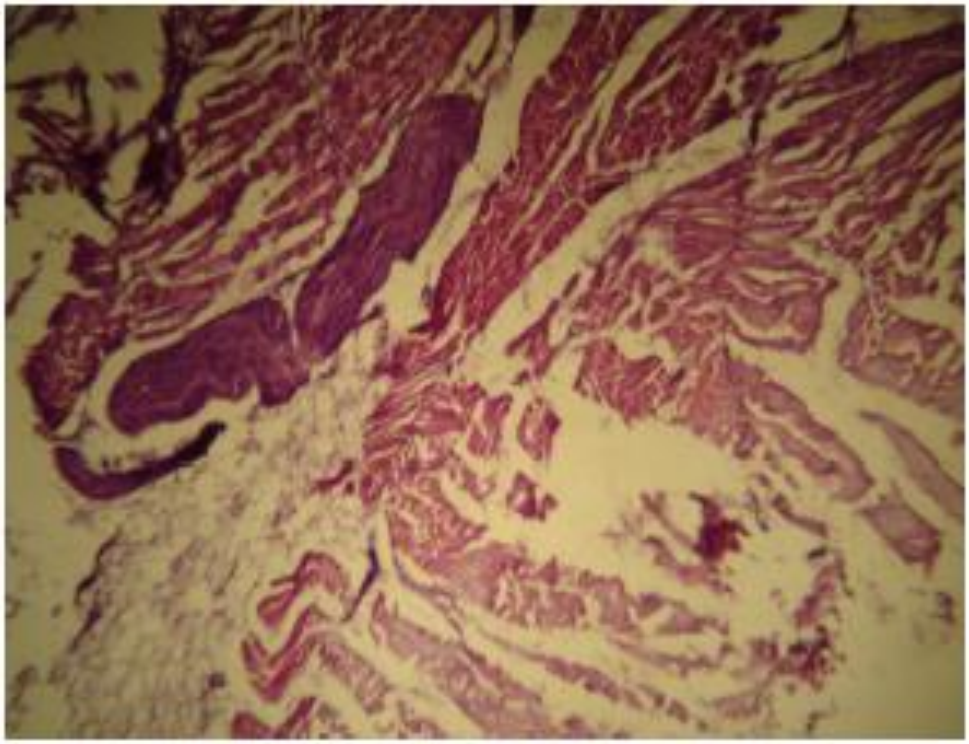


Fig.5:Skeletal muscle: This section show Erythrocyte and macrophge are expressed abundantly among the fragmented skeletal muscle fiber, degeneration and necrosis of the dermis and skeletal muscle fiber.

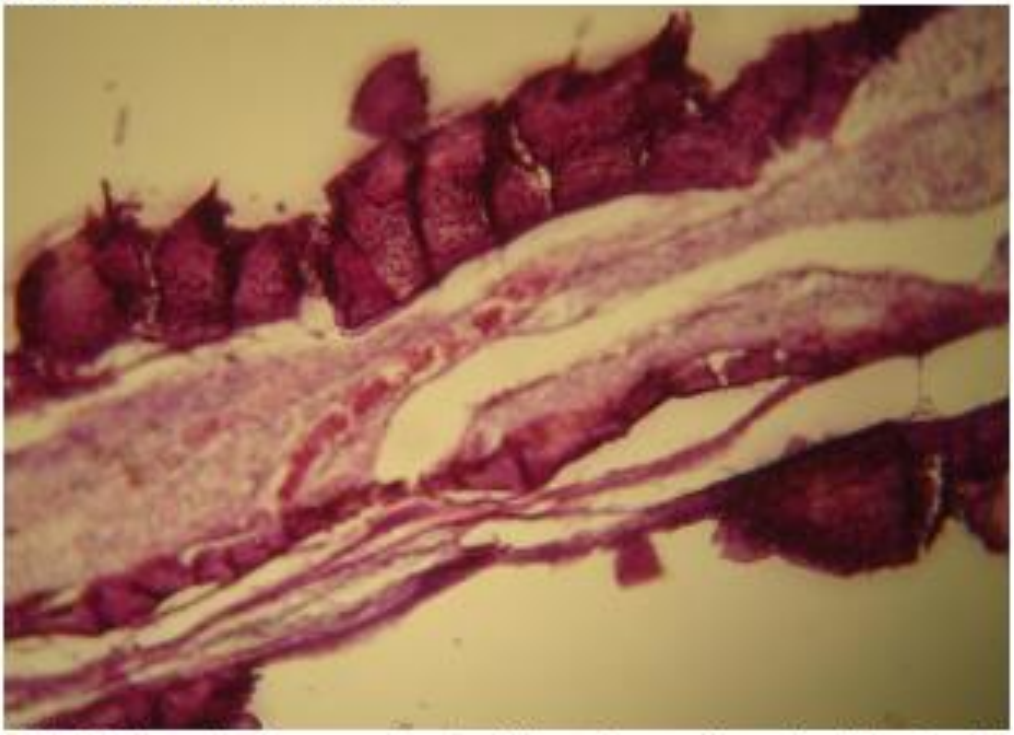


Fig.6:Fin: This section show no colonies of bacteria were detected. while, there is necrosis and hemorrhagic area.

Discussion:

V. parahaemolyticus is a Gram-negative, halophilic bacterium that thrives in warm climates with in marine or estuarine environments. It is commonly found free swimming, attached to underwater surfaces, or commensally associated with different shellfish species [6].

The bacteria which isolated from goldfish in the present study was a gram-negative , curved rod, green colony on TCBS agar, these results agreement with [7] ,also agreement with [8] which isolated the *V. parahaemolyticus* from fish samples in Kolkata. The clinical signs of infection in our study included darkened body color , skin lesion and haemorrhages in the skeletal muscle these result contrast with[9]. Histopathological examination of the tissue sections from skin and intramascular tissues of the infected fish revealed, degeneration and necrosis of muscles fiber represented by swelling, highly eosinophilic cytoplasm, loss of their striation and pyknosis of nuclei were noticed. Heavy inflammatory cellular infiltration of the dermal and the underlying muscular layers mostly lymphocytes and macrophages was seen (Fig. 4,5). Congestion of blood vessels and presence of multiple area of extravasation of erythrocytes were detected. Similar observations were also recorded in **[10]**.These pathological alterations in the skin and intramascular tissues could be attributed to the extensive bacterial multiplication and the *V. parahaemolyticus* possesses a powerful arsenal of potential virulence factors, including proteases, hemolysins and two type three secretion systems (T3SS1 and T3SS2) [11]. These T3SS, which are specially designed to inject effector virulence factors into eukaryotic host cells, play distinct and critical roles in the pathogenicity of the organism [12], these results agreement with **[13],** which showed the secretion of cytotoxin, haemolysin and extracellular proteases, esterases and hemolysins by *Vibrio anguillarum* that assists bacterial invasion, colonization and pathogenesis. Also the Proteases, phospholipase, haemolysins and other toxins may have important roles in the pathogenecity of *V. harveyi.* [13], Extracellular products (ECP) have been considered as a virulent factor of *V. harveyi*. [14]**.**

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