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SOME BACTREOLOGICAL AND HISTOPATHOLOGICAL STUDIES ON SHELL DISEASED OF CRAB

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Abstract

The study was conducted on hundred and fifty crab gathered from Suez Canal-El kantarah region, Ismailia governorate, Egypt. The clinical examination were recorded in situ, primarily showed swimming lazy, and when it walk toward the shore it unable to move, the swimming legs sometimes loss reflex and paralyzed with lost of one or two segments (propodus and dactyle), the eye stalks in some cases found destructed or unable to retract. Brownish or blackish spots distributed on the dorsal surface of the shell ranged from 1 or 2 mm up to 2-3 cm. In some cases, the spots fused to each other, so that the lesion expanded onto larger areas on different parts of the exoskeleton mostly the dorsal surface than the ventral. Postmortem examination revealed presence of gray black coloration of the gills. Bacteriological examination and identification was performed and resulted in 125 isolates of *Vibrio alginolyticus* by percentage of 49.02 %, 95 isolates of *Vibrio parahemolyticus* by percentage of 36.25% and 35 isolates of *Aeromonas hydrophyla* by percentage of 13.72 %. The antibacterial activity of lactobacillus acidophilus against the isolates strains resulted in inhibition zone 16, 14, and 12 mm diameter while the hemolymph inhibition zone was 12, 17, and 15 against *V. alginolyticus, V. parahemolyticus and A. hydrophila* respectively. The histopathological findings were described in details.

Key word: Crab, shell disease, bacteria, Egypt.

INTRODUCTION

Crabs represents a valuable component of small scale costal of fisheries in tropical and temperate latitudes including Egypt, its distribution extended from southern Mediterranean Sea , Red sea, Suez canal and the east cost of Africa and the across the Indian ocean to Japan and the west pacific ocean. Attention is focused on the marine animals as it plays role in environmental balance in marine aquaria, the production is increased in the later seasons since 1994 until now with continuous increase in all Mediterranean fisheries center of Egypt, GAFRD (2005). Crab and shrimp considered the most valuable fishery resources. Crustaceans, such as crabs, shrimp and lobster, provide a high quality protein and marine species also contain omega 3 fatty acids that afford potential health benefits. Shell disease is one of the most common problems affecting freshwater and marine crustaceans, it had reported in many feral populations of crustaceans Sindermann (1989) as crab, shrimp, crayfish and lobster. Shell disease is the progressive degradation of exoskeletal chitin accompanied by melanisation of the affected region. Therefore, it's known as black spot, rust spot or burned spot. The disease affects numerous crustacean species worldwide Getchell (1989). It manifests as necrotic lesions on the exoskeleton that caused by bacteria producing extracellular chitin digesting enzymes capable of degrading and digesting crustacean cuticle, this enzymes was a vital requirement for occurring the disease Rosen (1970). High prevalence of shell disease has been associated with stressful environments, this may occur by mechanical abrasion**,** Sindermann and Rosenfield (1967), Cook and Loften (1973), wounding, Dyrynda (1998), and- or microbial attack Baross *et al.* (1978), Cipriani *et al.* (1980), Smolowitz *et al.* (1992). Bacteria belonging to the genera reported as probable agents involved in the disease syndrome Getchell, (1989). Noga, *et al.* (2000) mentioned that blue crabs affected with shell disease were mainly due to aeromonads,

vibrios. Vogan *et al.* (2002) stated that Chitinolytic bacteria was the primary etiological agents of shell disease syndrome in marine crustaceans, and most of pathogenic bacterial causes was of the genus Vibrio isolated from the edible crab. So, this work aimed to isolate and identify the bacteria causing shell lesions in crabs and study the histopathological changes.

MATERIALS AND METHODS

Collection of samples:

One hundred and fifty shell diseased crabs were gathered alive from Suez canal-El kantarah region (Ismailia governorate), then transported alive in marine water by glass containers supplemented with aeration to the central laboratory for aquaculture. Handling the live active crab and postmortem examination carried out according to Ruppert *et al.* (2004) and Fox (2007).

Clinical examination:

Crabs examined for presence of any abnormal colorations of the epicuticle ranged from melanization, spotted areas of black or brown colure on the dorsal and ventral surface, and its distribution on the carapace and their legs, presence of pitting of the surface and their size and were graded due to the lesions founded. The external lesions then recorded and photographed.

Postmortem examination:

Anesthesia of crab and dissection done according to Ruppert *et al.* (2004) and Fox (2007).

Isolation and Identification of the causative bacteria:

The dorsal and ventral surfaces of the exoskeleton sterilized by ethyl alcohol and scrap from abrasions of both dorsal and ventral surfaces

lesions transferred into 600 µl sterile 0.5%. NaCl solution and was homogenized then inoculated into Nutrient broth media supplemented with sodium chloride 0.5%. then, the tubes incubated for 24 h. at 25 ^{o}C subcultures made from all broth cultures on nutrient agar supplemented by sodium chloride 0.5% then incubated at 25 ^{o}C and purified colonies inoculated onto slope nutrient agar for further identification. The biophysical and biochemical characters carried out according to Bergey's *et al.* (2004).

Preparation and propagation of Lactobacillus acidophilus cell free extract:

Lactobacillus acidophilus was isolated from cattle milk whey according to Savadogo *et al.* (2004) with some modifications.

Propagation was performed according to Ajitha *et al*. (2004); bacteria were grown aseptically in 10 ml of nutrient broth for 24 hrs at room temperature (28 ± 2°C). Five ml of log phase culture was then transferred under aseptic conditions into 250 ml of MRS broth and placed on a rotary shaker at 150 rpm for 24 hrs at 28 ± 2°C. The bacterial strain was harvested by centrifuging at 10,000 rpm under aseptic conditions for 15 min at 4°C. The cell free extract was saved, filtered and sterilized through Celtron filters of 0.2 mm pore size (Merck). PH adjusted to 6.8 by means of one mole NaOH. The cell free extract was stored at -70^{o}C till use.

Blue crab hemolymph serum:

Hemolymph was collected from blue crabs according to Noga *et al*. (1996 a&b) by inserting a 22 G needle attached to a 3 ml syringe into the arthrodial membrane of the swimming leg and gently aspirating into the syringe. The collecting hemolymph kept in Eppindorf tubes at 4^{o}C till clot completed. Frozen hemolymph was kept at room temperature to allow homogenize and break up the clot. It centrifuged at 50,000 rpm for 20 min. The hemolymph serum was pooled and stored at -70^{o}C until use.

Antibacterial activity:

Agar disc diffusion method of Bauer *et al.* (1966) was used for the assessment of antibacterial activity. Filter papers discs of 4 mm diameter were kept in aluminum foil placed in screw caped glass bottle and sterilize in autoclave; filter papers take two symbols (L and H) as abbreviations to *Lactobacillus acidophilus* cell free extract and hemolymph serum. The sterilized filter paper discs with symbol (L) were impregnated with sufficient amount of *Lactobacillus acidophilus* cell free extract for 2 h, discs with symbol (H) were impregnated with sufficient amount of blue crab hemolymph serum for 2 h. All discs were air dried under aseptic precautions. Agar plates were prepared, one plate seeded with *A. hydrophilia* and the second with *V. alginolyticus* and third with *V. parahemolyticus*. Under aseptic conditions, on the surface of each plate, two discs were gently fixed (L, H) and were incubated at 28-30^{o}C for 24 hrs and the results were recorded. Antibacterial activity was expressed in terms of diameter of inhibition zone in mm. Inhibition was indicated by the absence of bacterial growth around the test discs.

Histopathological examination:

Histological processing performed on the naturally infected crabs according to Noga *et al*. (2000); and Eddy *et al*. (2007). The exoskeletal lesions measured, photographed, and immediately fixed in Davidson's fixative solution. Tissues then decalcified in EDETA (PH 6) and 5µm sections stained with Haematoxylin and Eosin (H&E.).

RESULTS

Results of clinical examination:

The naturally infected crabs showed primarily swimming lazy, and when it walk toward the beach or shore it unable to move. The swimming legs sometimes showed leg paralysis or lost of the last two segments

(propodus and dactyle), the eye stalks in some cases found destructed or unable to retract with brownish or blackish coloration. Pitting of the topmost layer of the exoskeleton in the form of irregular sized pits ranged of few millimeters but due to coalescence or unite together to reach up for different centimeters. In some cases the pits extended toward the interior resulted in a form of foramen. The shells become soften and fragile so it was easily destructed, plate (1) photo (3, 4, 5, 6 and 7), and the most obvious lesions were graded into four grades: Very mild lesions: (Grade 1) lesions ranged from 1X1to2X2 mm, brown to black, pinpoint foci. Mild lesions: (Grade 2) lesions ranged from 1–8X1–20 mm, brown to black areas. The largest lesions appeared to arise from the coalescence of individual lesions. Moderate lesions: (Grade 3) lesions were 1–10X1–20 mm, round, oblong, or irregularly shaped, brown to black foci. They characterized by chronic erosion of the epicuticle, exocuticle and often all of the calcified endocuticle, exposing the surface of the underlying uncalcified endocuticle. The periphery of the lesions was often melanized. Severe lesions: (Grade 4) lesions, ranging up to 10-20 mm were of different shapes as round, oblong, or irregularly shaped, brown to black foci.

Results of postmortem examination:

The gills of affected crab became watery and muddy gray in color and hepatopancrease gray to black in color as showed in plate (1) photo (9 and 10). Hepatopancrease not showed obvious changes.

Plate (1): photo 1 and 2 normal crab, 3-10 showed abnormal external and postmortem lesions of affected crab.

Results of bacteriological examination:

Total number of 255 isolate were recoverd from 150 diseased crabs and was biochemically identified into *Vibrio alginolyticus* 125 isolates by percentage of 49.02 %, *Vibrio parahemolyticus* 95 isolates by ratio of 36.25% and *Aeromonas hydrophyla* 35 isolates by ratio of 13.72 %, table (1 and 2).

Table (1): Results of biophysical and biochemical characters of isolated bacteria.

Characters		*Vibrio Alginolyticus*	*Vibrio Parahaemolyticus*	*Aeromonas hydrophilia*
TCBS agar		Y	G	Y
***A hydrophila* base medium supplemented With ampicillin**		-	-	+
AGS		KA	KA	KK
Oxidase		+	+	+
Arginine dihydrolase		–	–	+
Ornithine decarboxylase		+	+	–
Gelatinase		+	+	+
Urease		–	V	–
Growth in (w/v)	**0% NaCl**	–	–	+
	3% NaCl	+	+	+
	6% NaCl	+	+	+
	8% NaCl	+	+	-
	10% NaCl	+	–	–
Growth at 42°C		+	+	-
Acid from	**Sucrose**	+	–	V
	Lactose	–	–	V
	Arabinose	–	+	V
	D-Mannitol	+	+	+
	ONPG	–	–	+
	V. P.	+	–	+
Sensitivity to	**10 µg O/129**	R	R	R
	150 µg O/129	S	S	R

Abbreviations: TCBS, thiosulfate-citrate-bile salts-sucrose; mCPC, modified cellobiose-polymyxin B-colistin; AGS, arginine-glucose slant; Y = yellow NG = no or poor growth S = susceptible nd = not done G = green V = variable among strains R = resistant P = purple, V= variable KK = Slant alkaline / Butt alkaline KA = Slant alkaline /Butt acidic, Ka = Slant alkaline/ Butt slightly acidic. V.P =Voges Proskauer

Table (2). Number of bacterial isolates and its percentages.

	V. alginolyticus	***V. parahemolyticus***	***A. hydrophila***
No of isolates	125	95	35
Percentages %	49.02	36.25	13.72

Results of antibacterial activity of lactobacillus acidophilus and crab hemolymph serum:

Antibacterial activity of lactobacillus acidophilus against the isolated strains resulted in inhibition zone measured 16, 14, and 12 mm diameter while the hemolymph inhibition zone measured 12, 17, and 15 against *V. alginolyticus, V. parahemolyticus and A. hydrophila* respectively, table (3).

Table (3). Antibacterial activity of LAB and hemolymph against isolated bacteria.

	V alginolyticus	***V parahemolyticus***	***A hydrophila***
Lactobacillus acidophilus cell free extract	16	14	12
Heamolymph cell free serum	12	17	15

Results of hitopathological examination:

The crab shell naturally infected by microorganisms of Vibrio, and Aeromonas sp. showed erosions of exocuticle, endocuticle with clavage formed extended to the membranous layer, hyperplasia of the epidermis with heavy haemocytic infiltration and proliferation of the membranous layer, in very mild, mild and moderate grades as shown in plate (2) photo (11, 12 and 13). In sever cases complete loss of the epicuticl, exocuticle endocuticle and epidermisas as shown in plate (2) photo (14). In chronic cases showed haemocytes infiltration often formed small aggregates with accumulations of melanized cells, indicating a chronic response and formation of pseudo membrane overlaid the exposed calcified endocuticle. New layers of uncalcified endocuticle membranous layer had been deposited by the epithelium between the older uncalcified endocuticle and the epithelium during the erosive process. Lesion with early pseudo membrane (PM) overlying a scalloped cuticular surface, the underlying epithelium showed moderately hypertrophic cells, with mild numbers of

granulocytes that migrating into the epithelial layer to be between the calcified endocuticle and the epithelium, plate (2) photo (15 and 16). The gills of affected crab became watery and muddy gray in color, as showed in plate (2) photo (9 and 10), this is due to an increase in the accumulation of dark material in their vacuoles in response to disease and a cuticular damage in the gills leading to the formation of haemocyte plugs formed nodules due to accumulation of nephrocytes within the branchial septa, enlargement of nephrocyte cells, vacuolation, and accumulation of a pigmented material inside, extended lesions over large areas of the gill filaments were associated with necrosis of the apical and medial parts of the gill filaments, plate (2) photo (17 and 18). Hepatopancreas of diseased crab under study plate (2) photo (19) showed destruction of hepatopancreatic tubules, infiltration of connective tissue with hemocytes and development of pigment granules, destruction of the plasmalemma, and coagulation of the cytoplasm, karyolysis and karyorrhexis of the epithelial cells, desquamation of the epithelial cells from the basal membrane observed, so only the basal membrane remained in the tubule. Sometimes we observed necrosis of the epithelial cells of all types and the basal membrane.

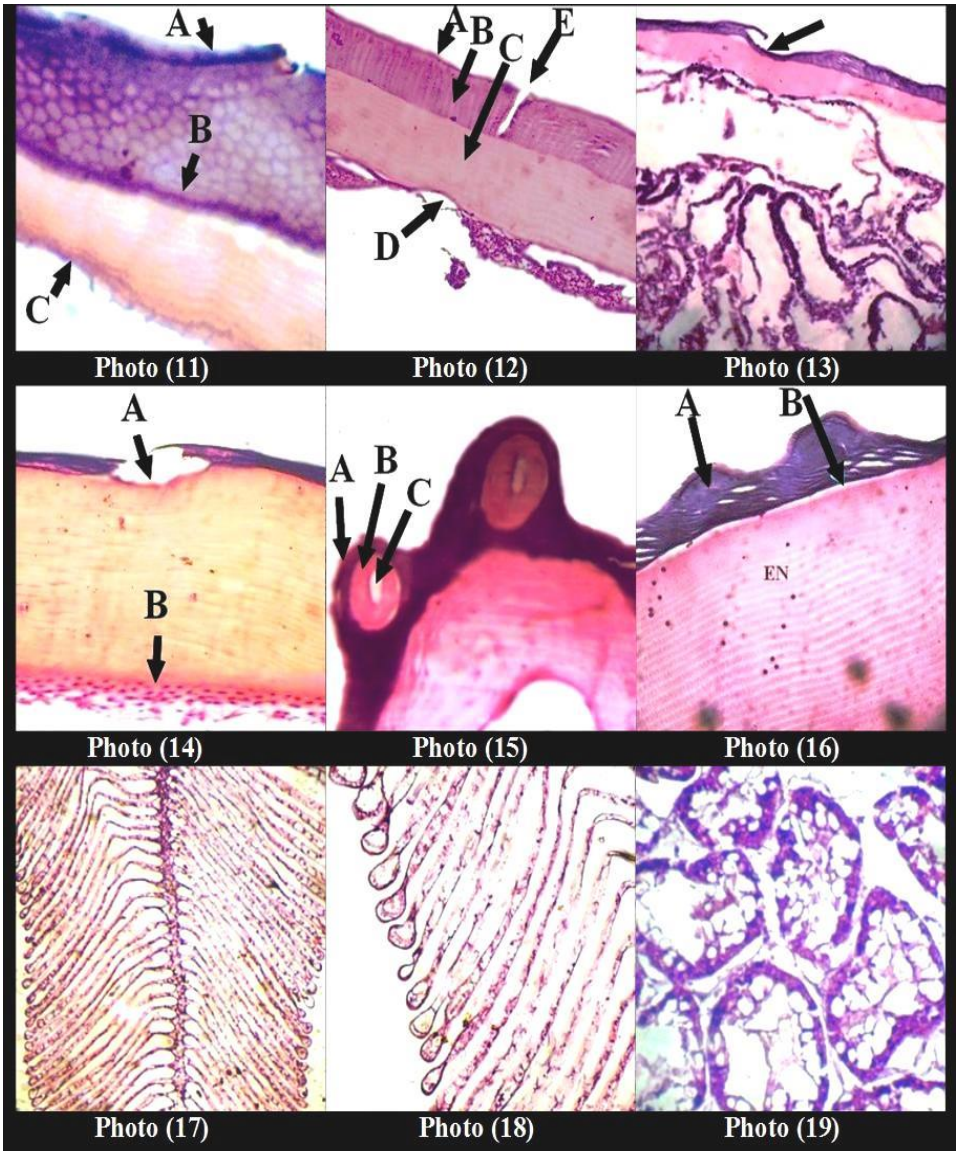

Plate (2): photo 11-19 histopathological changes in different organs of affected crab H&E.

DISCUSSION

Crabs live in different environments, marine, brackish and freshwater and no one could deny that marine environment by its water, clay, sand, rocks, and other marine animals are sterile. Chitin is an abundant polymer within the marine environment, thus chitinolytic bacteria are both common and vital to nutrient recycling Keyhani and Roseman (1999). Chitin degradation occurs to the exoskeletons (cuticle) of living crustaceans, the condition is known as shell disease or black-spot. The bacterial flora was abundant; some had beneficial effect, the others had harmful effect. In crab life cycle, it was exposed to several stages as ecdysis (molting), predatory or cannibalistic attacks Dyrynda, (1998), chemical attack Schlotfeldt (1972) or the abrasive action of sediment and /or articulated body parts Young (1991); Vogan *et al.* (1999), changes in chemicals and physical quality of water due to organic load Powell and Rowley (2005), all were play a stressful on crab ranged from very less to more effects.

The naturally infected crabs showed primarily swimming lazy, and when it walk toward the beach or shore it unable to move, this was due to the extracellular products (ECP) resulted from the growth of bacteria in the hemolymph and internal organs, these products has the ability to affect the nervous system of the diseased crab. The swimming legs sometimes showed leg paralysis or lost of the last two segments (propodus and dactyle), the eye stalks in some cases found destructed or unable to retract with brownish or blackish coloration. These finding similar to results of Costa-Ramos and Rowley (2004) they injected extra cellular products (ECP) of *Pseudoalteromononas* isolated from shell disease-infected edible crabs (*Cancer pagurus*) into healthy crabs causes the same symptoms and ended with death. Different discolorations of the epicuticle ranged from melanization, spotted areas of black or brown color on the dorsal surface of the crab sometimes distributed up to one

fourth to one third of the shell surface area, some crabs showed brown pigmentations on their legs. These results was explained by So¨derha¨ll and Cerenius (1998) and Lee and Söderhäll (2002) they mentioned that the black coloration of lesions is the end-result of the melanization reaction, a defenses response triggered by cuticular damage.

Pitting of the topmost layer of the exoskeleton in the form of irregular sized pits. In some cases the pits extended toward the interior resulted in a form of foramen. The shells become soften and fragile so it was easily destructed**.** These signs agree with those explained by Cipriani *et al.* (1980) who described that the disease commences with the removal of the non-chitin-containing outer layer of the exoskeleton, called the epicuticle, and may occur by lipolytic and proteolytic microbial activities**.** Some bacteria has the ability to digest chitin of the shell (carapace) that begins its action after the occurrence of abrasions due to stress factors and lead to soft shell**.** These symptoms and lesions agree with that resulted and discussed by Ryazanova (2005) who showed in heavy stage of the shell disease, numerous erosions and dark brown to black spots from 0.5 to 5 cm in size often with softening and ulceration in the center on different parts of the carapace, walking legs and chelae.

From a total number of 200 specimens collected from spot lesion, 150 isolates were obtained and were biochemically identified into 125 isolates of *Vibrio alginolyticus* by percentage of 49.02 %, 95 isolates of *Vibrio parahemolyticus* by percentage of 36.25% and 35 isolates of *Aeromonas hydrophyla* by percentage of 13.72 %. Shell disease experimentally induced by exposure of crustaceans to sewage sludge Young and Pearce (1975). Exposure to pesticides Weis et al. (1987) or heavy metals Nimmo *et al.* (1977) also produces shell disease-like lesions, suggesting that this syndrome may be a useful biomarker of environmental stress Sindermann (1989, 1990). Bacteria belonging to the genera Vibrio, Aeromonas, Pseudomonas, Alteromonas, Flavobacterium,

Spirillum, Moraxella, Pasteurella and Photobacterium were reported as probable agents involved in the disease syndrome, Getchell (1989), Özoğul *et al.* (2010). Noga *et al.* (2000) mentioned that blue crabs affected with shell disease due to aeromonads, vibrios. Vogan *et al.* (2002) stated that Chitinolytic bacteria were the primary etiological agents of shell disease syndrome in marine crustaceans.

Trial for controlling the pathogenic microorganisms was carried out by the use of safe, cheap, available, with no any harmful effect if it compared to that produced due to the chemotherapeutics use of drugs, it is the probiotic bacteria. By disc diffusion inhibition it was found that the inhibition zone diameter of lactic acid bacteria *(Lactobacillus acidophilus)* isolated from cattle milk whey was found16, 14, 12 and 16 mm against *V. alginolyticus V. parahemolyticus and A. hydrophila* respectively. The use of probiotic bacteria against some pathogenic bacteria as vibrios and aeromonads was conducted by researchers as Gildberg *et al.* (1995, 1998); Ajitha *et al*. (2004); Nogami and Maeda (1992) and others. Inhibition of *Vibrio* by the cell free filtrate of *L.acidophilus,* obtained in this study is in agreement with those obtained with LAB culture filtrate and by LAB in mixed culture against *A. salmonicida* (Gildberg *et al.*, 1995). We conclude that cell free extract of *Lactobacillus acidophilus* was effective against the isolated microorganisms by antagonism growth in vitro, while the live whole cell of LAB compete with pathogens for nutrients, this agree with the results performed by Ajitha *et al*. (2004) who studied the effect of Lactic acid bacteria (LAB) in vitro on some bacterial pathogens and cell free extracts of four strains of Lactic acid bacteria (LAB) *Lactobacillus acidophilus, Streptococcus cremoris, Lactobacillus bulgaricus* –56 and *Lactobacillus bulgaricus* –57 found to be inhibitor for growths of *Vibrio alginolyticus* in nutrient broth and confirmed by streak plating and suppression of growth of Vibrio was obtained. The antibacterial activity of probiotic

bacteria in vitro challenged the pathogens successfully as evidenced by the production of clear zones of inhibition against the growth of the target pathogens, Rahman *et al.* (2009), the bacillus propagated on selective basal medium were found most efficient against Vibrios and produce 20-30 mm zones of inhibition. The antibacterial activity of extracellular products from these probiotic bacteria rendered to rapid acidification through the production of organic acids, mainly lactic acid, Nogami and Maeda (1992), acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes is of importance in Lactic acid bacteria (LAB), De Vuyst *et al*. (2004).

Hemolymph of the brachyuran crustacean *Callinectes sapidus* possesses bactericidal activity which is highly inhibitory to Gram-negative bacteria including *Aeromonas hydrophila*, *Vibrio parahemolyticus*, *V. alginolyticus.* Antibacterial activity of the hemolymph owed to metabolites produced inside the humeral cells and released outside as a defense mechanism against invading microorganisms and its non specific in action, these results agree with that obtained by Enany *et al.* (2012) and Noga *et al.* (1996 a & b) antibacterial activity of the hemolymph appeared to be confined to the haemocytes. The initial investigations revealed that this antibacterial activity was proteinaceous (inactivated by proteolysis) and was found mainly within the hemocytes**.** In crustaceans, circulating hemocytes play significant roles in the innate immune response, including release of non self-recognition proteins, clotting proteins, antimicrobial peptides and prophenoloxidase (So¨derha¨ll and Cerenius, 1998 and Terwilliger, 1999).

Histopathological examination of the crab shell naturally infected by microorganisms of Vibrio, and Aeromonas sp. showed erosions of exocuticle, endocuticle with fissure formed extended to the membranous layer, hyperplasia of the epidermis with heavy haemocytic infiltration and

proliferation of the membranous layer. In chronic cases showed total loss of the epicuticl, exocuticle endocuticle and epidermis with haemocytes infiltration often formed small aggregates with accumulations of melanized cells, indicating a chronic response and formation of pseudomembrane overlaid the exposed calcified endocuticle**.** New layers of uncalcified endocuticle membranous layer had been deposited by the epithelium between the older uncalcified endocuticle and the epithelium during the erosive process. This resulted in thickening and mild parallel lamination of the uncalcified endocuticle layer, Lesion with early pseudo membrane (PM) overlying a scalloped cuticular surface, the underlying epithelium showed moderately hypertrophic cells, with mild numbers of granulocytes that migrating into the epithelial layer to be between the calcified endocuticle and the epithelium., these results agree with that described by Vogan *et al.* (2002), Lee and Söderhäll (2002) point out that shell disease characterized by external manifestation of black-spot lesions on the exoskeleton of crustaceans. The black coloration of lesions is the end-result of the melanization reaction, a defense response triggered by cuticular damage. The melanized encapsulations in the epithelium may have been related to stimulation of inflammatory cells by a bacteraemia or toxic products Johannson and So¨derhall (1989). For wound healing**,** since its principal targets are the circulating cells of the immune system, the hemocytes and healthy epidermal cells and fibrocytes. Massive migration of these cells occurs under the wound and their concerted efforts under ecdysteroid control are paramount to wound healing and repair. Vafopoulou (2009). The membranous layer showed epithelial hyperplasia associated with supra epithelial bullae packed with finely granular, eosinophilic matrix (presumably proteinaceous fluid) which may be a un- polymerized portion of the membranous layer or alternately an area of premature separation of the epithelium from the un-calcified cuticle. The gills of affected crab became watery and muddy

gray in color, this is due to an increase in the accumulation of dark material in their vacuoles in response to disease and a cuticular damage in the gills leading to the formation of haemocyte plugs termed nodules due to accumulation of nephrocytes within the branchial septa, enlargement of nephrocyte cells, vacuolation, and accumulation of a pigmented material inside, extended lesions over large areas of the gill filaments with necrosis of the apical and medial parts of the gill filaments, Similar to those reported by Vogan *et al.* (2001). Hepatopancreas of diseased crab under study showed destruction of hepatopancreatic tubules, infiltration of connective tissue with hemocytes and development of pigment granules, destruction of the plasmalemma, and coagulation of the cytoplasm, karyolysis and karyorrhexis of the epithelial cells, desquamation of the epithelial cells from the basal membrane were observed, so only the basal membrane remained in the tubule. Sometimes necrosis of the epithelial cells of all types and the basal membrane was observed. This was in agreement with the finding of Ryazanova (2005) and Vogan *et al.* (2001).

CONCLUSION

Crabs were infected by several bacterial pathogens such as aeromonas and vibrio can be a source of infection to human being through handling or insufficient coking, also it is a source of infection of most valuable marine and brackish fish and animals.

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دراســات بكتريولوجيــة وهســتوباثولوجية على مرض القشــرة فى الكابــوريا المعديــة طبيعيــا

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الملخص العربــى

اجريت هذه الدراسة على الكابوريا المأخوذه من قناة السويس منطقة القنطرة محافظة الاسماعيلية مصر. بالفحص الاكلينيكى لمائه و خمسون كابوريا بها عدوى طبيعيا عليها علامات اصابه بالقشرة مبدئيا كسولة العوم و عندما تمشى اتجاة الشط فهى غير قادرة علي الحركة، ارجل العوم احيانا تفقد رد الفعل و مشلولة مع فقدان قطعة او اثنين (الاولى و الثانية) ، وحامل العين وجد فى بعض الاحيان محطم او غير قادر على الانجذاب. انتشار بقع بنية او سوداء غلي السطح الظهري للقشرة تتراوح من ١-٢ ملليمتر و حتى ٢-٣ سنتيمتر. فى بعض الاحيان هذه البقع تندمج مع بعضها لتمتد العلامات لمساحات كبيرة على اجزاء مختلفة من الطبقة الخارجيه غالبا السطح العلوى اكثر من السفلى. بالفحص الداخلى نتج عنه وجد ان الخياشيم ذات لون رمادى مسود مع عدم وجود علامات على الاعضاء الداخلية الاخرى. اجرى الفحص البكتريولوجى والتصنف و نتج عنه ١٢٥ عزلة من الفيبريو الجينوليتكس بنسبة ٤٩.٠٢ % ، ٩٥ عزلة من القيبريو باراهيموليتكس بلسبة ٣٦.٢٥ %، ٣٥ عزلة من الايروموناس هيدروفيلا بنسبة ١٣.٧٢ %. النشاط المضاد البكتيري للاكتوباسيلس اسيدوفيلس ضد السلالات المعزولة نتج عنة منطقة تثبيط قطرها كان ١٦، ١٤، ١٢ ملليمتر بينما منطقة الهموليمف المثبطه قطرها كان ١٢، ١٧، ١٥ ملليمتر ضد الفيبريو الجينوليتكس، القيبريو باراهيموليتكس، الايروموناس هيدروفيلا على التوالى. النتائج الهستوباثولوجية كانت التهابات تتراوح من خفيفة جدا- خفيفة – متوسطة – خطيرة و مزمنة. تسلخات للطبقة الخارجية تمتد للطبقة الداخلية مع تكون شقوق تمتد للطبقة الغشائية، زيادة حجم الابيدرم انتشار لخلايا الهيموسايت و زيادة عددية لخلايا الطبقة الغشائية. فى الحالات المزمنة لوحظ فقد كل الطبقات اللتى تتكون منها القشرة مع انتشار لخلايا الهيموسايت غالبا متكونة من تجمعات صغيرة مع تراكم خلايا الميلانين موضحة استجابة مزمنة و تكوين غشاء كاذب يغطى الاندوكيوتكل الكلسى المكشوف. لوحظ تتكرز الخياشيم في الجزء القمى و الوسطى للخيوط الخيشومية مع انتشار خلايا الهيموسايت داخل تجاويف الخيوط الخيشومية و العمود المركزى و ظهور بقايا صبغية. لوحظ تتكرز للقنيات البنكرياسية للهباتوبنكرياس مع انتشار نسيج ضام بين خلايا الهيموسايت و تكون حبيبات صبغية وتكسير لغشاء البلازما القاعدى.