STUDY THE INCIDENCE OF FURUNCULOSIS IN SOME FARMS IN BASRA PROVINCE.

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ABSTRACT

During March to April 2016 many farms in Al_Qurna,Al_Zubair,and inside Al Basra city had thousands loses of *Cyprinus carpio* that had large furuncles on different sites of the skin ,these were examined clinically, microbiologically and histopathologically.The living infected fish appear with gasping , stop near the margin of the aquarium . The lesion was large circle with irregular margin, appears as open wound with clear haemorrhage ,lose of scales, pop eyes ,some had swelling abdomen. The biological examination revealed that *Aeromonas salmonicida* was the causative agent .Histopatholgically showed thickness of epiderms, increase alarm cells and mucin cells in the skin, odema in muscles, vacuolation of cortical tubules in kidney, vacuolation of hepatocytes, Testis with spermatogenesis and congested blood vessels. Gills with damage of secondary lamella. Intestine with dilated mucus glands . Eye inflammation with thickness of its layers.

INTRODUCTION

Furunculosis is a bacterial infection of salmonids and other fishes caused by *Aeromonas salmonicida* which is a Gram-negative, facultatively anaerobic, non-motile rod. The size is 0.8-1.3 mm (1),(2). Furunculosis is an acute to chronic condition, with a variety of clinical signs(3). The disease generally appears to develop as a septicaemia and is often fatal. Affected fish often show darkening of skin,

lethargy and in appetence. Haemorrhages may occur at the bases of fins and the abdominal walls, heart and liver. Enlargement of the spleen and inflammation of the lower intestine are common features of chronic infections, but in acute outbreaks fish may die rapidly with few signs. The disease is named after the raised liquefactive muscle lesions (furuncles) which sometimes occur in chronically infected fish (2).The major route of transmission appears to be via infected fish and contaminated water (3). Although the disease causes mortality of all ages, the most serious losses occurs during spring-autumn in the sea water farms. An important aspect of furunculosis is the carrier state, which is often established after the fish have been exposed to *A. salmonicida*. Clinical outbreaks and mortality appear to be triggered by stress factors such as crowding, poor water quality, fright, high temperature and physical trauma (4).Basra rivers were under long term chronic pollution(5). Shatt Al-Arab is polluted by some different source of pollutants including power station, paper industry, oil refineries ,petrochemical industry, chemical fertilizer companies and the sewage system and overfishing and the application of pesticides(6).

MATERIAL AND METHODS

Several live fish from each farm were received and immediately necropsies, and samples of water and fish for bacteriology test were taken, The latter consisted of swabs taken from the actual skin lesions and initially cultured on Trypton soya agar (TSA) and the plates incubated at 22° for 48 h., The identity of colonies was conducted principally by biochemical tests. As well as pieces of skin and muscle bordering the lesions, various internal organs, the eyes ,gills and the fins of the fish were also collected in 10% neutral buffered formalin for histopathological study .They were embedded in paraffin wax, sectioned and routinely stained with haematoxylin and eosin (HE).Some of these living infected fish were put in the glass aquarium to monitor the clinical signs.

RESULT

Clinical signs of living infected fish were less affective ,isolate from other fish in aquarium with difficult breathing ,large abdomen, pop eyes, The lesions were

ranging from small slightly raised furuncles (figure 1) to shallow ulcer and then developed to large ,deep judge ulcer including muscles with grayish -white necrotic zone with hemorrhage ,liquid &inflammation , shed scales ,fin rot (figure 2&3) . Some cases had darkening skin colure(figure 2) ,inflammation on mouth, paleness of gills(figure 4&5) .post mortem examination revealed yellow discoloration of the liver, splenomegaly with hemorrhage (figure 6&7), and slight enlargement of the kidney(figure 8) . The internal organs showed few gross changes only in heavy developed furuncle. There was fish with slight lesion had no changes remembered. The bacterioscopic examination resulted of Gram negative, rod-shaped, not motile bacteria (without cilia)), with length between 0,8 –1,2 μ, which led to their categorization into *Aeromonas* genus.The main biochemical tests for taxonomic categorization were: positive reaction to oxidase test-distinctive genus feature, lack of motility – distinctive feature for the species differentiation and brown pigment synthesis–characteristics of the S*almonicida* subspecies.The biochemical test and bacterioscopic exams conducted to the identification of the illness pathogenous agent, *Aeromonas salmonicida salmonicida*, which,in addition to clinical and anatomopathological examinations confirmed the diagnosis as furunculosis.The bacteria was sensitive to sulfanimid ,erythromycin, tetracycline and streptomycin.Histopathological exam showed thickening of fibrin layer,keratin like material,odematus like fluid with fibrin between skeletal muscle ,fibrile material like in the superficial part of skin,erosion like in the edge of the fin ,swelling and vaculated of fin cells, increase club cells (alarm cells) number in epidermal layer and mucin cells.Inflammatory cells with odema in the skeletal muscles ,kidney had localize hemorrhage, vaculation of cortical tubules ,evidence of hematopoises.Testis apperared with spermatogenesis &congested blood vesicles ,gills with damage of primary and secondary lamellae. Eye inflammation,thickness of retina ,choroid and sclera.

Figure 1: Small slightly raised furuncles.

Figure 2: Shallow ulcer

Figure 3: Deep judge ulcer, muscles With grayish –white necrotic zone inflammation and hemorrhage.

Figure 4:palness of gills with mouth inflammation

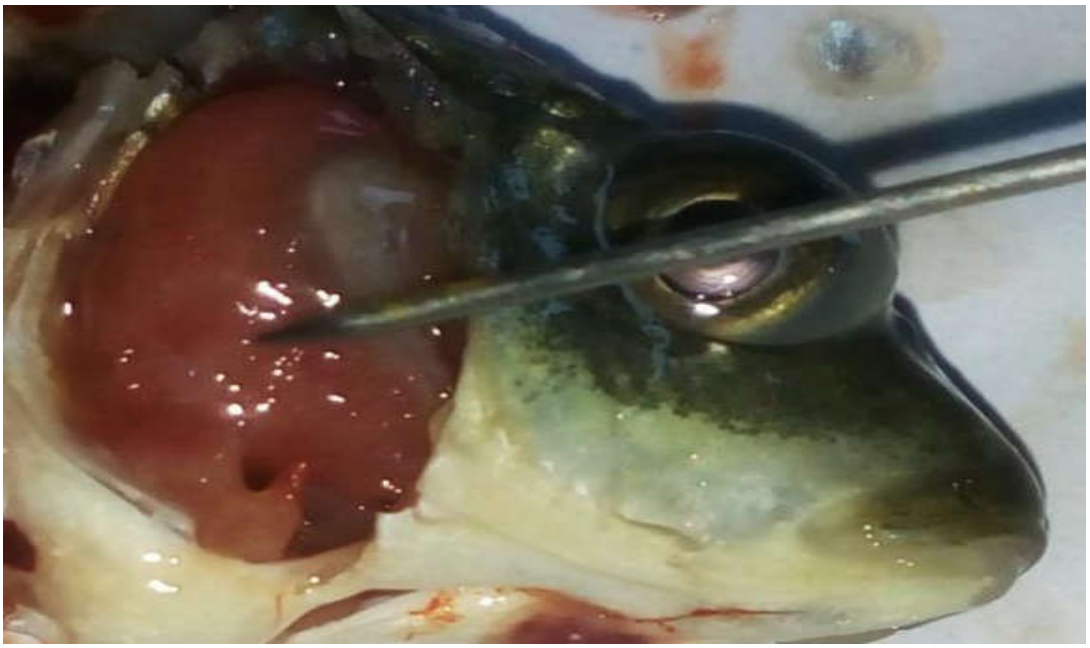

Figure 5:palness of gills with pop eyes

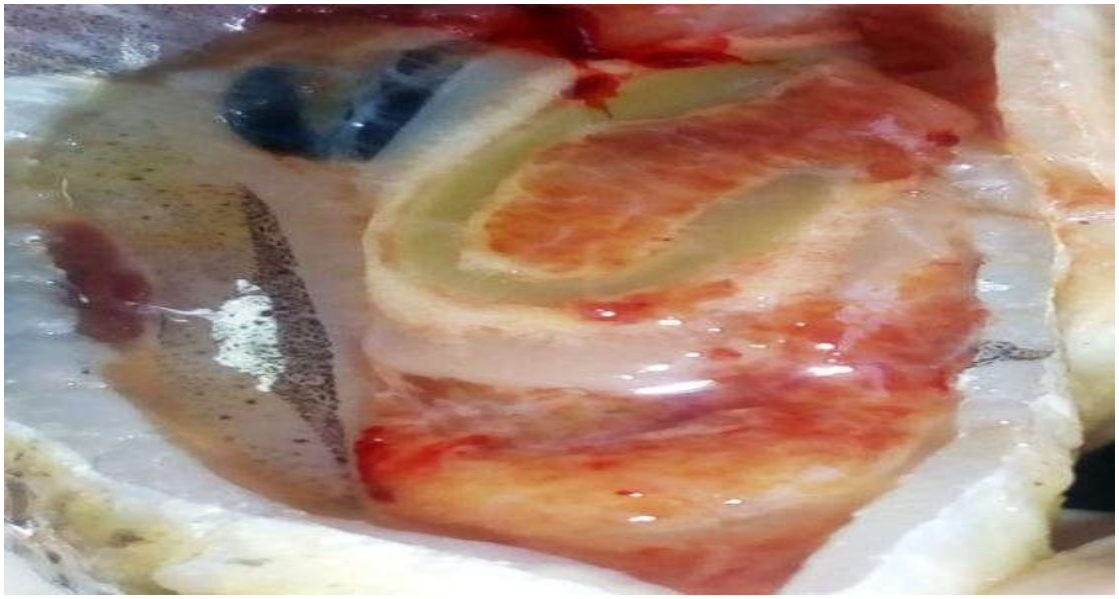

Figure 6: paleness of liver with slight splenomegaly.

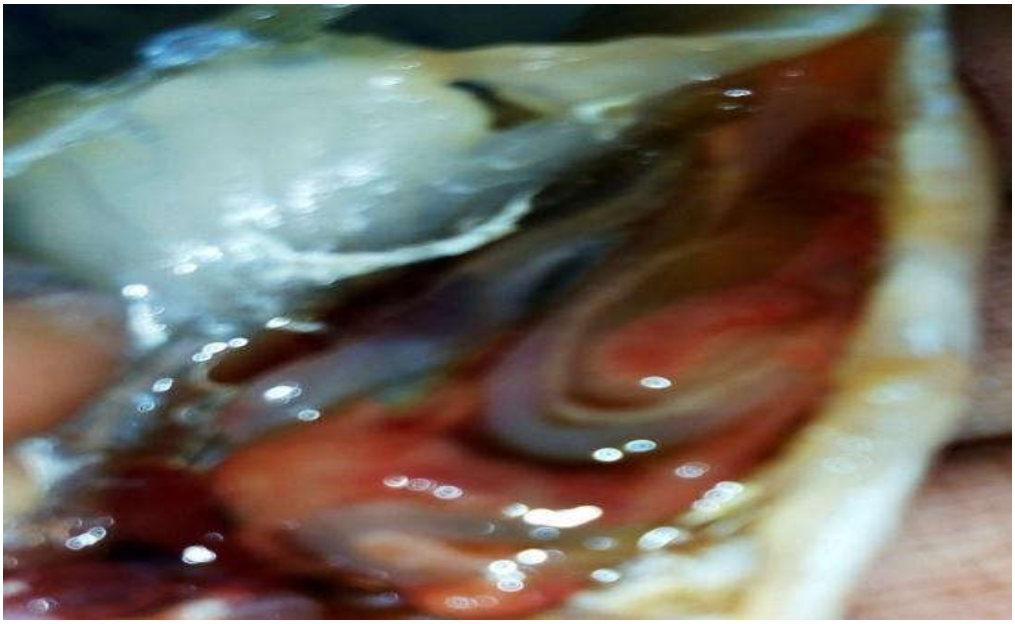

Figure 7: hemorrhage of liver and other internal organs

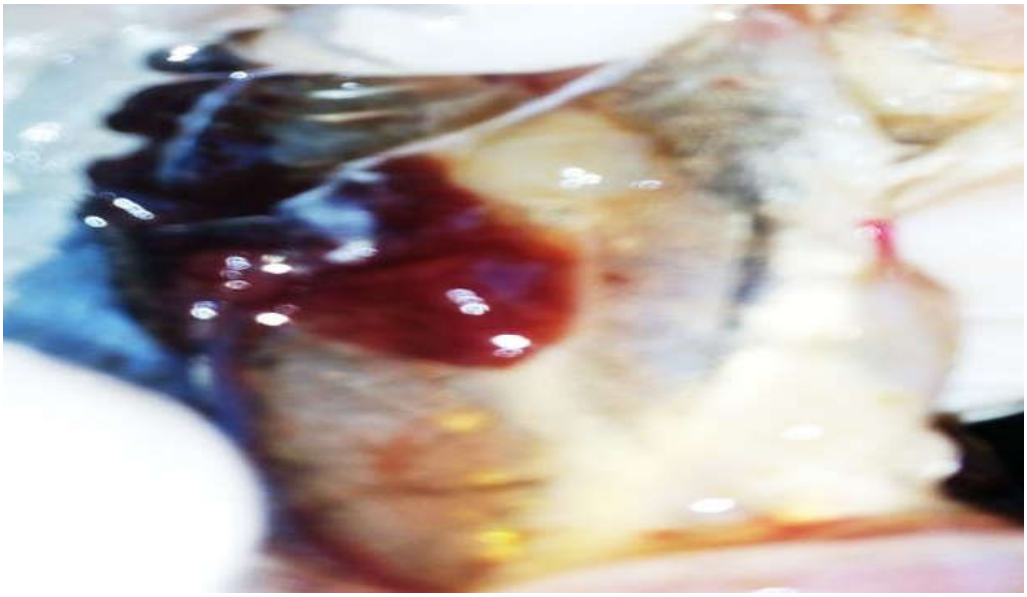

Figure 8: slight enlargement of kidney

Figure 9: Hepatomegaly.

Figure 10: Colonies of *Aeromonas salmonicida* on TSA.

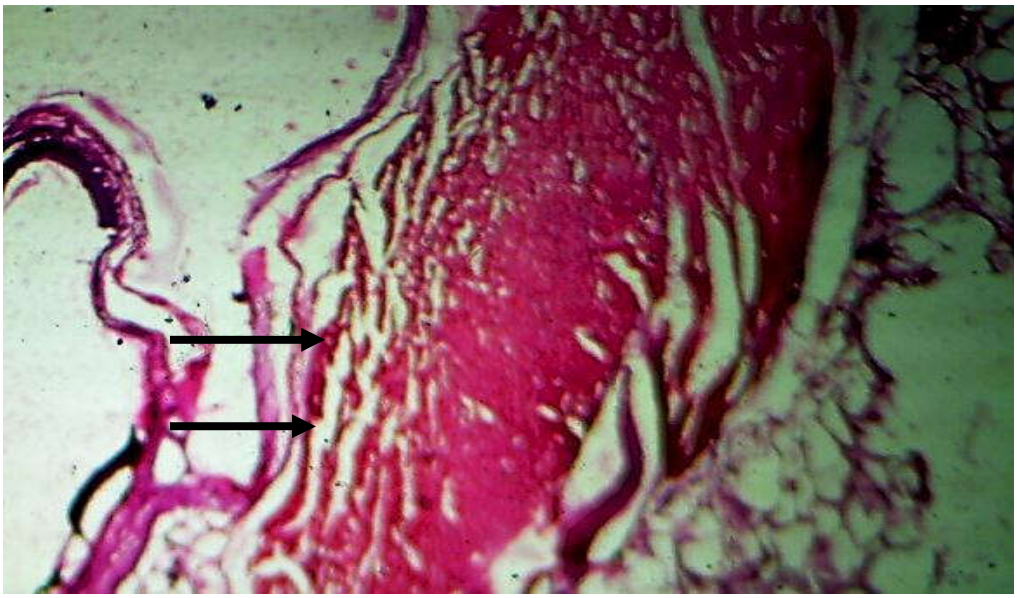

Figure 11: Fin and skin :Thickening of fibrin layer.
H&E(40X)

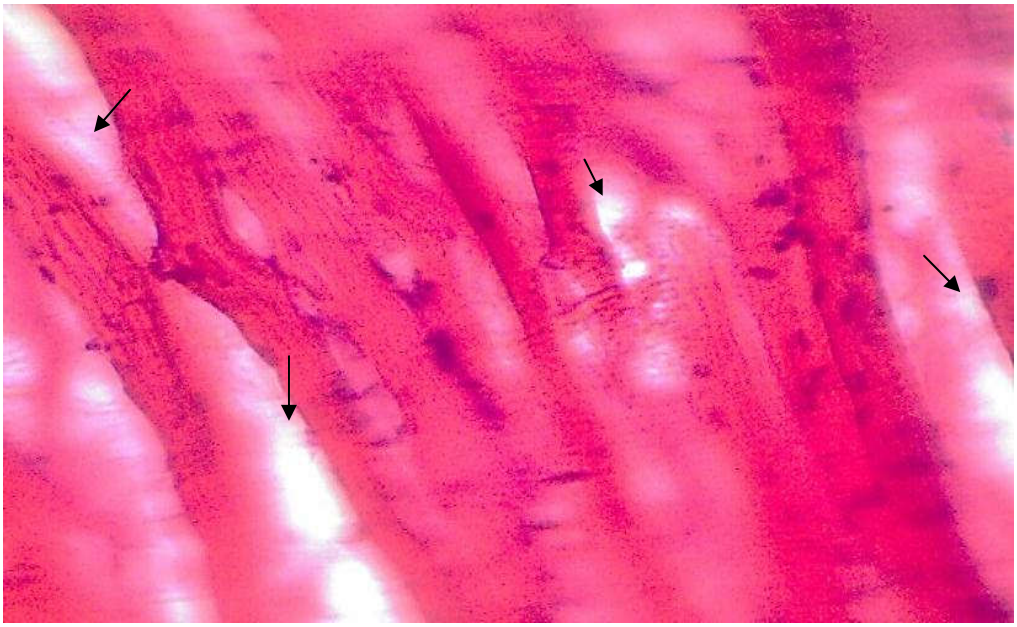

Figure12: Fin: Keratin likes material. H&E (40X).

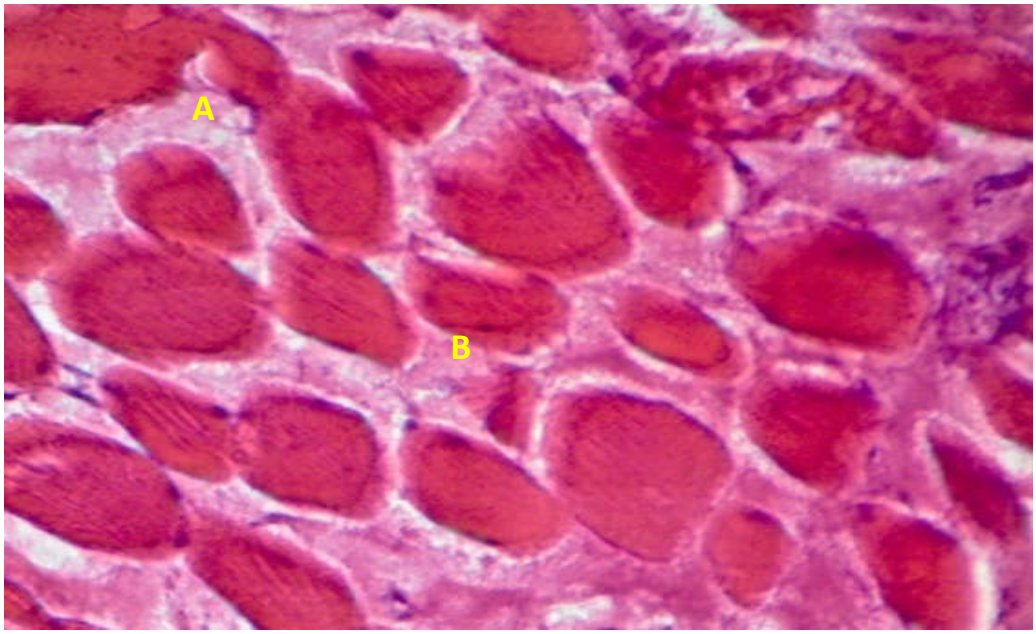

Figure 13:Skeletal muscles:Odematus like fluid(A) with fibrin between skeletal musclen(B) H&E.(40X).

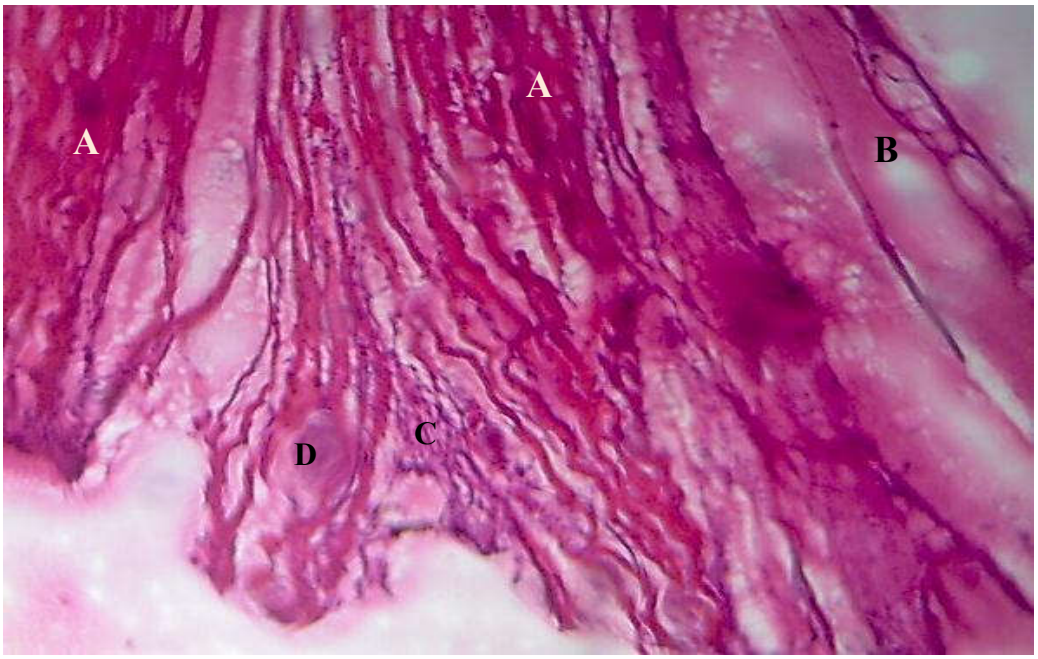

Fig 14:Skin:Fibrinous material in the superficial part of skin (A),Skin with fluid(B) ,Slight inflammation (C) and Mucine discharge(D) H&E(10X).

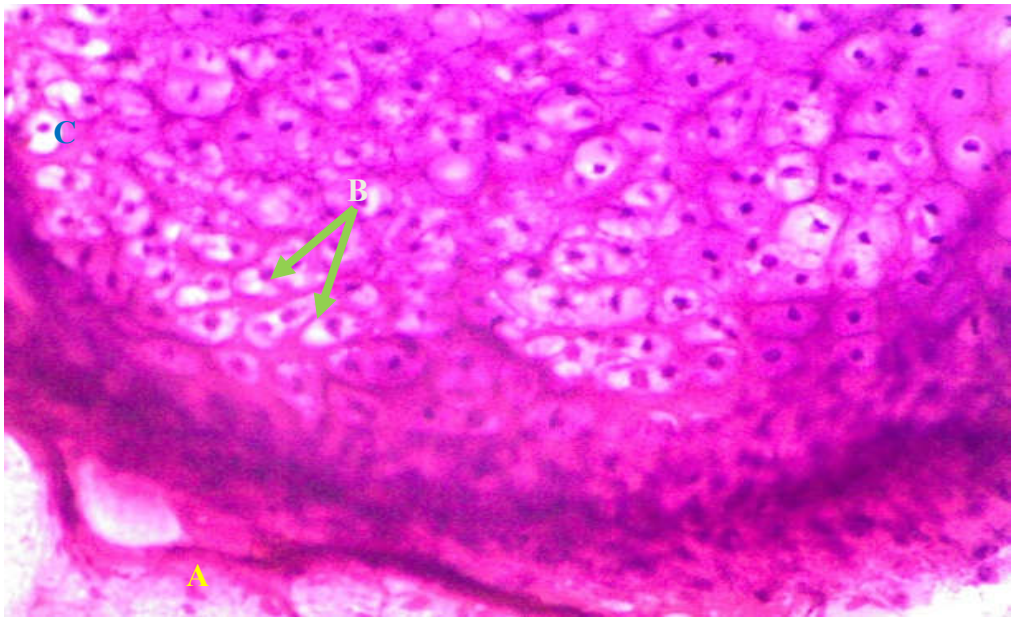

Figure 15:Fin: Erosion like in the edge of the fin(A),swelling(B)and vacuolated of fin cells(C).H&E.(40X)

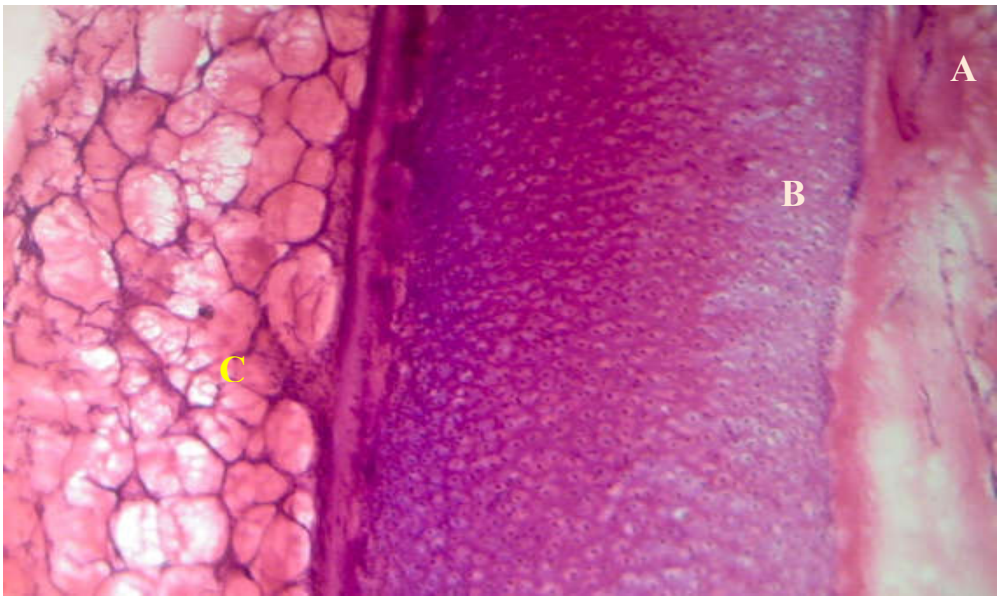

Figure 16: Skin with fluid(A) and fin layers, fin cartilage(B)and adipose tissue(c) . H&E(40X).

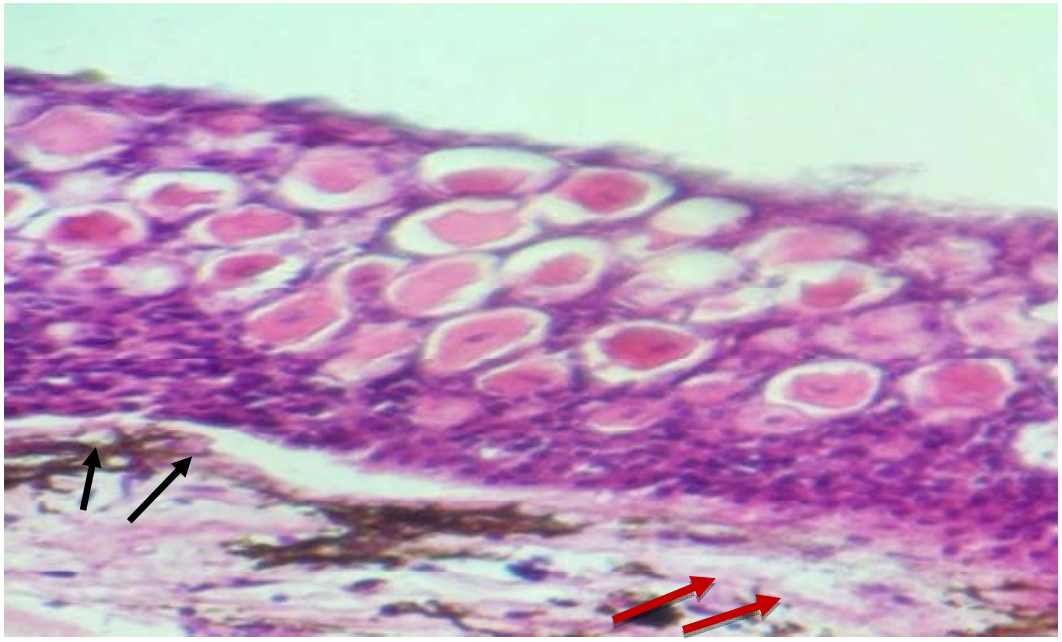

Figure 17: Skin: increase club cells (alarm cells) number in epidermal layer (black arrow) and mucin cells(red arrow).H&E.((X40).

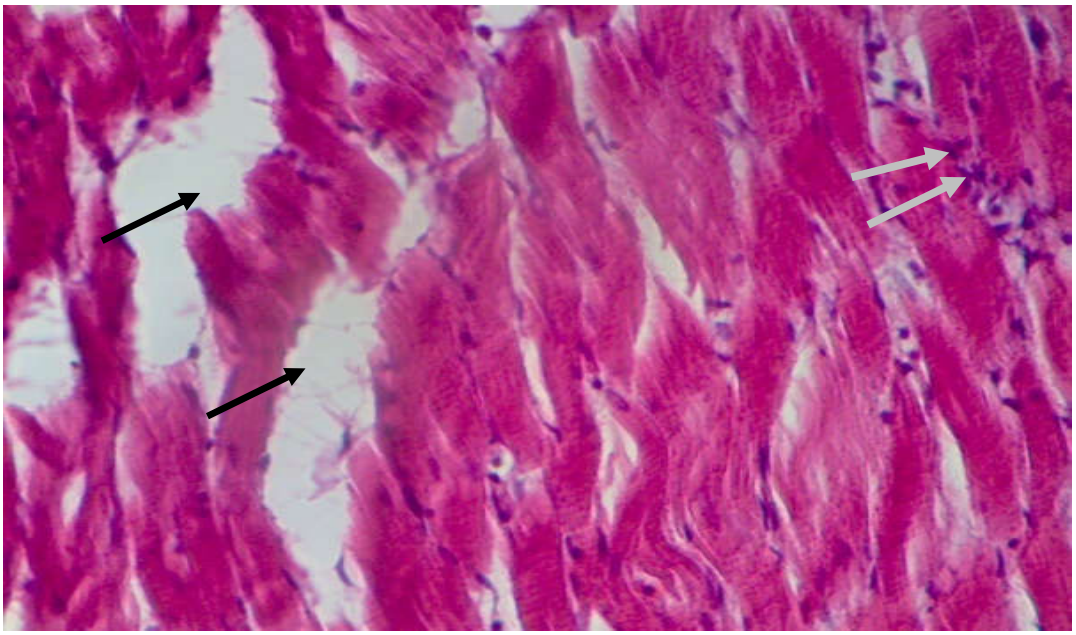

Figure 18 :Muscle :Inflammatory cells( )with odema(.)H&E(X40)

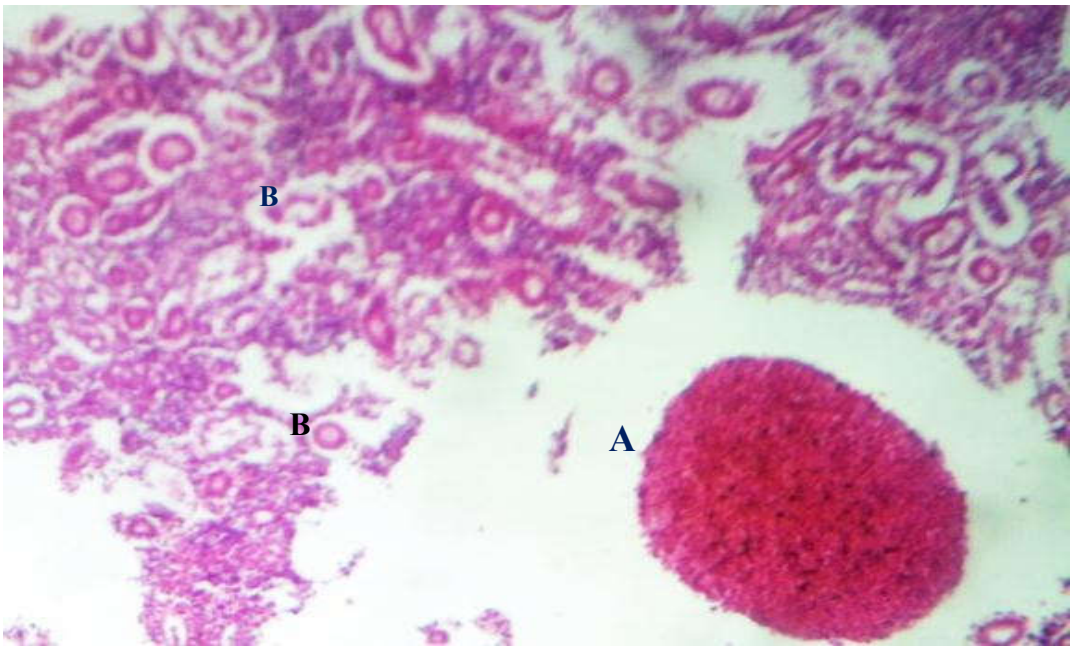

Figure 19: Kidney: localize hemorrhage(A) , vaculation of cortical tubules(B) , evidence of hematopoiesis . H&E(40X)

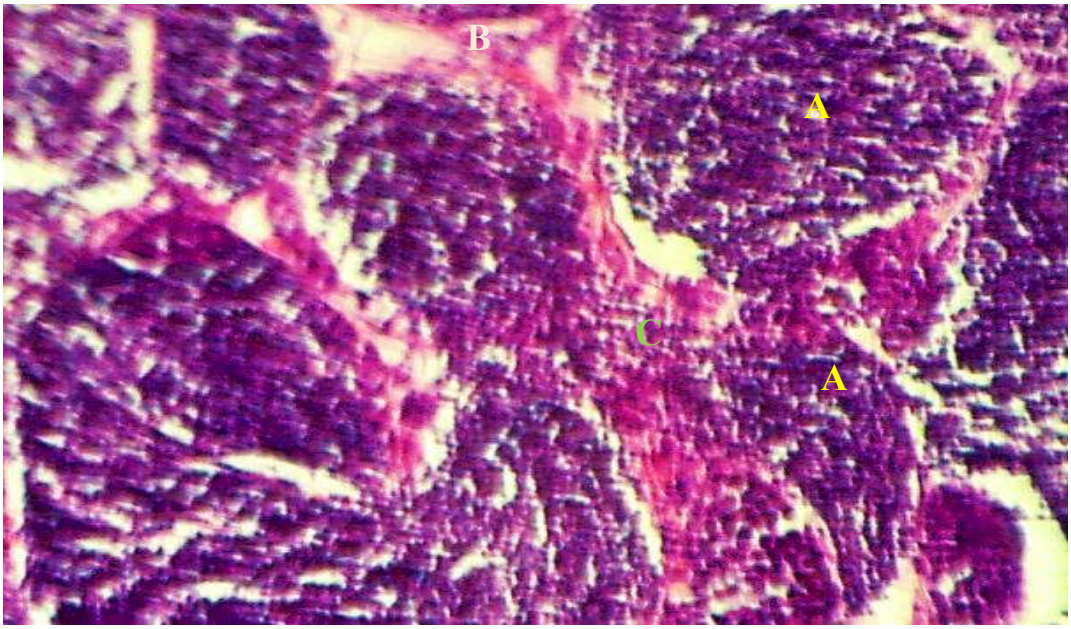

Figure 20: Testis with spermatogenesis(A) with fibrin(B) and congested blood vessels(C) H&E(10X)

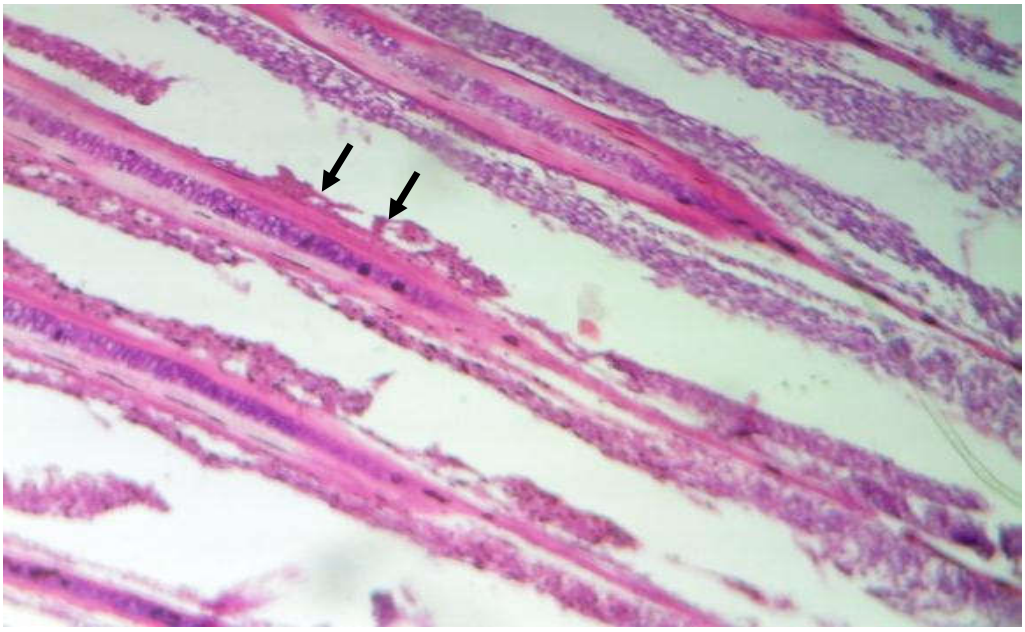

Figure 21: Gills with damage of secondary lamellae(40X).

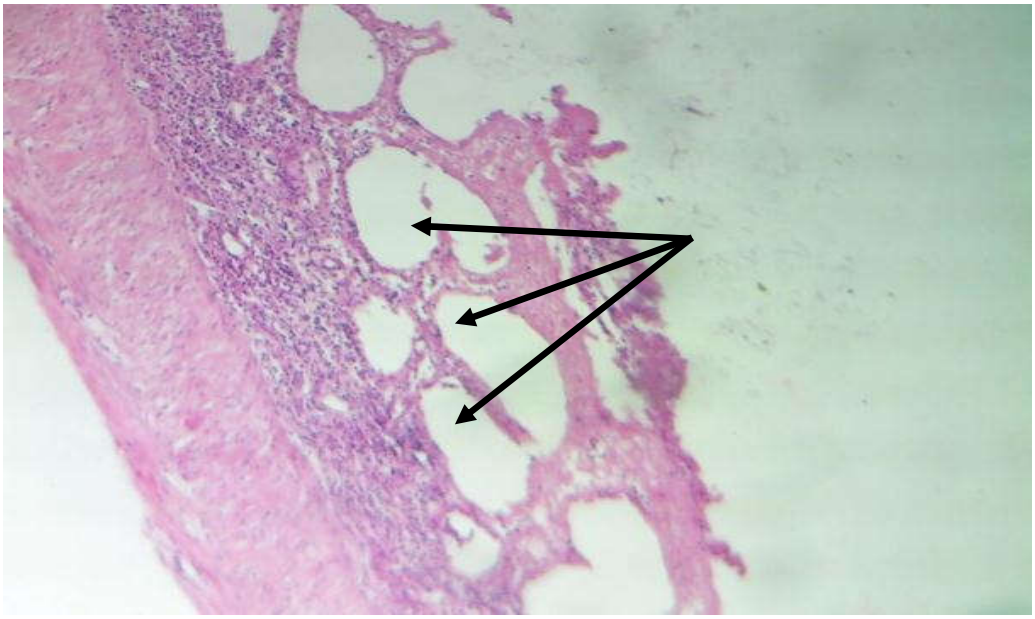

Figure 22: Intestine with dilated mucus glands. H&E(40X).

Figure 23: Liver with vacuolated hepatocyte(A) ,congested of central vein (B) H&E(40X)

Figure 24: Eye inflammation, thickness of retina(A) , sclera(B) and choroid(C) H&E. (10X).

Figure 25: Sensitivity test of *Aeromonas salmonicida* to antibiotics

DISCUSSION

In almost, most of fish examined had extensive skin damage in different sites of the body, the lesion had a furuncle like appearance resemble to those found by (7), (8) who found that in carp affected by Carp erythrodermatitis(CE), the ulcers can be found all over the body surface except on the head ,the scales of the fish have been reported to be initially surrounded by inflammation with infiltration of inflammatory cells,the scales are subsequently shed. The epithelium and corium become necrotized with marked hyperaemia surrounding the resulting ulcer. The inflammatory cells subsequently spread into the muscle(7),(8). (9) describe the first signs of CE appear as one or more small inflamed hemorrhagic areas or small white erosions surrounded by a narrow red zone and darkened pigmentation. Ulcers proliferate in the central necrotic areas. The causative bacterium is present exclusively in lesions between the dermis and epidermis (9).

Common carp with extensive lesions also show exophthalmia, have a distended abdomen and hemorrhages in the gills, and are anemic. In advanced cases, transudate is found in the abdominal cavity, and organs may be edematous (10). In present study only a few cases had the macroscopic lesion of internal organ. (11), and(8) reported that macroscopical signs of disease in internal organs of fish (silver bream *Blicca bjoerkna*, *carp, minnow,perch Perca fluviatilis*, yellow bass *Morone mississippiensis*) infected with atypical *A. salmonicida* were not observed, although(8) stated that a slight fatty degeneration was sometimes visible in carp affected by carp erythrodermatitis. According to (10), the internal organs of carp affected with small ulcers appeared normal, but in the terminal stages fluid accumulation was found in the abdominal cavity and the internal organs were edematous. In several studies no indication of pathological signs in internal organs of non-salmonids infected with atypical A. salmonicida have been reported (12), (13), (14).Additionally, enlargement of the hepatocytes, degenerative changes in the kidney, congestion and in some cases hyperplasia in the spleen were observed(15) and this is agree with the present study. Pathological signs of disease in the intestine (haemorrhage and hyperaemia) have been observed in captive sand-eels(16) and farmed common wolf fish *Anarhichas lupus* (17). Additionally, haemorrhages in the liver and in the musculature of infected sand-eels have been reported(16). In farmed wolf fish, neither a corresponding leucocyte reaction nor fibroblast encystment of atypical *A. salmonicida* was observed(17).Although for several years it has been widely accepted that a correlation exists between virulence and the possession of a cell-surface protein array, the A-layer, this was further questioned by the isolation of virulent strains lacking this A-layer as well as a virulent strains which retain the A-layer. Now it is widely accepted that although cell-surface characteristics can play a role in the pathogenesis of furunculosis, they are not the sole virulent determinants of *A. salmonicida*.(18).The "furuncle" in these fish consists of tissue fluid exudate, necrotic tissue, and some macrophages, and is different from true furuncles of homeotherm vertebrates, which are characterized by a necrotic mass of polymorphonuclear leukocytes.(19).In present study there is increase in alarm cells in epidermal layer.Alarm substance cells in fish may provide fitness benefits through defence against parasites (20), bacterial infection

(21). Knowing that a high level of pathogen exposure is correlated with high alarm cells densities(22).

Sensitivity test investigate that *Aeromonas salmonicida* was sensed to sulfanimid,tetracyclin,erythromycin and streptomycin, (19) remembered that sulfamerazine and oxytetracycline may be used for treatment of furunculosis and treatment must terminate at least 3 weeks before fish are to be marketed or stocked. It is well to remember that drugs are effective only in the treatment of outbreaks. Recurrences of furunculosis are likely to occur as long as *A. salmonicida* is present and environmental conditions are suitable for its growth. Some organotellurium compounds containing azo group were tested for antibacterial activity against some microorgasms isolated from *Cyprinus carpio*, thus, all compounds have high-moderate activity against *Aeromonas salmonicida* (23).The present study suggest more studies about furunculosis in Basra province because its high pollutions raised the possibility of recurrence of this disease ,also experimental examination for a chemical compound and natural plant against *Aeromonas salmonicida* and use polymerase chain reaction (PCR) for diagnosis to decrease loses of disaster.

دراسة حدوث الدمال في بعض المزارع في البصرة

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الخلاصة

خلال الفترة الممتدة من اذار الى نيسان عام ٢٠١٦ معظم مزارع القرنة ،الزبير ومدينة البصرة ظهرت فيها الاف الخسائر من اسماك الكارب الاعتيادي حاملة دمال كبيرة في مواقع مختلفة على الجلد .تم فحص الاسماك سريريا ومايكروبيولوجيا والفحص النسيجي المرضي .الاسماك الحية المصابة ظهرت عليها علامات صعوبة التنفس ،وقوفها قرب حافة الحوض،الافة عبارة عن حلقة غير منتظمة الحافات تبدو كجرح مفتوح مع نزيف واضح ،فقدان للقشور ،جحوظ العين ،بعضها منتفخة البطن.الفحص البايولوجي اثبت ان المسبب هو بكتريا *Aeromonas salmonicida* اما الفحص النسيجي المرضي اظهر تثخن في طبقة الادمة مع زيادة

خلايا الانذار وخلايا الميوسين في الجلد ، وذمة في العضلات، تفجي نبيبات قشرة الكلية،تفجي خلايا الكبد .الخصى ظهرت فيها عملية تكاثر الحيوانات المنوية مع وجود احتقان في الاوعية الدموية. تحطم الصفيحات الثانوية في الغلاصم ،توسع الغدد المخاطية في الامعاء.التهاب العين مع وجود تثخن في طبقاتها.

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