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STUDY OF THE CAUSATIVE AGENTS OF ULCERATED SKIN LESIONS OF CARP FISH PONDS AT SULAIMANI PROVINCE

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Key words: skin ulceration , bacterial infection ,protozoan parasite .

ABSTRACT

This study was conducted to detect the causative agents of skin ulceration in ponds carp fish in Sulaimani province. Samples were taken from 5ponds of common carp fish (*Cyprinus Carpio L.*) from different regions in Sulaimani province have ulcerated skin lesion .These pounds are available in Kfry, Kanarwe, Chwarta and 2 ponds in Dukan.

The results of the study revealed that the main causative agent of the skin ulceration was a bacterial infection and the main bacterial species were 9 species that were isolated from skin lesion, which were the following bacteria *(Pseudomonas spp*, *Citrobacter freundii, Citrobacter amalonaticus, Enterobacter aerogenes, Yarsina krestensia, Serratia odorifera, Escherichia coli, Proteus volgaris* and *Edwardsiella tarda*).

The high isolated bacterial prevalence in this study were *Pseudomonas sps* and *Citrobacter freundii (*19.35%) which have been isolated from 2 ponds. The rate of isolated *Edwardsiella tarda* was (12.89 %),while the rate of *Proteus volgaris Proteus volgaris, E.coli and Yarsina Kerstenia* were isolated separately with the same ratio 9.66 % .*Citrobacter amalonaticus, Enterobacter aerogenes* and *Serratia odorifera* were isolated with the same ratio 6.44% from the skin lesion.

There was only one complicated case (bacterial infection with parasite) the parasite was protozoan *(Chilodonella cyprinii*).

INTRODUCTION

Production of fish, gradually developed in the world as well as in Iraq, the history of fish farming in Iraq can be traced back to the mid of last century (1), while in Kurdistan region, the culture industry developed only during the last twenty years or so (2), as recently as 2004, two million fingerlings of common carp (*Cyprinus carpio L.*) were distributed by FAO to the Dukan hatchery to enhance production, and now spread to many farms in the region (3).

The main types of fish available in the fish farms of Iraq are common carp (*Cyprinus carpio L.*), silver carp (*Hypophthalmichthys molitrix*) and grass carp (*Ctenopharyngodon idella*), with a special fish farm which includes local fish species such as *Barbus sharpeyi, Barbus xanthopterus,* and *Barbus grypus* (4).

Fish diseases are one of the major problems in a fish farm (5), fish farms are affected by many pathogenic microorganisms including bacteria, virus, parasite and protozoa (6) and these farms could also be affected by noninfectious diseases (4).
Skin disorders in fish are especially harmful and any surface injury to the skin makes fluid balance more difficult and may lead to circulatory malfunction, so the skin layers are extremely important protective barriers for fish, and the mucus allows fish to slip through the water more easily, so less energy is used while swimming, also there are several protective compounds in the mucus that protect the fish from bacteria and other organisms in the water (7).

Skin ulceration in fish can has many different etiologies, including infectious agents, toxins, physical causes, immunologic causes, nutritional and metabolic disturbances (8).
Fish exactly swim in a sea of pathogens. Thus, any gap in the normal barrier function of the skin can allow colonization of the skin by infectious organisms, or invasion by microorganisms that normally colonize the skin, differential diagnoses for skin lesions in fish should include fungi, virus, bacteria, and parasitic organism (8) .
So the aim of this study was isolation and diagnosis of the infectious agents of ulcerative skin disease of the carp fish at Sulaimani pounds**.**

MATERIAL AND METHODS

1. Collection of Samples and Preparation

A collection of different cases of pond carp fish that have ulcerated skin lesions from different regions of Sulaimani province has been recorded. Total fish were collected from ponds during the period from beginning on October 1, 2014, till ending on May 30, 2015. Fish specimens were collected by nets that were used to capture these fish. Then each fish was rinsed with de-ionized water and the surface of the fish was decontaminated by dipping it in 70% ethyl alcohol.

2. Using some of the different isolated media, biochemical tests, laboratory chemical and stain reagents shown in (table.1)

Table (1) The table shows some of the different isolated media, biochemical tests, laboratory chemical and stain reagents

No.	Items	Note
1	Nutrient Broth	LAB M. LAB014
2	Nutrient Agar	LAB M. LAB008
3	MacConkey Agar	LAB M. LAB045
4	Blood Agar Base	LAB M. LAB015
5	Kliglar Iron Agar	LAB M. LAB059
6	Urea test	LAB M. LAB037
7	Simmons Citrate Agar	Accumix™ . BCM

3- Use of the Rapid Biochemical kits test (Remel one system) for bacterial diagnosis, which contains 20 rapid biochemical tests as shown in table (2).

Table (2). Remel one system contains 20 rapid biochemical tests

Remel rapid ™ one report form

Refrence #, NO.

Date.

reagent	NON				Rapid one reagent	non	Rapid spot Indol	
Positive	Red Or Violet	Bright purple or blue	Yellow	red	Violet, Purple, Red, dark pink	Yellow Or very light orange	Brown, black, purple	

Cavity# ,no	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	18	
Test code	URE	ADH	ODC	LDC	TET	LIP	KFS	SBL	GUR	ONPG	BGLU	BXYL	NAG	MAL	PRO	GGT	PYR	ADON	IND	OXL
Value	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2
result																				
Value total																				

Tech. Source

Identification

Microcode /

REMEL Inc 800-255-6730 printed in USA 04/1

Methods of this study

1. Gross Examination

The fish have been examined clinically according to the methods described by (7). It has been done immediately at the fish pond, and the skin lesions were carefully described.

2. Parasitic Investigation

Each fish had been observed by the naked eye for presence parasite (crustaceans), and then a thin mucus membrane scraping had been taken from the lesions and preserved in one ml of 70% methyl alcohol, till the laboratory examination for diagnoses of skin protozoa, according to the methods described by (9).

3. Bacteriological Examination

Swabs and a small piece of the ulcerated lesion, liver and kidney of infected fish have been taken. The samples were preserved immediately and disinfected in nutrient broth , after that have been preserved in a cool box till brought to the laboratory for bacterial isolation, and incubated at 25°C for 24hr, According to the methods described by (10).

Parameter of this studies:

1-Parasitic examination

The fish were freshly examined by the naked eye for presence parasite (crustaceans), and then a thin mucus membrane scraping had been taken from the lesions and preserved in one ml of 70% of methyl alcohol till the laboratory examined under a light compound microscope at 40-100x magnification, for detect the presence of skin protozoa. Parasite identification was achieved by consulting some concerned taxonomical accounts.

2-Bacterial isolation and identification

1- A loop full from each broth tube was streaked onto the following media: (Nutrient agar) for growth and characterized of bacterial colonies, (MacConkey's agar) selective media to the gram negative bacteria and (blood agar) for more purification of bacterial colonies that have different ability to hemolysis. Purified isolates were used as stocks for further morphological and biochemical identifications Morphological characterization of bacteria

Bacterial film was prepared from each suspected purified isolate and stained with Gram's stain (11) then examined under the bright field microscope with the oil immersion lens.

Biochemical characterization traditional methods

The separate colonies were subjected to biochemical identification by the following tests: triple sugar iron agar, urea utilization, and Simmon`s citrate agar, according to the biochemical identification keys of Practical Medical Microbiology (12).

2- Rapid biochemical kits test (Remel one system)

Biochemical profiling test was performed according to manufacturer's instructions. Finally isolates were stabbed into tubes containing semi-solid nutrient

agar medium and then incubated at 37°C for 18- 24 hrs. This test contains 20 biochemical tests as the following table (2).

RESULTS

Table (5)Shows different cases of ulcerated skin lesions with the infectious agents, and description of the lesions from different regions of Sulaimani province

The fish from Kfry pond were infected by bacteria, the external lesion of the infected fish have huge number of hemorrhagic spots on the fish's body, sloughing off the scales and a large detachment of the skin in the peduncle regions and small round ulcer on the body of the fish were present.

Kanarwe pond fish infected by bacteria that caused small hemorrhage of the skin of the mouth, erosion of operculum, cloudy eye, and exophthalmia.

Also, there were two cases of skin ulceration of fish from Dukan region at a different months. Both of them were in an earthen pond and the cause of infection was the bacterial infection ,they were characterized by a large ulcer in the peduncle region which were characterized by whitish lesion . Fish in the second pond of Dukan region infected at spring. The lesions which were present are hemorrhagic spots on the skin of the mouth, erosion of the operculum and pectoral and caudal fins, bilateral exophthalmia, skin ulceration at a different site of the body, especially in the dorsal part of body with whitish edges and loss of scales of infected fish.

Chwarta pond fish had a complicated case, protozoa and bacteria were present together ,the infected fish, the fish were infected with protozoa, *chilodonela cyprinidi*. This parasite caused secondary bacterial infection *(Edwardsiella tarda)* to the fishes. Infected fish were characterized by whitish, bluish in body tattered appearance to the skin, hemorrhage, loss of scales and small round ulceration of the skin of the fish's body, with present injury of the gill.

Table (4) Shows different cases of ulcerated skin lesions with the infectious agents, and description of the lesions from different regions of Sulaimani province

Location	Infectious agents	Description of the lesions
Kfry	Bacteria	patches of hemorrhage on the fish's body, sloughing off the scales. A large detachment on the skin in the peduncle region, and small round ulcer on the body of the fish
Kanarwe	Bacteria	Hemorrhage of the skin of mouth, erosion of operculum, cloudy eye and exophthalmia
Dukan 1	Bacteria	The large red ulcer in the peduncle region, which characterized by whitish edges, also present bleeding in the lesion.
Chwarta	Complicated cases: Parasite & bacteria	Were whitish, bluish in body tattered appearance to skin, hemorrhage, drop off the scales and small round ulceration of the body, with injury of the gill
Dukan 2	Bacteria	Hemorrhage of the skin of mouth, erosion of operculum and pectoral and caudal fins, bilateral exophthalmia. Skin ulceration at a different site of the body, especially in the dorsal part which characterized by whitish edges

Gross Examination

During this study, infected common carp fish in ponds were generally calm (quiet), and floating on the surface of the water, they also had lethargy, loss of appetite, weight loss and they didn't respond to external stimuli. Also, presence of

superficial skin hemorrhage, sloughing off the scales and a large detachment of the skin on the fish's body. The gross lesion of fish that are shows in table (5) were in Kfry's pond, patches of hemorrhage on the different site of fish's body, sloughing off the scales (Figure 1) and a detachment of the large skin piece in the peduncle region. (Figure 2).

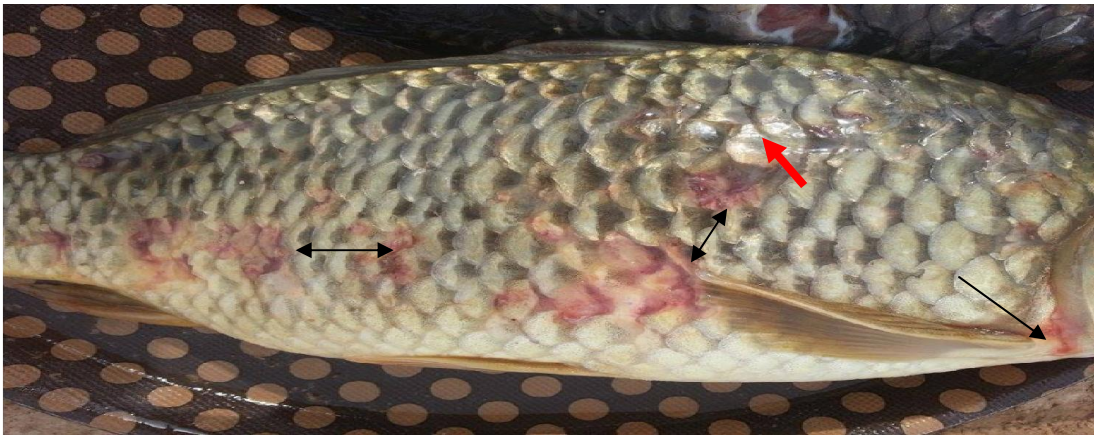

Figure 1 (Case of Kfry): The gross lesions show that there were patches of hemorrhage on the trunk of the fish's body and behind the operculum (black arrow) with sloughing off the scales (red arrow).

Figure 2 (case of Kfry): A large detachment of the skin in the peduncle region (red arrow) and small round ulcer on the body of the fish(black arrow).

The gross lesions which have been seen on the fish in Kanarwe pond were hemorrhage on the skin of the mouth, erosion on the operculum and, skin ulceration, loss of scales, exophthalmia (Figure. 3).

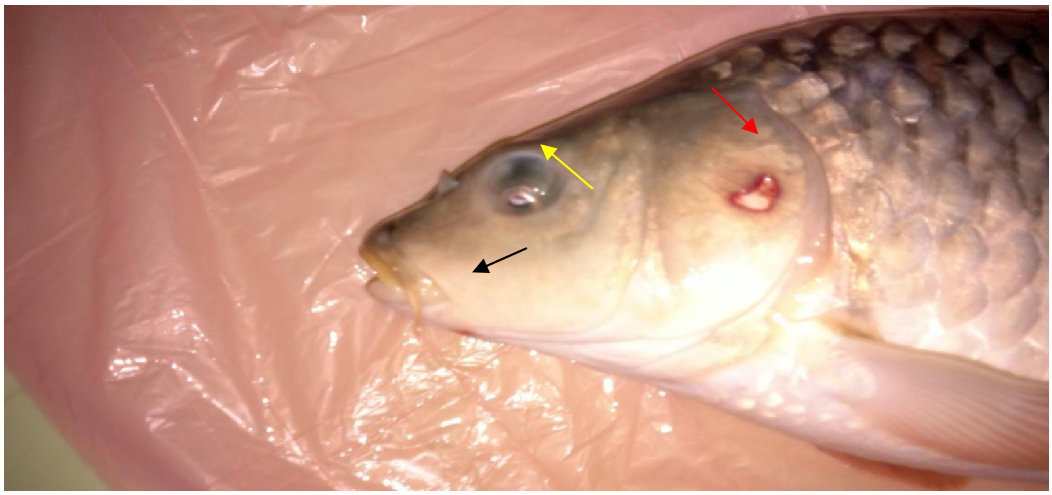

Figure. 3 (case of Kanarwe): Hemorrhage on the skin of the mouth (black arrow), erosion on the operculum (red arrow), and exophthalmia (yellow arrow).

The gross lesion in the first case of Dukan pond 1 where large skin ulceration in the peduncle region, which were characterized by whitish edges, also present of bleeding in the lesion (Figure 4) with swelling of the abdomen, inflamed vent with darkly pigmented.

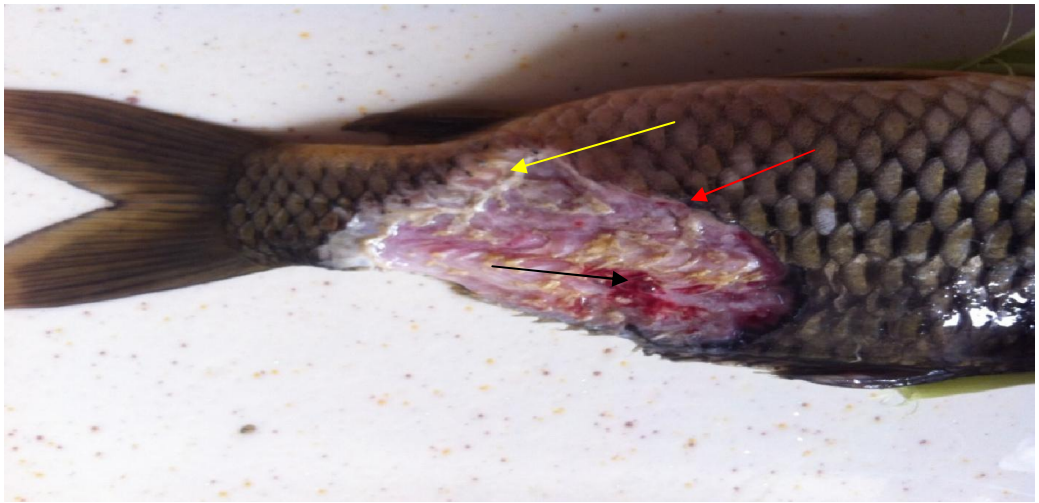

Figure 4 (case of Dukan pond1): A large red ulcer in the peduncle region (red arrow) which were characterized by whitish edges (yellow arrow), also present of bleeding in the lesion (black arrow).

The gross lesion of the second case of (Dukan pond 2) was an erosion of the pectoral and caudal fins that affected the vital activities: hemorrhage on the skin of the

mouth, erosion on the operculum, bilateralexophthalmia with skin ulceration at a different site of the body and loss of scales (Figure. 5).

Figure. 5 (case of Dukan pond 2): Erosion of operculum, pectoral and caudal fins (red arrow), bilateral exophthalmia (brown arrow), skin ulceration at different site of the body (black arrow).

There were typical lesion of protozoan ectoparasite (*Chilodonella ciprinii)* in the infected fish in Chwarta pond which were whitish,

Bluish in body tattered appearance of skin (Figure 6).

Figure. 6 (case of Chwarta): Hemorrhage with loss of the scales (red arrow) and discoloration of the body

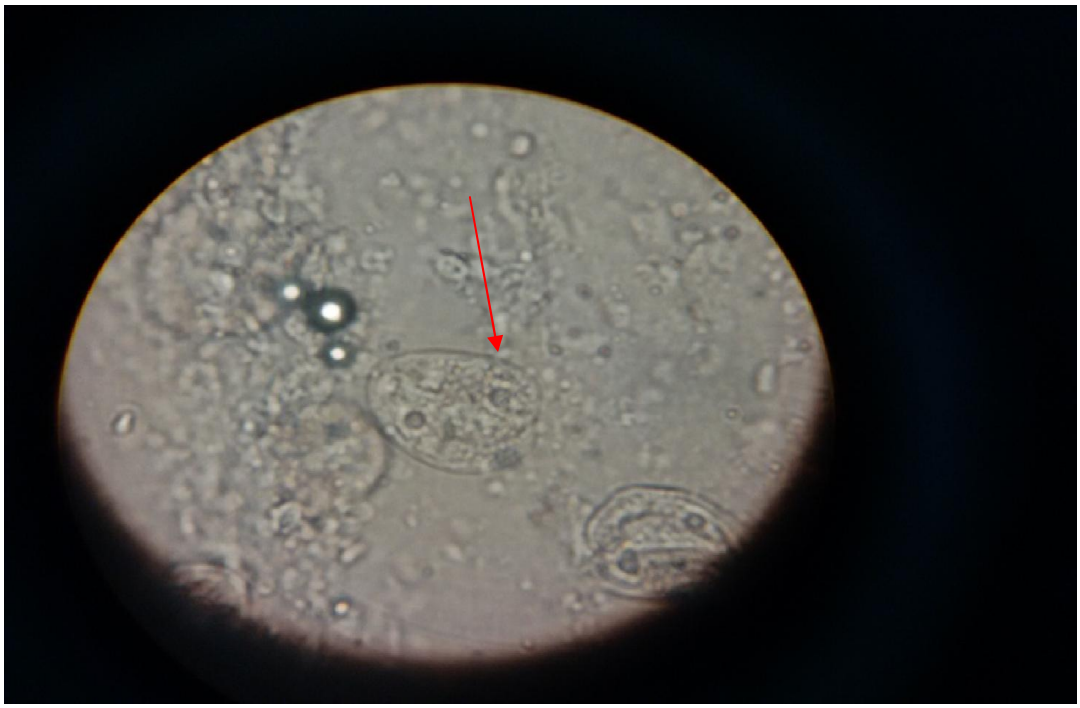

Figure 4.: *Chilodonella cyprini* showed as heart-shaped ciliated parasite (red arrow), under light microscopical examination 40X .

Bacteriological Examination

This study detects that the main causative agents of skin ulceration in fish are bacteria which was identified as (*Pseudomonas spp*, *Citrobacter freundii, Citrobacter amalonaticus, Enterobacter aerogenes, Serratia odorifera, Escherichia coli, Proteus volgaris, Edwardsiella tarda*.

A pure culture of isolated bacteria was successfully done from skin, of naturally infected common carp fish. All bacterial growth were gram negative and motile.

The biochemical patterns of representative of the recovered isolate are summarized in Table(6) the isolated bacteria from skin, of naturally diseased (*Cyprinus carpio L*) were *Pseudomonas spp (5500410), Citrobacter freundii (0031430), Citrobacter amalonaticus (4235131), Enterobacter aerogenes (0337330), Serratia odorifera (413143), Yarsina krestensia (0020501), Escherichia coli (4161002), Proteus volgaris (0602001) and Edwardsiella tarda (0602001).* All of them are under the Family of Enterobacteriaceae-Facultatively Anaerobic Gram-Negative Rods. According to the data shown in table 8, the origin of gram negative bacterial isolates tested were: *Pseudomonas spp* was isolated from 2 ponds (Kfry and Dukan1) in different site of

the fish samples: skin lesion, from Kfry's pond, also *Pseudomonas spp* was isolated from Dukan pond 1 from 3 sites of infected fish: skin lesion,. The species of *Proteus vagaries* were isolated from 2 ponds Kfry and Kanarwe, *Proteus volgaris* was isolated only from skin lesion in Kfry pond and from skin lesion and in Kanarwe's pond.

Citrobacter freundii had isolated from skin lesion, , from samples of both ponds Kfry and Dukan pond 2 Whereas the species *Edwardsiella tarda* was isolated from two pond Chwarta and Dukan pond 2, in Dukan pond 2 from fish skin, also in Chwarta pond .*Yarsina krestensia* have been isolated from skin, samples from Kanarwe pond. Also species *Serratia odorifera* were isolated from skin.

Table (5) shows bacterial isolates from skin of fish from different locations

location	organs	Bacteria								Total
Kfry	Skin	*Pseudomonas spp*	*Citrobacter freundii*			*Proteus volgaris*				3
Dukan 1	Skin	*Pseudomonas spp*		*Serrasia odenifera*	*E.coli*					3
Kanarwe	Skin				*E.coli*	*Proteus volgaris*	*Yarsina krestensia*			3
Chwarta	Skin								*Edward. tarda*	1
Dukan 2	Skin		*Citrobacter freundii*				*Enterobactor aerogenosa*	*Citrobacter amalonaticus*	*Edward. tarda*	4
	15	2	2	1	2	2	2	1	2	

Table(7) shows the number and percentage of the bacteria isolates from the skin lesions of fish at different region of Sulaimani province. Totally 9 species of bacteria were isolated from skin ulceration lesion. The *pseudomonas sps* 19.35% and *Citrobacter freundii* 19.35% has been a high bacterial prevalence of this study, each of them was isolated from 2 ponds, which had skin ulceration, also the ratio of the isolation of *pseudomonas sps and Citrobacter freundii* were present in the same percentage from of the infected fish. After that frequently rate of isolated *Edwardsiella tarda* was 12.89 % . *Proteus volgaris, E.coli and Yarsina Kerstenia* were isolated separately with the same ratio 9.66 %

Citrobacter amalonaticus, Enterobacter aerogenes and *Serratia odorifera* were isolated with the same ratio 6.44% from only one pond.

Table (7) Shows number and percentage of bacterial isolates from different region of Sulaimani province

Name of isolated bacteria	Number & Percentages of isolated bacteria.
Pseudomonas spp	(6) 19.35%
Citrobacter freundii	(6) 19.35%
Edwardsiella tarda	(4) 12.89%
Proteus volgaris	(3) 9.67%
E.coli	(3) 9.67%
Yarsina krestensia	(3)

	9.67%
Serrasia odenifera	(2) 6.44%
Citrobacter amalonaticus	(2) 6.44%
Enterobacter aerogenes	(2) 6.44%
	(31) 99.9%

DISCUSSION

Fish are in constant interaction with a wide range of pathogenic and non pathogenic microorganisms, in the aquatic environment (13), so fish suffer from various types of diseases and pathogens play an important role in affecting fish health, if the pathogenic load increases, it leads to disease (14). Fish are susceptible to a wide variety of bacterial pathogens causing large mortality in the aquaculture (15).

Enterobacteriaceae is a common water-borne bacterium that may be present in the tissues of apparently normal fish (16) whenever fish are revealed to environmental stress, or injury, it causes serious outbreaks of disease with mortalities (17). However, a major biological interface between a fish and its aqueous environment is the epidermis because its overlying mucous layer forms over the fish and playing a great role in protecting against injury, friction reduction and ion regulation (18, 19). The epidermis has a number of chemical defenses and mucous, such as complement, natural antibiotics, immunoglobulin, lysozyme and agglutinins, may support in the protection (20), and the continuous shedding of mucous may avoid microbial colonization.

However, exposure to environmental stressors may affect the epidermis and intervene with its protective role (21). Abnormal structure of both the epidermis and dermis led to an impairment of its functional activity as well as partially decrease the

respiration activity of the integument that is the main source of transported oxygen to the internal organs (15).

(22) state that a common fish disease is an epizootic ulcerative symptom characterized by the presence of severe, open dermal ulcers on the head, on the mid of the body, and on the dorsal regions of the fish. Ulcerative syndrome disease is still unknown; however, organisms belonging to the potentially fish-pathogenic genera *Pseudomonas, Vibrio, Aeromonas* and *Plesiomonas* were often isolated from the lesions (23).

In this study, at the level of macroscopic and microscopic examinations, only one parasite was observed, which was one species of protozoa (*Chilodonella ciprini*) as shown in (Fig 15 and 16), and has been isolated from the skin of diseased common carp fish from Chwarta pond and diseased fish had skin ulceration in different location of body surface, especially at the dorsal part as shown in (Fig 6), gill lesion, swelling of the abdomen, abnormal swimming behavior, including spiral movement and floating near the water surface, this agreed with (7) who detected that advanced *chilodonella* infestation are sometimes associated with skin ulcers, which may have tattered appearance.

If the ciliated parasites such as *Chilodonella ciprinii* are present in large numbers, their feeding activity on the surface of the fish can compromise the integrity of the tissues, this often results in osmotic stress and secondary bacterial infection (24). This is identical agrees with our result, because infestations of *Chilodonella ciprinii* caused secondary bacterial infection (*Edwardsiella tarda*), the same time during this study.

The result of this study indicates isolation of the many species of gram-negative bacteria from the infected fish are most frequently species of bacteria that are evreywhere in the aquatic environment acting as opportunistic pathogens and most of these bacterial infections of fish are caused by gram negative organisms and include the genera *Citrobacter, Pseudomonas, Edwardsiella, Aeromonas, Flavobacterium, and Vibrio*, which caused skin lesion in different species of fish (25). However, some species of gram negative and gram positive bacteria present as the bacterial flora of the digestive tract of healthy young tilapia and carp bred in a polyculture (26).

In this study, several members of family *Enterobacteriaceae* infected fish and clinically caused above external lesions. *Pseudomonas sps* was recovered in this

study; which is encountered in cases of spottiness of the skin and hemorrhagic septicemia (27, 28 (29). This bacterium have clinically shown hemorrhages, skin lesions, loosening of scales, and erosions at the bases of the fins and mucous secretion. Similar symptoms were also reported by (30), also, the other characteristic symptom of the diseased fish by Pseudomonas bacteria that have seen on diseased fish during this study were remarkable septicaemic hemorrhage on the skin of the mouth and ventral side of the body with sloughing off the scale and large skin ulceration at peduncle region, these results were similar to the ones by (28) who detect the clinical and post-mortem examination of both naturally and experimentally infected fishes by pseudomonas spp that showed hemorrhages all over the fish body, especially at the tail and fins rot, detachment of scales and skin ulceration.

دراسة أسباب افات التقرح الجلدي في أحواض اسماك الكارب في محافظة السليمانية

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الخلاصة

اجريت هذه الدراسة للكشف عن المسبب للتقرح الجلي في اسماك الكارب في أحواض تربية الأسماك في محافظة السليمانية .

أخذت العينات من 5 أحواض تربية اسماك الكارب الشائع (*Cyprinus carpio L*) من مناطق مختلفة في محافظة السليمانية مصابة بآفات تقرحية على الجلد وكانت هذه الأحواض موجودة في كفري وكناروه وجوارته وحوضين في دوكان.

أظهرت نتائج الدراسة أن المسبب الرئيسي للتقرح الجلدي هو الإصابة البكتيرية وعزلت 9 أنواع من البكتريا وهي *Pseudomonas spp*, *Citrobacter freundii, Citrobacter amalonaticus, Enterobacter aerogenes, Yarsina krestensia, Serratia odorifera, Escherichia coli, Proteus volgaris* and *Edwardsiella tarda*). وكذلك وكانت أعلى نسبة انتشار هي جراثيم *Pseudomonas spp* وبنفس النسبة 19.35% وكانت نسبة الإصابة بجراثيم *Citrobacter freundii* *Edwardsiella tarda* حيث كانت 12.89 % أما نسبة الإصابة بالأنواع *Proteus volgaris Proteus volgaris, E.coli*، *Yarsina Kerstenia* حيث كانت 9.66 % وكانت نسبة الإصابة بالأنواع *Citrobacter amalonaticus, Serratia odorifera*, *Enterobacter aerogenes* 6.44 % .

كانت هناك حالة واحدة الإصابة فيها مشتركة مع إصابة بطفيلي يعود إلى الاوالي وهو *(Chilodonella cyprinii*) .

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