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Isolation and Identification the Causative Agent of Bacterial Hepatitis in the Common Carp Cyprinus carpio (Linnaeus, 1758)

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Abstract
The present study was conducted to isolate and diagnose the bacterial infection of liver of common carp fish, with investigated the histopathological changes in effected organs. 102 common carp fish were collected from fish farms located in the area of the Abbasia, Najaf, Iraq, During six month of experimental period extended from 1st January to 1st July of 2016. The experimental period of current study was divided into two periods (three month each), first period was started from 1st January to 1st April and the second period was started from 1st April to 1st July . liver samples were collected for bacterial isolation and identification, with histopathological examination. Pathogenic agents were isolated and identified by using culture media (Nutrient agar, Salmonella - Shigella agar, MacConkey agar , pseudomonas agar and EMB agar), biochemical tests and Api20 test for confirmation.Result of this study showed differences between the bacterial isolates in first period from bacterial isolates in second period. The results of bacterial isolates in the first period were (*E.coli* 23.5%, *Enterobacter* 21.6% and *Aeromonashydrophila* 7.8% for the months January, February and March) , while results of the bacterial isolates in the second period were (*E. coli* 35.3%, *klebseliaoxytoca* 15.7%, *Salmonella Spp* 3.9%, *Pseudomonas aeruginosa* 7.8% , *K.pneumonia* 9.8%). Histopathological results show Vaculation, necrosis and nuclear condensation were observed in hepatocytes of effected livers. In conclusion, According to the results obtained in this study, we investigated that , the time period from April up to July pathogenic agent ratio was more than pathogens agent ratio that was isolated in the months of January, February and March.
Keywords: common carp, Bacterial disease ,*Aeromonashydrophila*, liver

1. INTRODUCTION

The fish have a key role in the human diet, as well as play an important role as samples mainly in medical research (1) there are problems and the many challenges facing the Fish. Shares Fish and interact with microorganisms pathogenic and started to cause disease (2). Catch fish in many diseases. Every fish catch pathogens and parasites as normal flora, and if the pathogen load increased that lead to the disease (3). Microbes play an important role in influeunce fish health.. The Microbes are movment and create a universal threat to the aquaculture and fish industry. The infected fish ultimately arrival to human food and poses a serious threat to public health. These infectious diseases may be caused by various pathogenic organisms that include bacteria, fungi, viruses and protozoa. These pathogenic microorganisms are present in the environment or transmitted by other fish (4) .

Bacteria comprise of the major group of the fish pathogens posing important threats to aquaculture and fish industry worldwide. Bacterial pathogens are smallest pathogenic agents causing devastating fish diseases where the pathogenicity is a characteristic feature associated with specific fishes as hosts (5) . It was recorded the spread of bacterial etiology in many types of farmed fish and wild(6). The spread of disease among fish in low productivity and increased mortality, and this in turn leads to fish farmers in economic losses, the cause of death was bacterial infections (3).The mortality was based on the concentration of bacteria and the occurrence of clinical signs in fish that the death occurred as a result of the large virulence factors, Where sucking mice are used to study the pathogenicity (2). In order to prevent and control of major diseases that limit the production of fish has been the work of the verification of the characteristics of microbial organisms (7).

The present study aimed to examine:

1. isolate and diagnose bacterial isolates encountered in fish ponds in the Abbasia AL- in the city of Kufa.

2. Sign Clinical signs and pathological changes as a result of bacterial infections in fish naturally.

2.Materials and methods

2.1.Sampling collection

In the current study , 102 fish sample were collected from fish ponds in AL-abbasia , Najaf city. These sample was divided into two parts , the first part(51 carp fish samples for each group) were take it in the period of (January, February and March) of 2016 while the second part were take it in period of (April , May and June) of 2016.

Use small sterile plastic bags for collection the samples , to avoid the contamination, these samples were placed in sealed containers containing ice cubes and transport to lab. of vet. Medical collage / Kufa university.

2.2. Isolation and identification of bacterial

The infected fish samples were separated to obtained the infected liver samples , the pathogenic strains were isolated by using a sterile swab and spread over the nutrient agar plates. These plates were incubated at 37°C for 24-48 hrs .

The morphologically different microbial strains were identified in bacterial plates. The colonies were isolated and purified by restreak method. The isolated colonies were streaked on (macConky agar ,Eosin Methylene Blue , Salmonella –Shigella agar , blood agar and Pseudomonas agar), incubated overnight at 37°C for identification of selected colonies that was isolated from the fish samples , then, the biochemical tests was carried out with APi20 bioMerieux to confirm the diagnosis.

2.3. Microscopy examination

The liver section was prepared for histopathological examination , fixed with Bouin 's liqued for 6 hours. After the complete the fixation in alcohol (70%) with routine dehydrated, the samples were processed in the recognized method of dehydration, cleared in xylene and finally embedded in paraffin wax before sectioned at 5 µm using a rotary microtome. The samples stained with hematoxylin and eosin..The prepared sections examined and photographically enlarged using light microscopy.

2.4. Statistical analysis

The bacterial infection of fishes were analyzed statistically by Chi-Square test using SPSS software version 23.

3.Results and discussion

Fish can live in an environment where the challenges facing many problems in the aquatic environment and the fish are in constant interaction with a group of microorganisms (2).Microbes play an important role in affecting fish health.

In the current study, bacterial isolates that was recorded in the months (January, February and March) *are E.coli* (23.5%),*Enterobacter* (21.6%) and *Aeromonashydrophila* (7.8%) table 1. While the results for bacterial isolates in the months (April , May and June) were *E.coli* (35.3%),*K.oxytoca* (15.7%), *Salmonella spp* (3.9%),*P.aeroginosa* (7.8%), *K.pneumonia* (9.8%) table 2.

Fishes in farms are susceptible to varies bacterial infections fundamentally When they are breeding in crowded conditions. Disease explosion among fishes elevate the mortality rate and decrease the productivity leading to high economic loss to fish plantation (3). *Aeromonashydrophila* is the major bacterial fish pathogens which is widely apportion in aquatic organisms in nature (8). Also these results agree with the finding of (9,10) who found that the bacterial load is higher in summer season in cultured fish. In the present study; *Aeromonas spp*. were isolated from the fish liver, similar finding was given by authors: (11,12,13).

These results agreement with (14) who observed ascites, internally the intestine was distended with clear, viscous fluid, hemorrhaging is common in the viscera and around the intestines, with enlargement of kidney , liver, and spleen due to isolation of *P.aeroginosa*. Also these results agreement with (15).In the previous study noted that considerable numbers of *Pseudomonas spp*. were found (16).Bacterial disease of fish caused by bacteria organisms causing infection or internal disorder. It is a complex interaction between a susceptible host, a pathogen and the environment. Bacteria diseases manifest in various method for the weakness of the normal physiology in the host (17).

The results of this present study was show that the percentage of infection in the period of (January, February and March) was 52.9% while in the months of (April , May and June) was 72.5% , The change in infection rates between the first and second group is due to the rise in temperature during these months, It`s regarded a stress factor on fish ,these results agreement with (18) who showed the total prevalence was 86.25% in the four months, also agreement with (19). The high temperatures as well as the transition season led to a further spread of the disease (20). The stress induced due to human activities, water diversion, changes in method of water and land empleyment had contributed to various impact on fish population(21).

The histopathological appearance of liver showed important alterations, The severity of histopathological changes increased with temperature .

Hepatic lesions in the liver tissues of fishes were characterized by cloudy swelling of hepatocytes, blood congestion in the central veins , vacuolar degeneration, karyolysis, karyohexis, dilation of sinusoids with cellular hypertrophy. The (22) described increased vacuolization of the hepatocytes a signal of degenerative process these t suggests metabolic damage, possibly related to exposure to contaminated water. Vacuole formation was considered by (23) as a cellular defense mechanism against substances injurious to hepatocytes and this mechanism responsible for collecting the injurious elements and preventing them from interfering with the biological activities of these cells.

Histopathological analyses of infected fish that exposure to *E.coli ,Salmonella spp* and *K.pneumonia* showed more congestion and dilation of sinusoid seen in (Fig A.).

The most important changes found in the liver of *E.coli*,*K.pneumonia* and *Pseudomonas spp ,* in which the histological alterations of liver were more evident and some other changes such as hepatocyte degeneration and vacuolar degeneration were detected (Figs C)

The majority of the these changes were presented in the liver (Fig D), in addition, vascular changes including hyperaemia and haemorrhages were marked ,hepatocytes exhibited enlarged cytoplasm with a granular appearance and cell borders were not differentiated in many of them. Necrosis with severe edema , which are more commonly observed in (Fig E). In samples with less severe necrosis, hepatocyte cytoplasms had dens eosinophilic appearance and their nuclei were picnotic whilst in samples with severe necrosis, disappearing hepatocytes were replaced by irregular, dark eosinophilic, and homogenous necrotic material (Fig A). During the summer this changes and the damage is severe due to warmer temperatures.

hepatic lesions duo to the liver was damaged by bacteria and its toxin ,the variation in lesions could be due to the vary in bacterial virulence and degree of individual resistance of fish. Histopathological studies are necessary for the description and evaluation of potential lesions in aquatic animals exposed to various infections and toxicants in aquaculture (24). the liver also showed changes and damage to the hepatic cells due to bacterial infection. Fish liver can be regarded as the body's detoxification organ and hence a target organ of various xenobiotic substances.

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Notes
No conflict of interest was declared by the authors.

Table 1. Bacterial isolates in the months (January, February and March).

Bacterial * samples Cross tabulation					
			samples		Total
			positive	negative	
Bacterial	E.coli	Count	12	39	51
		% within Bacterial	23.5%	76.5%	100.0%
	Enterobacter	Count	11	40	51
		% within Bacterial	21.6%	78.4%	100.0%
	Aeromonas hydrophila	Count	4	47	51
		% within Bacterial	7.8%	92.2%	100.0%
Total		Count	27		51
		% within Bacterial	52.9%		100.0%

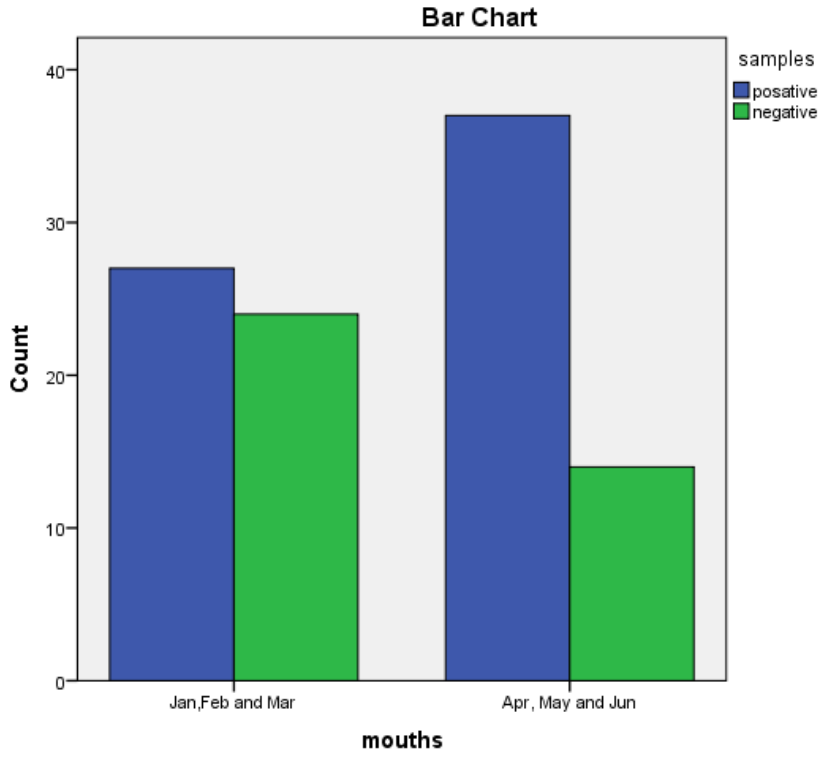
Table 2. Bacterial isolates in the months (April , May and June).

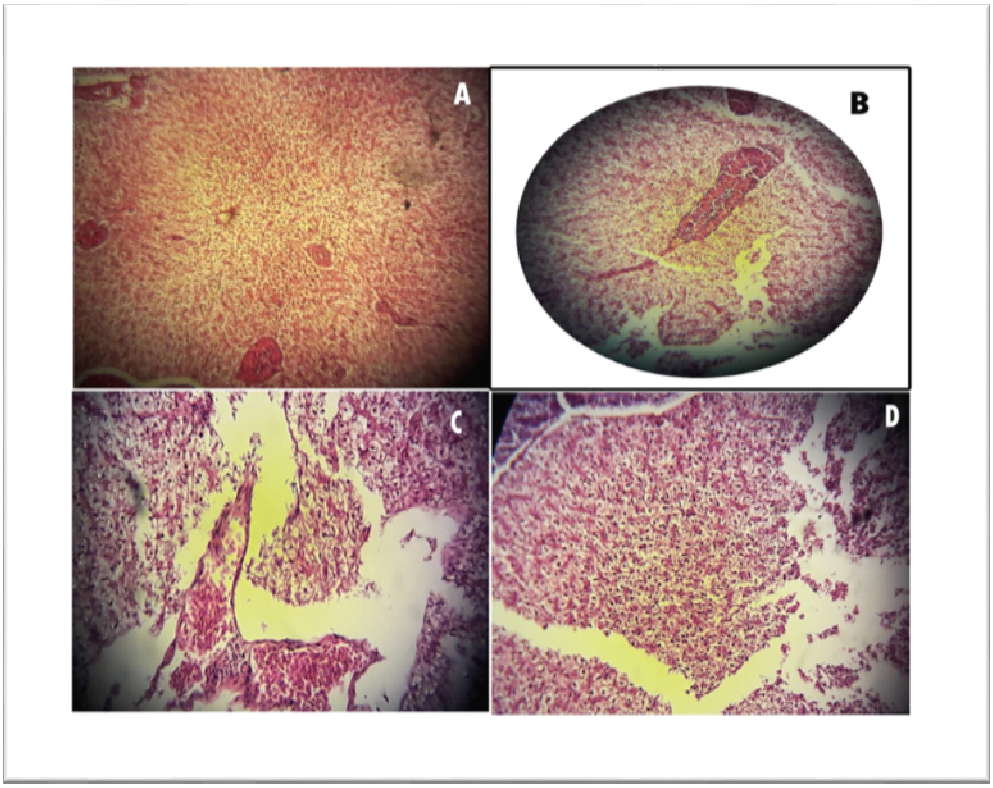
Bacterial * samples Cross tabulation					
			samples		Total
			positive	negative	
Bacterial	E.coli	Count	18	33	51
		% within Bacterial	35.3%	64.7%	100.0%
	K.oxytoca	Count	8	43	51
		% within Bacterial	15.7%	84.3%	100.0%
	Salmonella spp	Count	2	49	51
		% within Bacterial	3.9%	96.1%	100.0%
	P.aeroginosa	Count	4	47	51
		% within Bacterial	7.8%	92.2%	100.0%
	K.pneumonia	Count	5	46	51
		% within Bacterial	9.8%	90.2%	100.0%
Total		Count	37		51
		% within Bacterial	72.5%		100.0%

Table 3: the percentage of bacterial isolates according to the months.

mouths * samples Cross tabulation					
			samples		Total
			positive	negative	
mouths	Jan,Feb and Mar	Count	27	24	51
		% within mouths	52.9%	47.1%	100.0%
	Apr, May and Jun	Count	37	14	51
		% within mouths	72.5% *	27.5%	100.0%
Total		Count	64	38	102
		% within mouths	62.7%	37.3%	100.0%

* significant ($p \geq 0.05$) due to calculated Chi-Square(4.194) more than the table Chi-Square (3.84)





Histopathological section of A:liver; (a) necrosis in hepatocyte (H&E stain100X). B: dilation of the central venous sinus with congestion . (H&E stain100X) .C: degenerative changes (H&E stain 100X) D: liver; (D) hepatocytes show vacuolar degeneration .(H&E stain 100X) E: severe edema