

Some Hematological and Biochemical Parameter Accompanying with *Aeromonas Hydrophila* Infection Diagnosed Genetically in *Cyprinus Carpio*

Qusai Saleh Jumma¹, Thamer Jaddoa Shihab², Sabah Mahmood Hamad al-shammari³

¹Department of Pathology and poultry disease / College of Veterinary Medicine / University of Tikrit, Tikrit, Iraq

²Department of Pathology and poultry disease / College of Veterinary Medicine / University of Tikrit, Tikrit, Iraq

³Department of Biotechnology / College of Sciences / University of Diyala, Diyala, Iraq

qusaisaleh@tu.edu.iq, <https://orcid.org/0000-0002-6366-0415>

dr.thamer.vet@tu.edu.iq, <https://orcid.org/0000-0002-7285-0157>

dr.sabahmahmoud@sciences.uodiyala.edu.iq, <https://orcid.org/0000-0002-2248-1592>

Abstract

The current study aimed to find the effect of infection with *Aeromonas hydrophila* in some haematological and biochemical parameter in *Cyprinus Carpio*. For this, 70 samples of fish (50 fish suspected with *Aeromonas hydrophila* and 20 healthy fish) were in Babylon provenance from June-July 2021. The results of the current study showed that *Aeromonas* spp. detected in a rate of 88.5% according to results of culture and 80.6% of them belong to *Aeromonas hydrophila* according to PCR test. In addition, there is a significant decrease in the level of (red blood cells, packed cell volume and haemoglobin concentration) in the infected group compared with the healthy group, Whereas there is a significant increase in the level of white blood cells in the infected group in comparison with the healthy group, regarding biochemical tests we noted a significant decrease in the level of (total protein, albumin and globulin) in the infected group compared with a control group. We conclude from this study that infection with *Aeromonas hydrophila* can induce alteration in the level of blood and some biochemical parameters and causes economic losses in this field of production.

Keywords: hematological parameter, biochemical parameter *Aeromonas hydrophila* infection, *Cyprinus Carpio*

Introduction

In recent years, fish farming has been increasing in Iraq, This increase will certainly lead to an increase in health problems that occur by bacteria, and many zoonotic bacteria have the ability to cause diseases in fish and humans. (1,2). *Aeromonas hydrophila* is gram-negative bacilli, present in the gastrointestinal tract as normal flora, it is conceded as opportunistic pathogen that may be caused Hemorrhagic Septicemia, Ulcer Disease and Red-Sore Disease, which is characterized by loss of appetite, pale gills and skin ulcerations. Alternatively, maybe affect other organs such as the gills, kidneys, liver, spleen, pancreas, and skeletal muscle. (3, 4)

Materials and methods:

- A- Fish sample : the current study conducted in aquarium in Babylon provenance 50 fish suspected with *Aeromonas hydrophila* and 20 healthy fish were targeted in the current study
- B- Detection of *Aeromonas hydrophila*: sample from eyes, ulcerative skin areas, kidney, liver and spleen were collected and homogenized then divided in to part , one cultivation on Aeromonas Agar (for bacterial isolation) and other cultivation on for DNA extraction, positive results from any specimen consider as positive case
- 1- Bacterial isolation : bacterial isolation were conducted by use of Aeromonas Agar (RYAN) with ampicillin selective supplement (Thermo fisher- UK) prepared according to company manifestation , agar plate Incubate plates aerobically at 37°C for 18-24 hr., any dark green colony suspected with *Aeromonas spp.*
 - 2- DNA extraction: blood, liver, spleen, skin and muscle were and cultivated on brain- hart infusion broth (HIMEDIA-India) at 28c for 24hours, DNA extraction by use of DNA extraction kit – BIORON-Germany.
 - 3- Primer used for detection of *Aeromonas hydrophila*:
 F: 5'-AACCTGGTTCGCTCAAGCCGTTG-3'
 R: 5'-TTGCCTCGCCTCGGCCAGCAGCT-3'
 - 4- Reaction mixture and thermocycler program : according to that give 760 bp
- Complete blood picture: samples of blood collected from caudal veins then divided in tow tube first one with EDTA tube for blood picture and other without anticoagulants for biochemical parameters (5).

Counting blood parameters

We used in our study Vet. Hematology coulter to count complete blood picture.

Counting biochemical parameters

- **Determination of Total protein concentration in blood serum :**
We estimate Total protein by using specialized Kit supplied from Randox Company.
- **Determination of Albumin n in blood serum:**
We estimate Albumin by using specialized Kit supplied from Syrbio company.
- **Determination of globulin concentration in blood serum:**
We estimate globulin by using modulated method described by (6).

Results:**Number and rate of *Aeromonas spp* isolation**

Results of *Aeromonas spp* isolation: according to the result of bacterial culture, *Aeromonas spp* was isolated at rate of 88.5% as in Table 1. Table 2 showed that *Aeromonas spp* was isolated from skin and eye and from internal organs at rates of 70.9% and 74.1% respectively.

Table (1): No. and rate of *Aeromonas spp* isolation

Fish group	Case no	No. of <i>Aeromonas spp</i>	Rate of <i>Aeromonas spp</i>
Suspected infected fish	50	50	100%
Healthy fish	20	12	24%
Total	70	62	88.5%

Table (2): No. and rate of *Aeromonas spp* isolation according to isolation site

Organs	Rate and number <i>Aeromonas spp</i> isolation		
	Suspected infected fish	Healthy fish	Total
Skin and eye	(38:50)76%	(11:12) 91.6%	(49:62) 79.0%
Internal organ (liver, spleen and kidney)	(42:50)84.0%	(4:12) 25.0%	(46:62) 74.1%

Number and rate of *Aeromonas hydrophila* detection

Result of PCR test showed that of *Aeromonas hydrophila* detected in rate of 80.6% from total of *Aeromonas spp.* as in Table (3) and Figure (1).

Table (3): Number and rate of *Aeromonas hydrophila* detection.

Fish group	No. of <i>Aeromonas spp</i>	No. of <i>Aeromonas hydrophila</i> isolate	Rate of <i>Aeromonas hydrophila</i> isolation
Suspected infected fish	50	43	86.0%
Healthy fish	12	7	58.35%
Total	62	50	80.6%

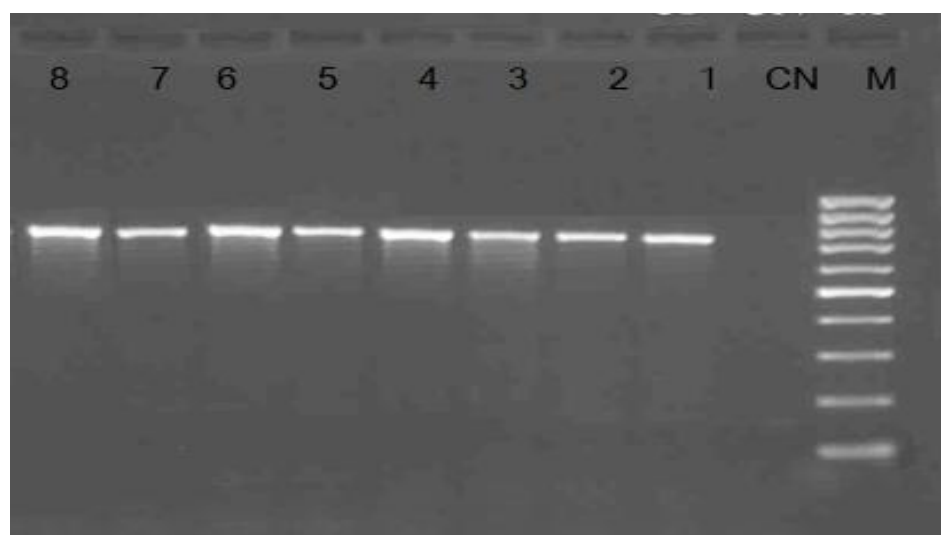


Figure (1): showed positive results of PCR test, M: 100bp DNA markers, CN: control negative, lens (1-8) positive samples for *Aeromonas hydrophila* which band in size 760bp.

Blood parameters

Table (4) showed significant differences between blood parameters in infected fish in comparison with healthy fish. In addition, we noted that there is a significant decrease in the level of (red blood cells, packed cell volume and haemoglobin concentration) in the infected group compared with the healthy group, while there is a significant increase in the level of white blood cells in the infected group in comparison with the healthy group.

Table (4): Blood parameters in the infected fish and healthy fish

Blood Parameter	Infected fish	Healthy fish
RBCs count (10^3 per mm^3)	0.9±0.05 b	2.7±0.09 a
WBCs count (10^9 cells/L)	11.5±1.92 a	7.7 ± 2.31 b
PCV%	19.5±1.92 b	38.7 ± 2.31 a
Hb g/dL	6.2±1.92 b	11.3±1.72 a

- Values represent mean ±SE.
- The difference between the values marked with various letters in the same line is significant ($P \leq 0.05$).

Biochemical parameters

We noted that there is a significant decrease in the level of (total protein, albumen and globulin) in the infected group compare with control group.

Table (5): biochemical parameters in the infected fish and healthy fish

Biochemical Parameter	Infected fish	Healthy fish
Total protein (g/ dl)	3.5 ±0.09 b	4.1±0.2 a
Albumin concentration	1.0±0.1 b	1.2±0.1 a
Globulin concentration	2.5±0.02 b	2.9±0. 2 a

- Values represent mean ±SE.
- The difference between the values marked with various letters in the same line is significant ($P \leq 0.05$).

Discussion

The results in Tables 1, and 3 showed the wide spread of *Aeromonas* spp. Chiefly *Aeromonas hydrophila* this agreed with the study (7, 8, 9) with the difference in detection rate, these differences may be due to differences in the geographic location of the study, the season in which the study conducted, breeding system and clinical signs. Table (2) showed that internal organs in better than the skin and eye for detection of *Aeromonas* spp. due to their pathogenesis design and their ability to cause bacteremia and coverage in these organs (10, 11). These results of decreased (RBCs, HB, and PCV) in the experimental group may be the result of the swelling of RBC as well as poor mobilization of haemoglobin from the spleen and other haemopoietic organs due to infection with bacteria (12). During the experimental period, the WBC count also increased in the experimental groups compared to the control. This result were supported by the study of (13) who found that the WBC count was increased in the infected group as an immune response to infection. The levels of WBC was significantly increased compared to control values, indicating suppression of the growth of the pathogen. However, the (RBCs, HB, and PCV) levels in the infected group were significantly decreased compared to the control in the study. Monitoring these values and profiling leucocytes provides information about the general immune status of the fish and the destruction of RBCs leading to anaemia (14). This decrease in these parameters levels is thought to be associated with a stronger innate immune response in fish (15). On the other hand, our results agree with (16) who reported that many fish diseases were found to alter the concentration of total protein, albumin and globulin in the infection cause decrease in

the level of it in the fish infected with *Aeromonas hydrophila*. (17) Found that increase in the level of total protein, albumin and globulin level in healthy group may be due to the good nutritional status of the cultured common carp. (18) revealed the decreasing in the concentration of total protein in many disease statuses is due to Similarly stress and infection can reduce appetite of fish and thus affect the serum protein level decrease and capacity of synthesis and reduces absorption or protein loss through hemodilution. (19) Reported that blood proteins act as buffer to maintain hydrogen ion concentration and osmotic pressure, The concentration of total protein decreased in many diseases status due to decreased capacity of synthesis, reduced absorption or protein loss.

Conclusion

We infer from this study that *Aeromonas hydrophila* infection can cause changes in blood levels and other biochemical indicators, as well as economic losses in this sector of manufacturing.

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Conflict of interest

The authors state that there are no conflicts of interest in the publication of this work.

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Article highlights

- 1- *Aeromonas hydrophila* bacteria effect on level of (red blood cells, packed cell volume and haemoglobin concentration).
- 2- *Aeromonas hydrophila* causes economic losses in this field of production.