

HAEMOTOLOGICAL, IMMUN AND BIOCHMICAL CHANGES IN SOME INFECTED FISH WITH PARASITES IN TIGRIS RIVER AT SALAH AL-DIN PROVINCE, IRAQ

Al-Shaima M. Jasim¹, Abdullah H. Abdullah²

¹College of Science, University of Tikrit, Iraq

²College of Education for Women, University of Tikrit, Iraq.

alshima.jasim@tu.edu.iq

Abstract

The currently study has been conducted during the period from February 2021 to the end of January 2022, a total of 90 fish specimens belonging to seven families and 16 species from Tigris river at Tikrit city. The results revealed that infection with sixteen species involved 12species of genes *Myxobolus* (*M.bouixi* , *M.bramae*, *M. branchiophilus*, *M. cyprinicola* , *M. dispar*, *M. ellipsoides*, *M. infundibulatus*, *M. koi*, *M. magnus*, *M. molnari*, *M. musculi*, and *M. shaerica*) and 4 species of Cestoda (*Caryophyllaeus brachyoclois*, *Eubothrium salvelini*, *Ligula intestinalis* and *Schyzocotyle acheilognathi*). This study also found out that the effect of infection external and internal parasites in some standards of blood, immune and biochemical in some fish species.

Key words: Haematological, Immun, Myxobolus, Cestoda,

Introduction

Fish parasites can be ectoparasites which are parasites that live on the surface of the host or endoparasites which live in the hosts interior organs. Fish are infected with different groups of parasites including Protozoa, Termatoda, Cestoda, Nematoda, Acanthocephala and Crustacea. There are too much information about fish parasites around the world, the study of fish parasites is important to rise pond productivity and improve commercial stocks value (1).

Phylum myxozoa are protozoan parasites, and are very common in freshwater and marine fishes, divided into two class including: class Myxosporidia which parasitize of vertebrates particularly fishes, often toxic imports for their host (2), and class Actinosporidia which parasitize of invertebrates specially Oligochaetes and Polychaetes (3).

Myxosporidia are carefully important group of parasites, which cause disease in a large variety of commercially important fishes, and are responsible for enormous losses of eggs, fries and fingerlings of freshwater fishes, and cause high mortality rates in farmed fish. Also, they cause production losses and some fishes do not have to be consent because they are disgusting and not considered to be suitable for human feeding (4).

Cestoda are also commonly called as tapeworms, it is a class of parasitic worms in the flatworm phylum (Platyhelminthes). All cestodes are generally flat, segmented and ribbon-like. The body consists of a scolex (head, a short neck and strobili (segmented body) formed of proglottids. The scolex is used by the worm to anchor itself on the host. The length of these worms might arrive 10 meters in some species. Since cestodes are endoparasites, they can cause severe damage for the infected fish, especially by large helminthes. These worms have no gut or mouth; therefore, they get their food by absorbing nutrients from the hosts alimentary tract via particular cuticle which can also protects the parasite from digestive enzymes of the host (5).

Materials and Methods

After fish brought to the laboratory, they left in the containers to stabilize form 15- 30 minutes in the dark place. Since strongly light affect bloody measurement. Fish alive were caught carefully by a piece of clothing to avoid stress. Then hitting on the head to be ready to examine morphological characteristics, bloody measurements and diagnosis parasites. Besides, these fish were determined according to their scientific names (6 , 7).

Scales have been removed by scarper from tail of fish, after that place was cleaned from water and mucus by dry piece of cloth. Then vertical cut has been done in the area of anus and fins. Pressure on its slid where it has been observed that blood was flowed from blood-vessel. Samples of blood were taken directly and divided in to two tubes: gel and Ethylene diamine tetra acetic acid (EDTA) tubes.

Result and dissection

1- Hematological study :

The obtained data shows in the(Table 1) revealed decreases in blood values (Hb, PCV) in infected fish with *Myxobolus* and Cestodes , except the W.B.C. counts were lower than those of healthy fishe.

Significant differences were noticed in $P \leq 0.05$ the blood parameters of infected fishes with *Myxobolus* (b)and Cestodes (c) , except WBC Counts were higher than infected fish compression with non-infected ones . the WBC counts in group C were higher thin group B .

Table (1) : Mean of Some blood parameters of uninfected (healthy) and infected fish (± S.E.) .

Group	No. of fish Examined	H.B. g/100m	P.C.V.%	W.B.C count 10*3
A	20	10.490±0.144 a	31.570± 0.435 a	3.709 ±0.170 c
B	15	7.347± 0.378 b	22.080±1.13 B	9.517±0.357 b
C	8	5.900±0.475 c	17.113±0.991 c	13.116 ±0.675 a

Means ± (SE) within same column followed by different superscript small letters are significantly different at ($P < 0.05$) based on t-test.

* Group A = **Healthy fish**

* Group B = ***Myxobolus* infection**

* Group C = **Cestodes infection**

Table (1) explained the declining in Hb, PCV, values in infection with *Myxobolus* and cestodes, when compared with uninfected fish except WBC counts which increased in the infection with these parasites. Infections with parasites have higher effect on blood parameters. The present results agree with the results obtained by (8) who recorded that mixed infections with parasites have higher effects on blood parameters than single infections of different fish species and this phenomenon may be attributed to the interaction between asphyxia and anemia. (9) the following conclusions are the hematological parameters of common carp were affected by parasites (single, and mixed infection), and the rates of all blood parameters Hb, PCV, RBC counts decline in infected fishes except the WBCs counts were higher than those of healthy fishes. Also, (10) recorded significant reduction in Hb, PCV and RBC counts in infection fish with *P. spindletruncatus* compared with healthy fish. The similar results were found by (11) in catfishes from Anambra River, Nigeria, The haematological manifestation of the infected fishes showed marked decrease in the content of haemoglobin concentration (Hb), packed cell volume (PCV) and red blood cells. However, the infected fishes had higher content of white blood cell (WBC) than the uninfected.

The difference in environmental characteristics, despite the stability of the geographical location, led to variations in both fish blood analysis and parasitic infestation level in the study sites. Therefore, fish and parasites are evidently used as biological biomarkers to assess their environment (12).

The haematological profile in fish farms may indicate physiological status and fish health, so that alternative standard blood diagnostic methods are used to establish and assess stress and / or the influencing performance of the disease state (13).

Parasites are important components of societies and make up a large part of the biodiversity found in ecosystems, providing valuable information about their hosts and the environment in which they live. Most parasite species rarely cause problems in the natural environment but in aquaculture, parasites often cause serious outbreaks of disease (14).

Hematological parameters signify vital tools in defining the sublethal levels of contaminants like heavy metals in fish. Hematological variables are very necessary tool for the estimation of fish health status. The changes in blood parameters depend on numerous factors including: species, age, the cycle of the sexual maturity of spawners, and diseases. That stress of the fish is nonspecific response and the fish need any demand made upon it. Since the Stress in fish may be induced by various abiotic environmental factors such as changes in water temperature, pH, oxygen concentration and water pollutants including pesticides (15).

The high effect of *A. parasiluri* on the numbers of W.B.C. counts in contrast with *E. mosulensis* may be attributed to the high immunological effects of this parasite upon its host. Therefore, this monogenean has a high feeding activity on its host tissues and its excretory products may have a

high toxic effect. The lowering of the blood parameters in mixed infection than that of a single infection may be attributed to the interaction between asphyxia and anemia (16).

2- Immunity study:

The obtained data shows in the (Table 2) revealed The mean of antigens were higher in all infected fish when compared with uninfected.

There are significant differences ($P \leq 0.05$) in all infected groups fish, The mean of (A) were lower than another groups while group (D) was creased of antigens. The highest values of antigen (IgG) in strong infection, then in medium infection and lower values in group A. while the highest of antigen (IgM) in simple infection, then in group (D) and lower values in healthy fish.

Table (2):Mean of level of Immunoglobulin (IgG, IgM) in fish(\pm S.E.)

Means \pm (SE) within same column followed by different superscript small letters are

Groups	No. of fish Examined	IgG	IgM
A	20	2.113 \pm 0.118 d	337.1 \pm 8.99 C
B	7	5.016 \pm 0.201 c	725.0 \pm 163 a
C	5	7.786 \pm 0.289 b	429.9 \pm 39.0 bc
D	11	10.531 \pm 0.374 a	501.3 \pm 38.2 b

significantly different at ($P < 0.05$) based on t-test.

*Group A = **Healthy fish (no infection)**

*Group B = **Simple infection(one parasite)**

*Group C= **Medium infection (two parasites)**

*Group D =**Strong infection(3-4 parasites)**

The results obtained in table(2) revealed The antigens (IgG , IgM) were higher in all infected fish when compared with uninfected. The ability of fish to produce antibodies depends on factors such as the ambient temperature; pollution; antigen concentration; route of administration of antigen; use of adjuvant ; physiological differences in individual species of fishes; the geographical location of the fishes, the water sediment and the water quality. the factors influencing the production and functioning the humoral factors in genetic constitution, age, sex, nutritional status and hormonal balance of the fish with the additional impact of environmental variables, both the natural and man-induced. The antigen-antibody balances in the environment are integral factors of biochemical interactions which build up a fish population. These populations are of importance to the commercial fisheries, the sport fisheries, as well as the hobbyists dealing with exotic tropical fishes (17).

The B cell receptor and secreted antibody are at the nexus of humoral adaptive immunity. Immunoglobulins or antibodies, which play a vital role in adaptive immune responses, are heterodimeric glycoproteins belonging to the broad Ig superfamily (IgSF). Antibodies were first reported by von Behring and Kitasato in 1890 as an agent in the serum that could neutralize diphtheria toxin (18, 19).

The major component of fish specific humoral defence is immunoglobulin M (IgM), although IgD and even IgZ and IgT have also been recently described. Clear seasonal changes of plasma IgM levels were found to be related to water temperature and/or gonad maturation. The production of specific immunoglobulin against gill monogeneans or other helminths was observed. Specific antibodies play an essential role in cytolytic or cytotoxic mechanisms, such as in the activation of the complement system (classical pathway) or helping leukocytes to adhere to the parasite surface, presumably through Fc-like receptors (20).

3- Biochemical study:

Table (3) shows the mean of some biochemical parameters (Total protein, Albumin and Globulin) in infected with *Myxobolus* and Cestodes were lower than those of uninfected (healthy fishes).

The statistical analysis indicated that significant differences ($p \leq 0.05$) were noticed among proteins, Albumin and Globulin in infected with *Myxobolus*, Cestodes and healthy fishes (a,b), but there was no significant differences ($p \leq 0.05$) among three blood proteins in the infected (b). While the results of the globulin showed that differences were significantly ($p \leq 0.05$) in infected with Cestodes fishes.

Table (3) : Relationship among Mean of serum proteins with uninfected (healthy fish) and infected.

Groups	No. of fish Examined	Total protein	Albumin	Globulin
A	20	3.953±0.141 a	2.379 ±0.177 a	1.574±0.169 a
B	15	2.346±0.136 b	1.576±0.157 b	0.768±0.167 b
C	8	2.409±0.863 b	1.208±0.398 b	1.201± 0.545 ab

Mean SD ±., the different letter(a, b) reference of Significant different ($p \leq 0.05$).

* Group A = **Healthy fish**

* Group B = *Myxobolus* infection * Group C = **Cestodes infection**

The results obtained in table(3) The biochemical parameters were affected by parasites (*Myxobolus* and Cestodes) infection, since the total protein ,albumin and globulin were decreased during the case of infection with parasites compared uninfected fishes, agree with results obtained by (21) who found reduction in the concentration of total protein and albumin in *C. gariepinus* infected with *Trypanosoma mukasai* compared with the healthy fishes and (9) ConclusionsThe biochemical parameters of common carp were affected by parasites (single and double) infection, since the total protein ,albumin and globulin were decreased during the case of infection with

parasites compared unification fishes. Also, these results will agree with the results noted by (10) chemical characteristic of fish muscle reported generally a significant decrease in protein content of the muscles of all *P. spindletruncatus* infected fish types compared with healthy fish.

(22) recorded that decrease in the concentration of total protein in many disease statuses in *C. carpio* from Romania, and regarded this decreases to the capacity of synthesis and reduces absorption or protein loss through hemodilution. However, blood proteins act as buffer to maintain hydrogen ion concentration and osmotic pressure. the results recorded by (23) who did not find any difference in total serum protein level and albumin in *C. carpio* infected with four different groups of ectoparasites including Ciliophora (*Ichthyophthirius multifiliis*), Monogenea (*Dactylogyrus* spp.), Digenea (*Ascocotyle coleostoma*) and Crustacea (*Dermostogaster* sp.). The no significant differences in the blood total protein in the same length of common carp, noted in the present study, may be reflects to the good nutritional conditions of the cultured common carp as suggested by (21).

The reduction in the level of total protein in numerous diseases statuses due to decline ability of creation reduced absorption or protein charge through hemodilution (22). Radovan *et al.* (2006 24) studied the biochemical parameters of common carp and found that the total protein and albumin decreases in infected fishes with cyanobacteria. (21) found by clinical examination of naturally infected catfish (*Clarias gariepinus*) which showed a marked decrease in total protein, albumin and Globulin.

Reference

- 1- Tessema, W. (2019). Review on parasites of fish and their public health importance, a seminar paper for the course seminar on current epidemiologic topic (Vep- 752). Res., 11 (10): 71-81.
- 2- Bassey, S.E. (2011). A Concise Dictionary of Parasitology. 1st Edn. Zetus Concepts, Port Harcourt. 115pp.
- 3- Lom, J. and Dykova, I. (2006). Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. Folia Parasitol. 53: 1-36
- 4- Kaur, H. and Gupta, A. (2017). Morphological, histopathological and molecular characterization of *Thelohanellus muscularis* n. sp. (Cnidaria: Myxosporea) infecting head muscles of *Labeo rohita* from Ranjit sagar wetland, Punjab (India). J. Appl. Biol. Biotechnol., 5(1): 21-28.
- 5- Kuchta, R. ; Basson, L. ; Cook, C. ; Fiala, I. Bartosovs-sojkova, P. and Rehulkova, E. (2018). Asystematic survey of the parasites of freshwater fishes in Africa. In: Scholz, T. ; Vanhove, M. P. M. ; Smit, N. ; Jayasundera, Z. and Gehnar, M. (eds). A guide to the parasites of African freshwater fishes. Brussels: R. Belg. Inst. Nat. Sci., 135-360.
- 6- Froese, R. and Pauly, E. (2017). Fish Base. World Wide Web electronic publications, www.fishbase.org.
- 7- Eschmeyer, W.N. (2018). Species by family/ subfamily in the Catalog of fishes <http://research.calacademy.org/research/ichthyology/Catalog/SpeciesByFamily.asp>. (Updated 2 July 2018).

- 8- **Jori, M. (2006).** Parasitic study on the asian catfish *Silurus triostegus* (Heckel, 1843) from Al-Hammar marshes, Basrah, Iraq. Unpublished Ph. D. Thesis, Coll. Educ., Univ. Basrah:192pp.
- 9- **Rasool, A. H. (2017).** Detection of Some Parasites and Study Their Effects on Certain Blood and Biochemical Parameters of *Cyprinus carpio* in South of Iraq. M. Sc. Thesis, Vete. Med., Univ., Basrah: 102pp.
- 10- **Al-Kakayi, S. A. G. (2019).** Comparative study on meat characteristics and blood biochemical parameters of some non- infected and infected fishes with the acanthocephalan *Pomphorhynchus spindlitruncatus*. M.Sc. Thesis, Coll. of Agri., Univ. Kirkuk,73 pp.
- 11- **Nnabuchi, U. O.; Ejikeme, O. G.; Didiugwu, N. C.; Ncha, O.S.; Onahs, S. P. and Amarachi, A. C. (2015).** Effect of parasites on the biochemical and haematological indices of some clariid (Siluriformes) catfishes from Anambra River, Nigeria. *International Journal of Fisheries and Aquatic Studies*, ; 3(2): 331-336 .
- 12- **Omar, R. H; Hagra, A. A.; El-Naggar, A. M and Mashaly, M. I. (2021).** Ecological, Hematological and Parasitological Studies on *Oreochromis niloticus* Linnaeus 1757 in the Nile Delta Region, Egypt. *Egyptian Journal of Aquatic Biology & Fisheries*. Vol. 25(1): 795 – 819.
- 13- **Fazio, F. (2019).** Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture*, 500(1): 237–242.
- 14- **Roberts, R. J. (2012).** *Fish pathology*. 4th edn. Wiley Blackwell, Oxford: 597pp
- 15- **Witeska M., Knodera E., Lugowski K. (2010).** The effects of Ichthyophthiriasis on some hematological parameters in common carp. *Turk. J. Vet. Anim. Sci.* 34 (3): 267–271
- 16- **Jori, M. M. (1998).** Study of the parasites of two mugilid fish species and the effect of some on the blood parameters. M.Sc. Thesis, Univ. Basrah., 136 p. (In Arabic).
- 17- **Magnadottir, B. (2010).** Immunological Control of Fish Diseases. REVIEW, *Mar Biotechnol* 12:361–379
- 18- **Yu, Y., Wang, Q., Huang, Z., Ding, L. and Xu, Z.(2020).** Immunoglobulins, Mucosal Immunity and Vaccination in Teleost Fish. *Frontiers in Immunology*, 11: 567941 .
- 19- **Mashoof, S. and Criscitiello, M. F. (2016).** *Fish Immunoglobulins*. Biology (Basel), 5(4): 45.
- 20- **Rohlenová, K.; Morand, S.; Hyršl, P.; Tolarová, S.; Flajšhans, M.; and Andrea Šimková, A. (2011).** Are fish immune systems really affected by parasites? an immunoecological study of common carp (*Cyprinus carpio*). *Parasites & Vectors*, 4:120.
- 21- **Osmani, H.A., Fadel, N.G. and Ali, A.T. (2009).** Biochemical and histopathological alternations in catfish *Clarias gariepinus* infected with trypanosomiasis with special reference to immunization. *Egyption J. Comparative Pathology and Chemistry*, 22(3): 164-181.
- 22- **Patrich, T.; Patriche, N.; Bocioc, E. and Coadă, M.T. (2011).** Serum biochemical parameter of farmed carp (*C. carpio*) *International J. the Bioflux Society*, 4(2):131-140.

- 23- **Ahmed, S. M. and Ali, A. H. (2013).** Serum proteins and leucocytes differential count in the common carp (*Cyprinus carpio L.*) infested with ectoparasites. Mesopot. J. Mar. Sci., 28(2): 151 – 162.
- 24- **Radovan K.; Jan, M.; Miroslava, P.; Koval, S.; Zdenefl, K.; Andrea, Z.; Jana, K. and Ludefl, K. B.(2006).** Biochemical parameters of blood plasma and content of microcystins in tissues of common carp (*Cyprinus carpio L.*) from a hypertrophic pond with cyanobacterial water bloom. Aquaculture Research, 40: 1683- 1693.