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RESEARCH ARTICLE OPEN ACCESS

Detection of Blood Parasites in Fish

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Abstract

Fish are often infected with different blood protozoa of the genera *Babesiosoma, Trypanosoma*, and *Hemogrigarina*. These blood parasites match the genera which infect the blood of mammals. A total of 120 samples from five different fish species (*Barbus grypus, Barbus sharpyi, Liza abu, Carasobarbus luteus,* and *Aspius vorax*) were collected from the Khazar River in Ninevah Governorate, Iraq at weekly intervals during the period from April 2022 to October 2022 for the detection of blood parasites and hematological analysis. Thin and thick Giemsa stained blood smears revealed significant infection with *Trypanosoma, Babes iosoma,* and *Haemogregarina* which were recorded in 32%, 16%, and 8% of *Carasobarbus luteus* samples, respectively; 30%, 15%, and 10% of *B. sharpyi* samples, respectively; and 23.3%, 16.7%, and 6.7% of *B. grypus* samples, respectively. No infection with *Trypanosoma* or *Babesiosoma* were recorded in *L. Abu,* and no infection with *Babesiosoma* or *Haemogregarina* were recorded in *A. vorax*. Hematological parameters of blood samples from infected fish showed a significant decrease in hemoglobin concentration, total red blood cells, and stacked cell size along with a significant increase in the number of inflammatory cells including lymphocytes, eosinophils, and basophils. This is the first study to identify blood parasites in fishes and to monitor the changes of hematological parameters in blood samples of infected fishes from the waters of Mosul, Iraq.

Keywords: *Trypanosoma, Babesiosoma, Haemogregarina*, Fish, Hematological Parameters, Iraq

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INTRODUCTION

Fishes are considered as an important source of nutrition for humans and livestock as they are rich in protein, vitamins, minerals, and serve as a good source of omega-3 fatty acids.[1,2] Like all animals, fishes can be infected with many parasites leading pathogenic diseases and economic losses due to decreases in the weight and nutritional value of infected fish.[3] Blood protozoa, or haemaprotozoans, such as *Trypanosoma, Babesiosoma*, and *Haemogregarina* are of great interest to researchers due to their variety and pathogenicity in fishes. *Babesiosoma* parasitize both circulating erythrocytes and erythrocytes from reticuloendothelial tissues and, to complete their lifecycle, require leeches as an intermediate host. Trypanosoma are haemoflagelates that resemble the genus that infects the blood of mammals.[4-6] Parasites in the blood stream can cause a range of symptoms from mild anemia, associated with low levels of parasitemia, to severe pathological changes, due to a heavy parasite burden, and serious illnesses that can result in anorexia, ascites, exophthalmia, with edema and splenomegaly, and even death.[7,8]

Haemogregarina protozoa have a sausage-like shape and are a malaria-like parasite of fish erythrocytes. *Haemogregarina* species are poorly studied parasitic sporozoans of the family Haemogregarinidae.[9] The development of haemogregarines is complex and occurs through two types of hosts: blood-sucking invertebrates and vertebrates, including fish.[10]

Blood parasites are of veterinary interest because their host populations can be human food resources. Additionally, there is limited information available on these parasite in Iraq,[7] thus the present study was designed to study and report the blood parasites of the genera *Trypanosoma, Babesiosoma*, and *Haemogregarina* parasitizing freshwater fishes in Mosul, Iraq. Further, their effects on the hematological profiles of infected fishes were analyzed.

MATERIALS AND METHODS

Fishes

A total of 120 samples from five fish species (*Barbus grypus, Barbus sharpyi, Liza abu, Carasobarbus luteus,* and *Aspius vorax*) were collected from different locations in the Khazar River in Ninevah governorate at an at weekly intervals and brought to the Lab of Parasitological Research, Microbiological Department, College of Veterinary Medicine, University of Mosul, Iraq.

Blood sample collection

Blood samples were obtained from the caudal artery and heart of fish and thin and thick blood smears were immediately prepared. The smears were air dried, fixed in 95% methanol for five minutes, then stained with Giemsa. Prepared slides were examined under a microscope using a 100X oil immersion objective lens for the detection of blood parasites. Images were taken using a digital camera.[11] To measure hematological parameters, approximately 1 mL of blood was placed in tubes with the anticoagulant EDTA. Hemoglobin (Hb) concentration, total red blood cells (TRBC), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), and total leucocyte count (TLC) were measured. The percentage of each white blood cell type was calculated.[12,13]

Statistical analysis

Statistical analysis was performed using the Chi-square test and SigmaStat software V3 (Systat Software, San Jose, CA, USA).

RESULTS

Parasitological examination of thin and thick blood smears stained with Giemsa revealed that fishes were infected with three genera of blood parasites (*Trypanosoma, Babesiosoma*, and *Haemogregarina*) as shown in Figures 1, 2, 3, and 4. Of the 30 examined of *B. grypus* fish, 7 (23.3%) were infected with *Trypanosoma*, 5 (16.7%) were infected with *Babesiosoma*, and 2 (6.7%) were infected with *Haemogregarina*. Of the 20 examined *B. sharpyi*, the infection rates with *Trypanosoma, Babesiosoma*, and *Haemogregarina* were 6 (30%), 3 (15%), and 2 (10%) respectively. No infections with *Trypanosoma* or *Babesiosoma* were recorded in the 25 examined *L. abu*, but 1 (4%) was infected with *Haemogregarina*. In the 25 examined *C. luteus* fish, 8 (32%), 4 (16%) and 2 (8%) were infected with *Trypanosoma,*

Babesiosoma, and *Haemogregarina*, respectively. Of the 20 *Aspius vorax* fish examined, 3 (15%) were infected with *Trypanosoma*, but no *Babesiosoma* or *Haemogregarina* infections were recorded (Table 1).

Blood Hb concentrations, TRBC, PCV, MCV, MCHC and TLC all decreased significantly in fish infected with blood parasites, while the number of inflammatory cells (eosinophils, lymphocytes and basophils) were significantly increased in infected fish as shown in Tables 2 and 3.

DISCUSSION

In this study, three blood parasite genera (*Trypanosoma, Babesiosoma*, and *Haemogregarina*) were recorded in different

Table 1. Prevalence of blood parasites according to species of fish

Species of fish	No. of fish examined	*Trypanosoma*		*Babesiosoma*		*Haemogregarina*	
		No. of fish infected	Prevalence %	No. of fish infected	Prevalence %	No. of fish infected	Prevalence %
Barbus grypus	30	7	23.3a	5	16.7a	2	6.7a
Barbus sharpyi	20	6	30a	3	15a	2	10a
Liza abu	25	0	0	0	0	1	4a
Carasobarbus luteus	25	8	32a	4	16a	2	8a
Aspius vorax	20	3	15b	0	0	0	0

Different letters vertically indicate significant differences at $p \leq 0.05$

Figure 1. *Babesiosoma* parasite in fish blood smears stained with Giemsa stain 100X

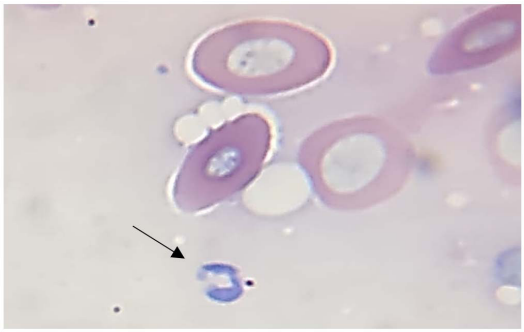

Figure 2. *Trypanosoma* parasite in fish blood smears stained with Giemsa stain 100X

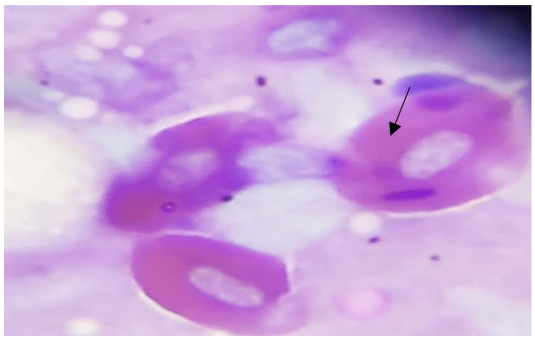

Figure 3. *Haemogregarina* parasite in the erythrocytes of fish blood smears stained with Giemsa stain

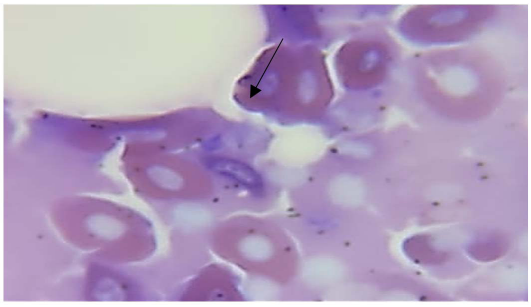

Figure 4. Free *haemogregarina* parasite in fish blood smear stained with Giemsa stain 100X

Table 2. Hematologic parameters of infected fishes with blood parasites

Parameters	Mean ± standard error	
	Control fishes	Infected fishes
RBC x 10^6 microliter	1.2 ± 0.05	0.6 ± 0.2
Hb g/100 ml	8.2 ± 0.2	5.5 ± 2.31
PCV %	30 ± 0.79	25.62 ± 5.62
MCV %	260.9 ± 16.83	450.75 ± 11.30
MCHC g/100 ml	70.8 ± 2.80	120.06 ± 50.21
TLC x 10^3 microliter	36.0 ± 6.0	90.85 ± 75.35

Table 3. The percentage of leukocyte cells of fishes infected with blood parasites

Cell type x 10^3 ml	Mean ± standard error	
	Control fishes	Infected fishes
Neutrophils %	8.21 ± 0.12	2.54 ± 0.02
Lymphocyte %	40.81 ± 0.25	60.65 ± 0.18
Eosinophil %	8.93 ± 0.05	11.51 ± 0.07
Basophils %	1.05 ± 0.03	1.20 ± 0.05
Monocytes %	20.81 ± 0.18	20.05 ± 0.14

species of fish. The highest infection rates were observed in *C. luteus, B. sharpyi*, and *B. grypus* which may have been due to pollution, habitat preferences, or the availability of intermediate hosts for the parasites. [14] *Trypanosoma* infection was observed in 23.3%, 30%, and 32% of *B. grypus, B. sharpyi*, and *C. luters* samples, respectively. The results of this study were similar to those obtained by Jarallah[6] who found that *Trypanosoma* infection rate in fish was 41.4%.

The high prevalence of *Babesiosoma* infection in fish observed here is in agreement with Shahi et al.[15] who reported a *Babesiosoma* infection rate of 16.6% in fish. The observed mixed infection with *Trypanosoma* and *Babesiosoma* has also been previously described.[16] The prevalence of blood parasites in fish are often the result of interactions with leeches.[17] The infection rate of *Haemogregarina* was low compared to a previous report[18] that recorded a *Haemogregarina* infection rate of 14.9 %.

Hematological parameters revealed a decrease in red blood cell count, hemoglobin value, and packed cell volume in infected fishes, which are conditions that often lead to anemia.[19] Parasitic infection causes stress that leads to the release of catecholamine which can mobilize RBCs from the spleen. Entry of fluid into the leukocyte cellular compartment has also been shown to increase in infected fishes. The results reported here align with these prior reports.[20,21] Another study indicated that *Trypanosoma* causes damage to the hematopoietic tissues, vascular system, and kidneys. Fish with a heavy infection have sunken eyes and become lethargic, anemic, and emaciated with damage to the excretory part of the kidney, causing osmoregulatory problems.[22]

While the infection rate with *Trypanosoma* was lower in *A. vorax* (15%), which is similar to the infection rate obtained by Jarallah[6] (12.28%), in this case, the low infection rate may have been due to improved host immunity. Leukocytes are considered to be the first line of defense for the immune system[6]; surveying the changes in the number and distribution of leukocytes is a good indicator to the presence of infection. It has been previously reported,[23] and this study confirmed, that leukocytes are directly related with an increase in parasitemia, especially lymphocytes and eosinophils, during *Trypanosoma* infection in fishes.

CONCLUSION

To the best of our knowledge, this is the first study investigating the prevalence of blood parasites in fishes of Mosul, Iraq. The evaluation of blood parameters could be used to determine the health status of fish, establish the degree of damage to tissues, and determine the severity of the infection. This study has reported the presence of three blood parasites: *Trypanosoma, Babesiosoma*, and *Haemogregarina*. It is recommended that further research on fish parasites be performed to investigate the occurrence of parasites in aquatic systems and provide information on the potential hazards to fish health. This is especially important with the growing demand for fish as a source of animal protein for humans and livestock.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

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