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Study of haemoflagellates *Trypanosoma* sp. infection in some fish of Iraq marshes and relationship of leukocytes with inflammatory response

H M Jarallah ^{1*}

¹ Marine Science Center - Basrah University- Iraq

* Corresponding author: hind.jarallah@uobasrah.edu.iq

Abstract

Fresh water fishes infected with different species of genus *Trypanosoma* parasite that is similar to that same genus in blood of mammals. Trypanosomiasis or sleeping sickness disease that causes by protozoa flagellate parasites which live inside cell called *Trypanosoma*. Fish parasites play an important role in regulating the population of their hosts. This study was implemented to verify the infection with *Trypanosoma* sp. report the cellular intervening through inflammatory response in blood smears of infected fishes. A total of 190 fishes of three species *Carasobarbus luteus*, *Aspius vorax* and *Liza abu* were collected from Al- Hamare and Al-Chibayish marsh south of Iraq. The flagellate parasite *Trypanosoma* sp. observed in blood of *Carasobarbus luteus* 41.42% and *Aspius vorax* 12.28% fishes, while no infection was recorded with *Trypanosoma* sp. in *Liza abu* fishes. Prevalence and mean intensity of infection were calculated. The different counts and various types of inflammatory cells were monitored, there are increased in lymphocytes in the infected fishes. Statistically, there are significant differences ($p < 0.05$) among species of infected fish with *Trypanosoma* sp.

Keywords

Fish, Parasites, *Trypanosoma* sp., Iraq marshes.

1.Introduction

Fresh and marine water fishes infected with different types of parasites genus *Trypanosoma* which is similar to that same genus in blood of mammals by a blood sucking insect tsetse fly (1). Trypanosomes are haemoflagellates having a single free flagellum at the anterior end of their body (2). *Trypanosoma* infection prevails against the host immune system resulting in pronounced diseases conditions and ultimately death of the host (3, 4). Fish health sector is considered as one of the main principal sectors that most of Arab regions adopt it, it is a necessary factor in the strategy of Arab food safety. lately, the Arab Organization for Agricultural Development (AOAD) Tackled, for the first time the problem of fish diseases aiming at improving and developing these important natural resources (5). Like all animals, fish can infect with diseases, the ecological factors and human interference would allow for entry of pathogens (6, 7). Olsen demonstrated that the worms belong to genus *Piscicola* and *Hemicleipsis* were transferring the infection among fresh water fish, while the worms belong to genus *Pontobdella* and *Trachobdella* were transferred the infection among marine water fish (8). There is



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cellular intervention in the inflammatory response, neutrophils followed by macrophages (9). All non-mammals such as fishes have nucleated erythrocytes, while the leukocyte is similar to birds and mammals cells. Eosinophils number is thought to be associated with parasitism defense (10, 11, 12). There are few studies on fish protozoa parasites, therefore, the present study was carried out to investigate the infection with *Trypanosoma* sp. parasite in some fish in Al-Hammar marsh and to report the relationship between infection and inflammatory immune response through blood smears of infected fishes.

2. Materials and Methods

2.1. Fishes

One hundred ninety (190) of three species fishes *Carasobarbus luteus*, *Liza abu* and *Aspius vorax* were collected from Al-Hamare and Al-Chibayish marsh south of Iraq during October 2018 to November 2019 and brought to lab of Marine Science Center-Basrah University in plastic aquariums.

2.2. Blood sample collection and experiment

Blood samples (1ml blood by using 1 ml a syringe) were collected directly from heart of fishes and then stored in tubes with anticoagulant (EDTA) to determine hematological values. Blood smears were fixed in 95% methanol for 5 min. and stained with Giemsa stain (13). Photos were taken under compound microscope. Fish blood examined by oil immersion, showed the organisms to be *Trypanosoma*. The relative number (percentage) of each white blood cell type was calculated based on the number of cells of that type counted divided by the total number of leukocytes counted (100 fields of view had been examined). Scientific photos were taken under compound microscope.

2.3. Statistical analysis

The parasites distributions were described using prevalence and mean intensity (14) of infection were calculated. Statistically, the data were given as Mean. The differences were recorded as significant whenever probability (p) was less than 0.05 SPSS, SPSS (15).

3. Results

Among 70 *Carasobarbus luteus* there are 29 were infected with the protozoa parasite *Trypanosoma* sp., the intensity of infection was 0.31, while the infected fish was 7 from 57 in *Aspius vorax* within intensity of infection 0.28. No infection was recorded with *Trypanosoma* sp. in *Liza abu* fish Table 1. The flagellated parasite *Trypanosoma* sp. observed in blood of *Carasobarbus luteus* and *Liza abu* fishes. It appears as monomorphic like S and C letters Figure 1.

Table 1. Prevalence and intensity infection with *Trypanosoma* sp. in some fish of Iraq marshes.

Fish	Total No. of Fish		Total No. of parasites	%	Mean intensity of infection
	Exam.	Infec.			
Carasobarbus luteus	70	29	77	41.42	0.31
Aspius vorax	57	7	11	12.28	0.28
Liza abu	63	0	0	0	0

(p>0.05) Methodology

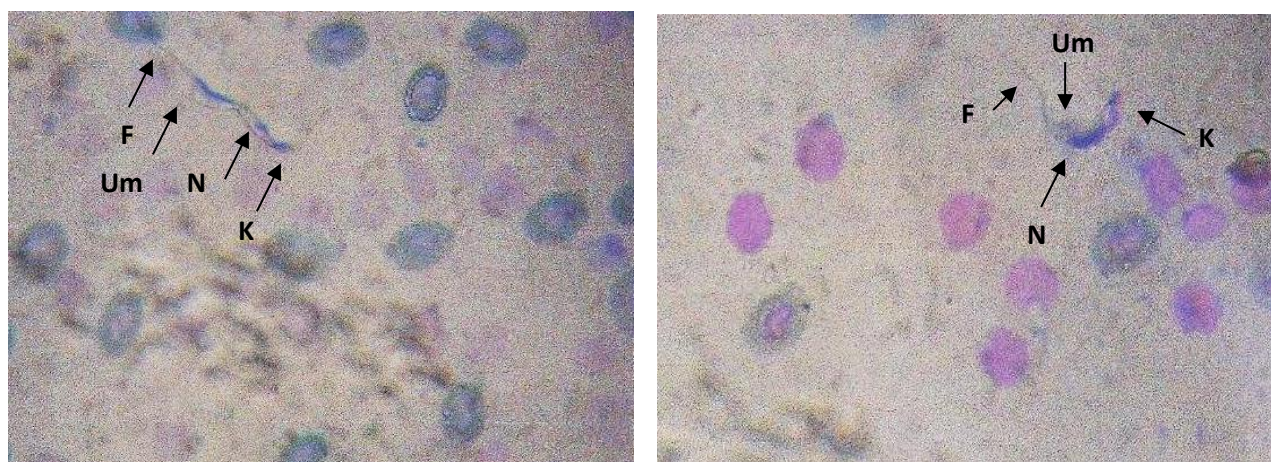


Figure 1. *Trypanosoma* parasites in blood smears, stained with Giemsa's stain. F: flagellum, N: nucleus, K: kinetoplast, UM: undulating membrane.

The inflammatory cells have been seen to increase in number in infected fishes, different count and various types of inflammatory cells were monitored, there are increased in Lymphocytes in the parasitized fishes. The mean values and the prevalence of the leukocytes for infected species of fishes are shown in Table 2. Lymphocytes, monocytes, neutrophils and eosinophils were found in Figure 2. Statistically, there are significant differences ($p < 0.05$) among species of infected and different kind of leukocytes in blood smears in infected fish with *Trypanosoma* sp.

Table 2. The number and percentage of different type of leukocytes (WBC) in blood smear in some fish infected with *Trypanosoma* sp. (50 fields were scanned).

Leukocytes types	Fish type					
	Carasobarbus luteus		Aspius vorax		Control	
	No.	%	No.	%	No.	%
Monocytes	41	25.7	19	24.05	21	32.81
Lymphocytes	92	57.86	51	64.55	30	46.87
Basophils	3	1.88	1	1.26	1	105
eosinophils	12	7.54	6	7.59	7	10.93
Neutrophils	11	6.91	2	2.53	5	7.81

($p < 0.05$)

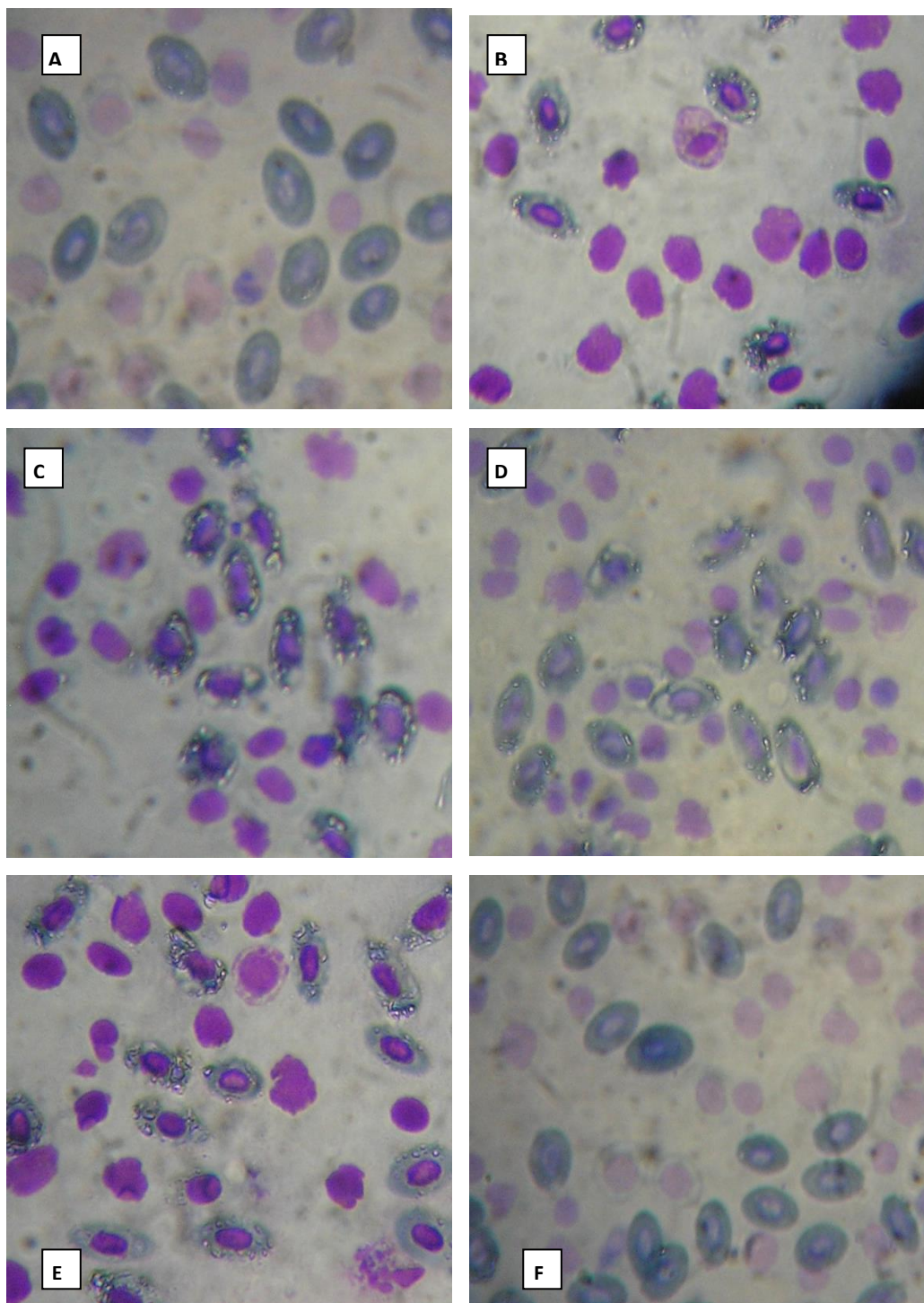


Figure 2. Smears of carp fish blood cells *Carasobarbus luteus* stained with Giemsa's stain (A: Erythrocyte, large lymphocyte and monocyte, B: Monocytes, C: Large lymphocyte and basophil, D: Neutrophil, monocyte and small lymphocyte, E: Eosinophil, large and small lymphocytes, F: Eosinophil).

4. Discussion

Trypanosomiasis or sleeping sickness disease that caused by protozoa flagellate which live among blood cells called *Trypanosoma* [16]. Fish parasites play an important role in regulating the populations of their hosts [6, 17]. Some of parasites consist of communicable diseases between mammals and fish which called zoonosis [7]. This study shows an increase in the prevalence was

accompanied with increase mean intensity of infection and when decreased in prevalence of infection that perform to decreased in mean intensity of infection [18, 3, 12]. This study indicated Avery interesting features that accounts for the restlessness of infected fish and the different types of changes in haematological parameters in fish, these results are agreement with Ramkrishanan which were quite comparable to those observed in mammals including man [19]. Fish suffer from diseases and parasites like human and other animals, but the automatism of fish immunity is similar to nethermost vertebrates for that if pathogens reach the defenses of fish; Parasites induce inflammation and modification of construct and functionality of fish [20, 21, 22].

The infection with parasites *Trypanosoma* sp. was slight in blood fishes of *Aspius vorax*, 7(12.28%), in this case the slight infection may be due to good healthy of hosts fishes. This result was indicated by Post [23] and Al-Daraji [24]. While the infection with these parasites *Trypanosoma* sp in *Carasobarbus luteus* blood was 29 (41.42%). Many investigations led to the demonstration of *Trypanosoma* sp. Infection in several species of fish. The first record of *Trypanosoma* sp. in fish blood of *Aspius vorax* in Iraq by Al-Daraji [24]. Leukocytes are the first line of defense for immune system; immune defense provide an important symbolizing of cells defense throughout the body [25, 26, 12]. The some more primary routes to evaluating the immune protocol is by surveying changes in the number of fish distribution leukocytes. This study has confirmed the previous findings that leukocytes are directly related with the increase in parasitemia during the *Trypanosoma* infection in fishes [4]. Also, these results were reported by recent study in rats infected with *Plasmodium* parasites [27]. Immune functionality may be reflected by the lymphocytes and neutrophils counting because of important cell types within the fish immune system, lymphocytes and neutrophils cells have been seen to increase in numeral during infected fish and it constitute the primary immune system.

Fish can develop inflammatory responses that increase the flow of blood to infected areas and deliver erythrocytes that attempt to destroy the pathogens. The inflammation consists of mechanisms involving the immune, nervous and circulatory systems that contribute in the defense system against health and environmental factors [28, 21, 29]. Fish have relatively simple system for that they are used as model for studying vertebrate immune systems [7]. Descriptions of effects of protozoa flagellates on fish are rare and limited observations include the definition of the inflammatory cells associated with host reaction [30]. Eosinophilic granule cells are morphologically and functionally similar to their mammalian counterparts. Eosinophils number is thought to be associated with parasitism defense [6, 11]. Statistically, there is no significant differences $p > 0.05$ were noticed in the percentage of prevalence and mean intensity of infection of all species of fish. More studies are needed in this scope of research for knowledge the associating of these granulocytic in immune defense against protozoa infection in fish.

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