Impact of trypanosomiasis on the blood parameters and growth factor (K factor) on freshwater fish *Heteropneustes fossilis* (Bloch)

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Abstract
Like other living organisms, fishes could host internal or external parasites that, while may not be usually fatal, thrive at the host's expense, causing pathological and detrimental effects. Research on the pathological effects of Trypanosoma infections in fish remains limited, highlighting the need for further studies to understand their impact on fish health and physiology. This study investigates the pathological impact of Trypanosoma sp. infection on fish, focusing on inflammatory responses and physiological changes in blood parameters. Trypanosoma sp. infection was confirmed through blood smear analysis, which revealed significant alterations in hematological indices. Hemoglobin levels decreased markedly from 13.8% in uninfected fish to 9.50% in heavily infected fish. Erythrocyte counts also declined significantly from 1.80 × 10^6/Cumm, while leucocyte counts increased from 21,400/Cumm to 26,400/Cumm as parasite load intensified.

Mean corpuscular hemoglobin (MCH) values exhibited an initial rise during mild infections (60 Pg) but fell to 52.77 Pg during severe infections. Growth indices were notably impacted, dropping from 107.07% in uninfected fish to 77.73% in cases of heavy infection, correlating with increasing parasite density. These findings highlight the detrimental effects of Trypanosoma sp. on fish health, indicating reduced metabolism and suppressed growth in infected individuals. Blood diagnostics provide valuable insights into the physiological and pathological status of fish under parasitic stress, emphasizing the need for effective management strategies.

Keywords: *Trypanosomiasis*, Blood parameters, Growth factor (K factor), *Heteropnetes fossilis*.

Introduction

Fishes are the most diverse group of aquatic creatures known as cold-blooded vertebrates which are adaptive in swimming and breathing with specific characteristics. Various fish species have scales to protect themselves and an effective body for realistic swimming[33]. Of approximately 28,900 fish species found worldwide, 13,000 are classified as freshwater, with 170 families and 2,513 genera and are found in rivers and lakes, which make up 1% of the world's total water supply. The remaining 16,000 species are found in saline environments, which make up 70% of the planet's surface[29]. Furthermore, fish is regarded as a vital component of human diets and contributes significantly to the economies of many nations across the world[4,9].

Numerous parasites have an impact on fish either directly or indirectly, leading to a high rate of death in this species. Protozoa (*ciliates, flagellates, microsporidians* and *myxozoans*), platyhelminthes (*monogenean, digenean* and *cestodes*), nemathelminthes and acanthocephala are the four main types of parasites that infect fish. Numerous deadly diseases that cause mass mortality are made easier by the physiology of fish[2,28].

Additionally, gastrointestinal (GI) helminths, which are major fish parasites and cause significant losses to the fish industry, are among the many parasites that live in fish. The nutrition, metabolism and secretory processes of the digestive system are all impacted by parasites, which seriously harm the nervous system and prevent fish from reproducing normally. The primary cause of the decline in fish populations is parasite threats[22]. Among the several protozoan groups, ciliates and flagellates primarily infect pond water fish and have a direct life cycle. Conversely, microsporidians are intracellular and need the host's tissue to reproduce (FAO 2015). Consuming spores from diseased fish or food sources, the fish contracted the infection.

Fish's physiological state and health can be assessed using a variety of techniques including hematological assessment[6,7,11,17,24]. Numerous details on fish oxygen transport ability, immunological potential, stress level, illness, intoxication, nutritional state etc. can be found using hematological and biochemical indicators. Routine hematological analyses include measurements of biochemical indices, such as plasma component concentrations or activities, as well as evaluations of blood cell counts and other cell-related factors. Because of the shape of blood cells (all cells are nucleated), manual procedures are primarily used in fish hematology. As an alternative to manual approaches, attempts have recently been made to do automatic hematological examinations of fish blood[11,13,14,35,37].

Haemoflagellates with a single free flagellum at the front of their bodies are known as trypanosomes. *T. trichogasteri var. fasciatae*[21] and *T. piscidium*[18,19] were the trypanosomes identified from fish blood through previoous researches in India. From moderate anemia linked to low parasitaemia levels to severe pathological alterations brought on by a high parasite burden, the symptoms of piscine trypanosomiasis can vary widely[23,26].

The important parasites of fish grown in intensive culture are protozoa when the host fish is overcrowded, these parasites can multiply, causing emaciation, weight loss, mortality and ultimately causes the economic losses[16,30]. *Trypanosoma carassii danilewsky*i was identified as the trypanosome causing the infection in blood parrot cichlids fish through morphological, genetic investigation and histological examination revealing a significant cause of anemia, anoxia and steatosis in the liver as well as a pigment accumulation in the kidneys and spleen that was probably caused by melanomacrophages[25]. Above finding express that the haemoparasites stress supress the haemtological profile and the piscine growth index (k factor) has not yet been studied. Therefore, the goal of the current study is to discover and document the trypanosoma, the blood parasites that affect population of freshwater fish in the Ganga River in the Kanpur area. So it is a modest attempt to investigate the aforementioned facets of fish trypanosomiasis.

Material and Methods

The catfishes *Heteropneustes fossilis* (10-25 gms.) were used for pathogenesis studies. The experimental fishes were collected from Vijay Nagar Fish Market, Kanpur district. A total of 39 fish of different sizes and body weights were scanned for studies. The haematological investigation were conducted by collecting blood from the caudal vein of fish with the help of anticoagulated syringe. Heamoglobin (Hb%), Total Erythrocyte Counts (TEC), Total Leucocyte Counts (TLC/cumm) and MCH (Mean Corpuscular Heamoglobin), were estimated in infected fishes. Giemsa stain was applied to blood smears after they had been fixed in 100% methanol for five minutes[39].

Under a compound microscope, *Trypanosomes* were identified as the organism with non-infected (No *Trypanosomes*/100 RBC), mild infection (01*Trypanosomes*/100 RBC), moderate Infection (02*Trypanosomes*/100 RBC) and heavy Infection (03-04 *Trypanosomes*/100 RBC) and the pictures were captured when fish blood smear was tested using oil immersion. In other investigations the fisheries biologists usually use the link between length and weight, also referred to as the growth factor or K factor, as an index to measure the condition of well-being in the workplace. Fish that have a high k factor, are heavier than their length and vice versa[38].

Results

A parasitological analysis for growth index and haematology and trypanosomiasis showed that total out of 39 experimental fishes, 28% were non-infected (Fig. 1) and 72 % (Fig. 2: Mild infection, Fig. 3: Moderate infection and Fig. 4: Heavy infection) were infected, so these fishes were categorised in four different groups. Fishes without infection show a healthy status of growth index and blood profile in which the growth index significantly falls as well as the number of trypanosomes increases in the blood.

The value of the growth index was significantly higher in (105.2) noninfected group of fishes and further decreased (89.37 to 77.72) from mild to heavy infected fishes (Table 1). The percentage changes in growth index showed an increase in their values which is 15.54% (Mild infection) to 26.11 (Moderate infection) and 26.55% (Heavy infection) as in table 2 and graph 1.

Haemoglobin values were 13.8 gram (Noninfected) which showed a significant reduction 9.5 gram (Heavy infection of trypanosome (Table-1; fig.3) Subsequently the percentage changes increased from 10.86% to 31.15% from mild to heavy infection (Table 2; Graph 2). The total erythrocyte counts also showed drastic changes in their values which were reduced from 2.58 X 10^6 Cumm in non-infected to 2.05 X 10^6 Cumm to 1.80 X 10^6 cumm from mild infection to heavy infection (Table 1; Fig. 3). The percentage changes also increase from mild (20.54) to heavy infection (30.24) (Table 2; Graph 3).

The TLC counts significantly increased from 21400 (Non-infected) to 26400 (Heavy Infection) their numbers as well as the number of trypanosomes increased in blood per 100 of RBC (Table 1). The percentage changes decreased from 14.01 (Mild infection) 23.36 to (Heavy infection) (Table 2; Graph 5). The percentage changes in MCH were highly effected form from infection, it increased 10.88 % (mild infection) to 0.62% (Heavy infection) as compared to no infected group of fishes (Table 2; Graph 5). In the present work the erythrocyte membrane damage occurs at the site of contact when trypanosomes and RBC come into close contact (Fig. 5).

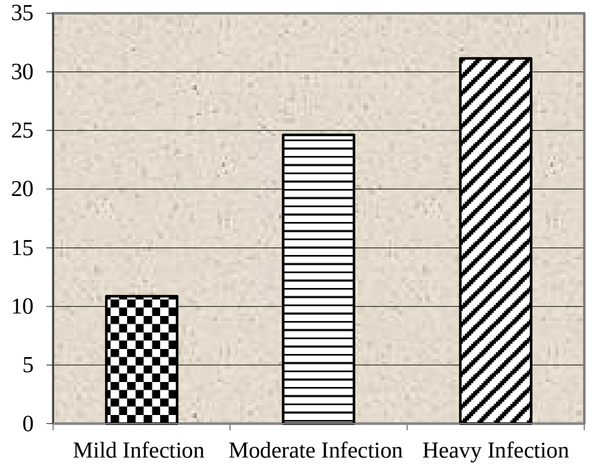
Discussion

The present study investigates the impact of trypanosomiasis on the blood parameters and growth factor (K factor) of the freshwater fish *Heteropneustes fossilis*. The findings highlight significant alterations in haematological indices and a noticeable decline in the condition factor, suggesting severe physiological and metabolic disturbances caused by the parasitic infection[20,31]. Haematological parameters serve as crucial indicators of the health status of fish. Infected *H. fossilis* exhibited a marked reduction in red blood cell (RBC) count, haemoglobin concentration (Hb) and haematocrit (Hct) values when compared to healthy controls[1,5].

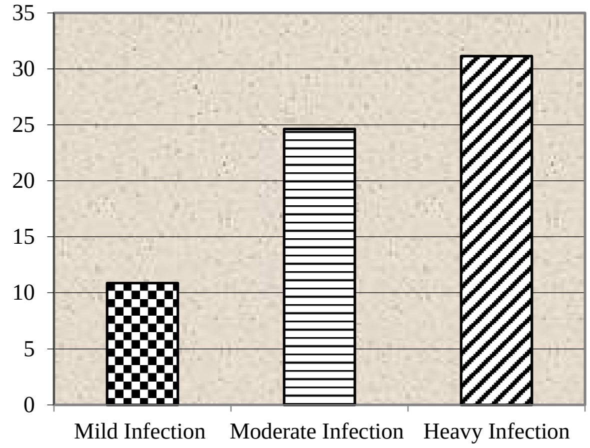
These findings align with previous studies, which attribute such declines to haemolysis and impaired erythropoiesis caused by trypanosome parasites[15].

Table 1
The mode of trypanosomiasis on the blood parameters and growth index of freshwater catfish *Heteropneustes fossilis*

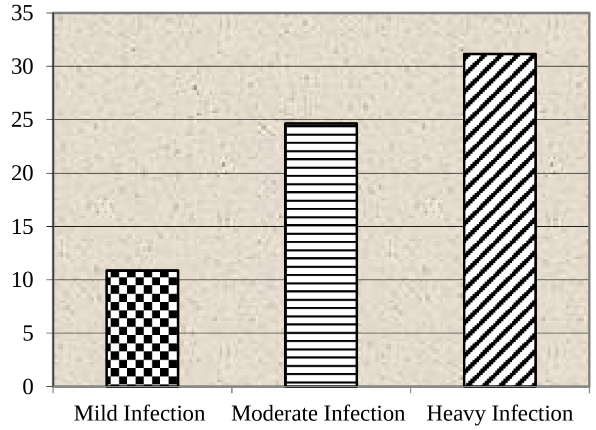
S.N.	Length (Cm)	Weight (Gram)	Growth index (K) Factor		Mode of Infection		No. of Pathogen /100 RBC	Haemoglobin		Total Erythrocytes Counts		Total Leucocytes Counts		MCH	
				Mean				Percentage %		TEC $x10^6$/Cumm				Pg	Mean
1	14	15.66	111.9	105.82	No Infection	---	0	13.9	*13.8*	*2.80*		21900		49.64	54.11
2	11	10.28	93.45			---	0	14.3		*2.36*		20300		60.59	
3	14	15.57	111.2			---	0	13.8		*2.75*		20600		50.18	
4	16	20.34	127.1			---	0	13.4		*2.80*		23000		47.85	
5	13.4	12.17	90.82			---	0	13.5		2.39		22100		56.48	
6	15	15.7	104.7			---	0	13.7		2.45		21500		55.91	
7	15.1	15.16	100.4			---	0	13.7		2.48		21400		55.24	
8	14	14.12	100.9			---	0	13.9		2.65		21100		52.45	
9	16	15.78	98.62			---	0	14.0		2.38		22600		58.82	
10	16	18.1	113.1			---	0	13.9		2.54		21000		54.27	
11	14	15.66	111.9			---	0	13.9		2.80		21900		49.64	
12	11	9.1	82.72	*89.37*	Mild Infection	+	1	12.5	12.30	2.24	2.05	24700	24400	55.8	60.00
13	16.7	22.78	136.4			+	1	12.6		1.89		24000		66.66	
14	11.2	9.2	82.14			+	1	12.0		2.11		24900		56.87	
15	11.9	9.3	78.15			+	1	12.4		2.07		25900		59.9	
16	13	10.22	78.61			+	1	12.1		1.90		23200		63.68	
17	13	10.17	78.23			+	1	12.2		2.09		23700		58.37	
18	13.2	10.4	78.78	*78.18*	Moderate Infection	++	2	10.2	10.40	1.99	1.89	25900	25100	51.25	55.02
19	13.5	10.5	77.77			++	2	10.7		2.32		25400		46.12	
20	13.4	10.23	76.34			++	2	10.9		1.91		24900		57.06	
21	12.8	9.68	75.62			++	2	9.9		1.86		23600		53.22	
22	13	11.02	84.76			++	2	10.4		1.72		24900		60.46	
23	12	9.1	75.83			++	2	10.2		1.94		24300		52.57	
24	12.2	9.16	75.08			++	2	10.8		2.04		24800		52.94	
25	13.3	10.21	76.76			++	2	10.4		1.59		24500		65.4	
26	11.8	9.76	82.71			++	2	10.0		1.64		27600		60.97	
27	14	10.96	78.28	*77.72*	Heavy Infection	+++	3	10.2	9.50	1.71	1.80	28800	26400	59.64	53.77
28	19	17.69	93.1			+++	3	9.3		2.16		28500		43.05	
29	12	8.59	71.58			+++	3	10.5		2.13		27900		49.29	
30	14.5	11.1	76.55			+++	4	9.2		1.68		29300		54.76	
31	9	5.42	60.22			+++	3	9.3		1.96		27900		47.44	
32	10	7.83	78.3			+++	3	9.8		1.57		27300		62.42	
33	11.2	8.1	72.32			+++	3	9.3		1.51		28200		61.58	
34	17	19.1	112.4			+++	3	9.2		1.47		28900		62.58	
35	13	10.3	79.23			+++	3	9.9		1.93		29900		51.29	
36	14	10.8	77.14			+++	3	9.0		1.42		27800		63.38	
37	14.2	10.14	71.4			+++	3	9.0		2.16		29800		41.66	
38	14.6	11.02	75.47			+++	3	9.4		1.93		28900		48.7	
39	10	6.44	64.4			+++	3	9.5		1.77		28800		53.67	



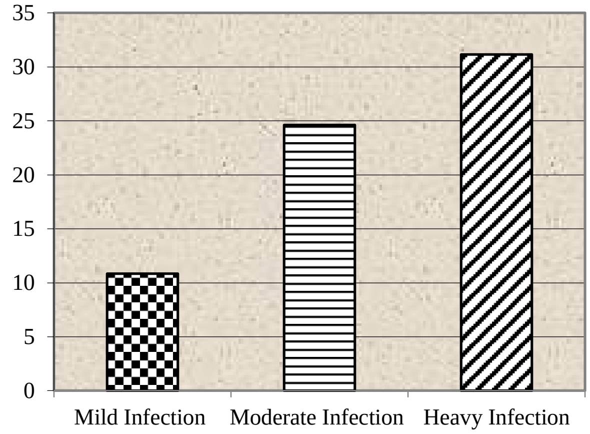
Graph 1: The percentage changes in growth index (K factor) of trypanosome-infected freshwater fish *Heteropneustes fossilis*



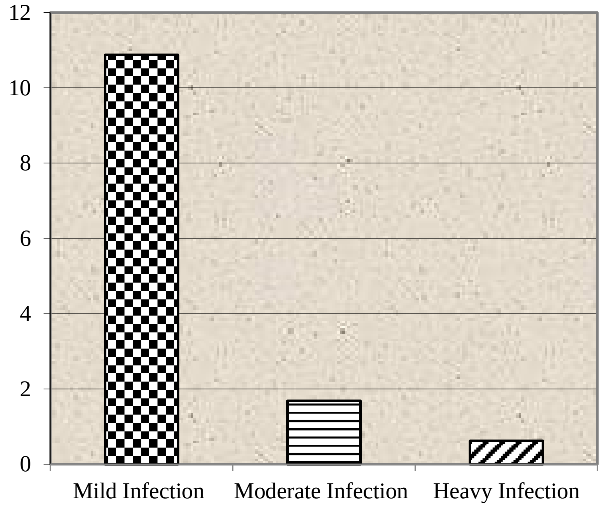
Graph 2: The percentage changes in haemoglobin percentages (Hb%) of trypanosome-infected freshwater fish *Heteropneustes fossilis*



Graph 3: The percentage changes in total erythrocytes count (TECx10^6/Cumm) of trypanosome-infected freshwater fish *Heteropneustes fossilis*



Graph 4: The percentage changes in total leucocytes counts of trypanosome-infected freshwater fish *Heteropneustes foss*



Graph 5: The percentage changes in total MCH (Pg) value of trypanosome-infected freshwater fish *Heteropneustes fossilis*

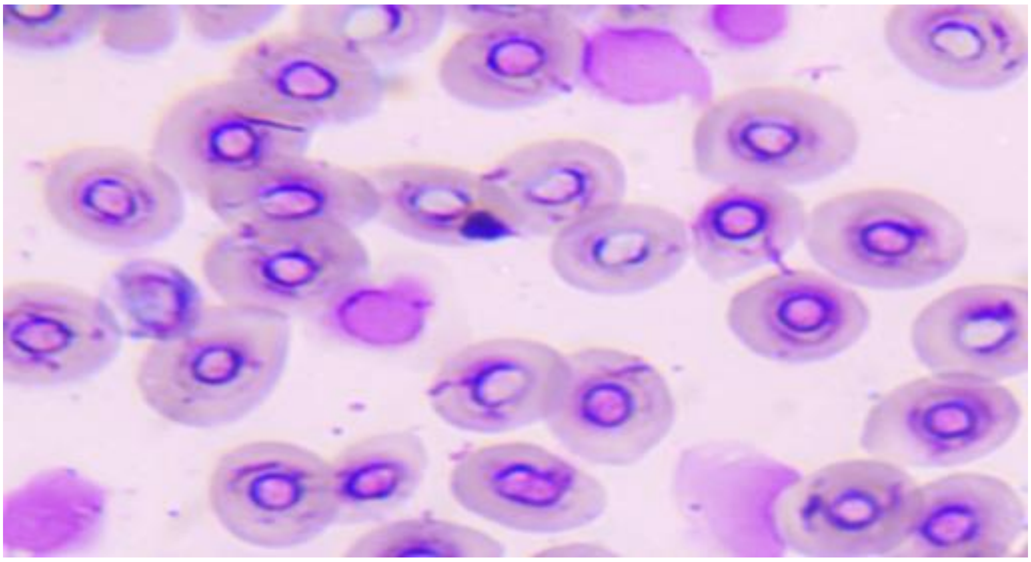


Fig. 1: Microphotograph shows the noninfected blood/100 of freshwater teleost *Heteropneistes fossilis*

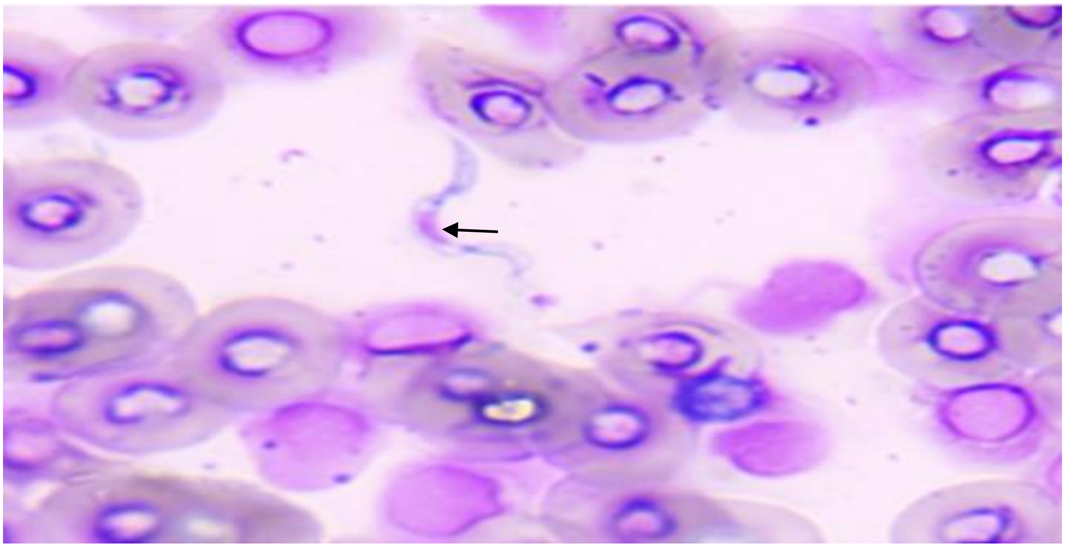


Fig. 2: Microphotograph shows the noninfected blood/100 of freshwater teleost *Heteropneistes fossilis*

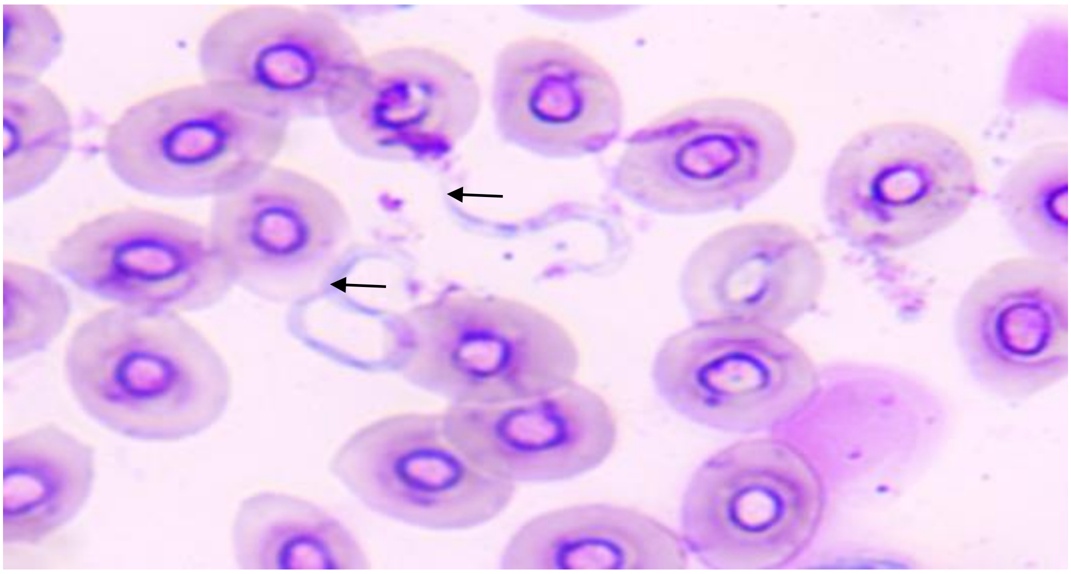


Fig. 3: Microphotograph shows the noninfected blood/100 of freshwater teleost *Heteropneistes fossilis*

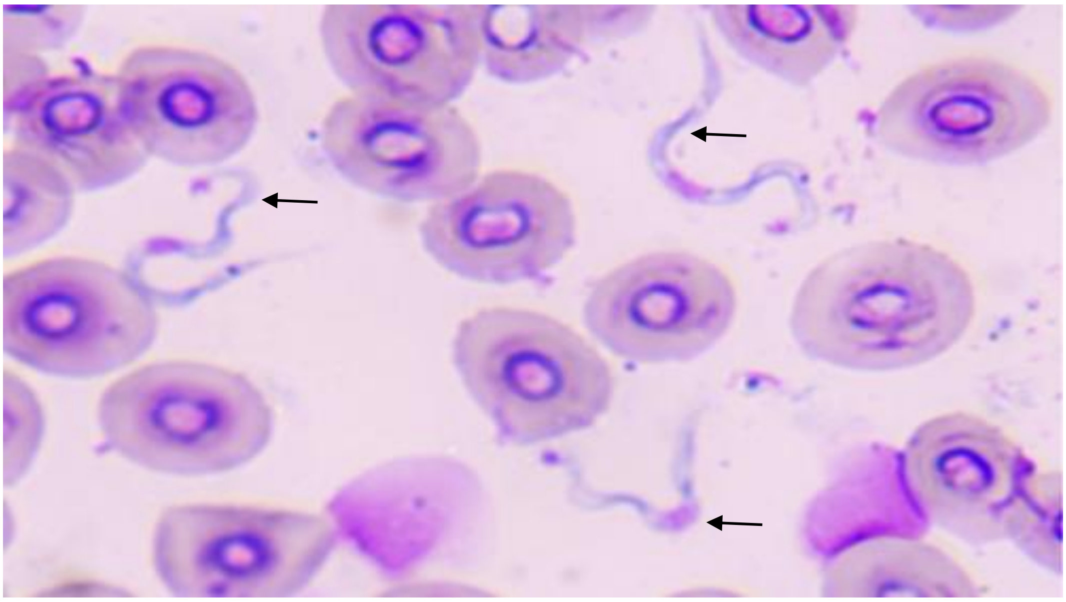


Fig. 4: Microphotograph shows the three trypanosomes /100 of freshwater teleost *Heteropneistes fossilis*

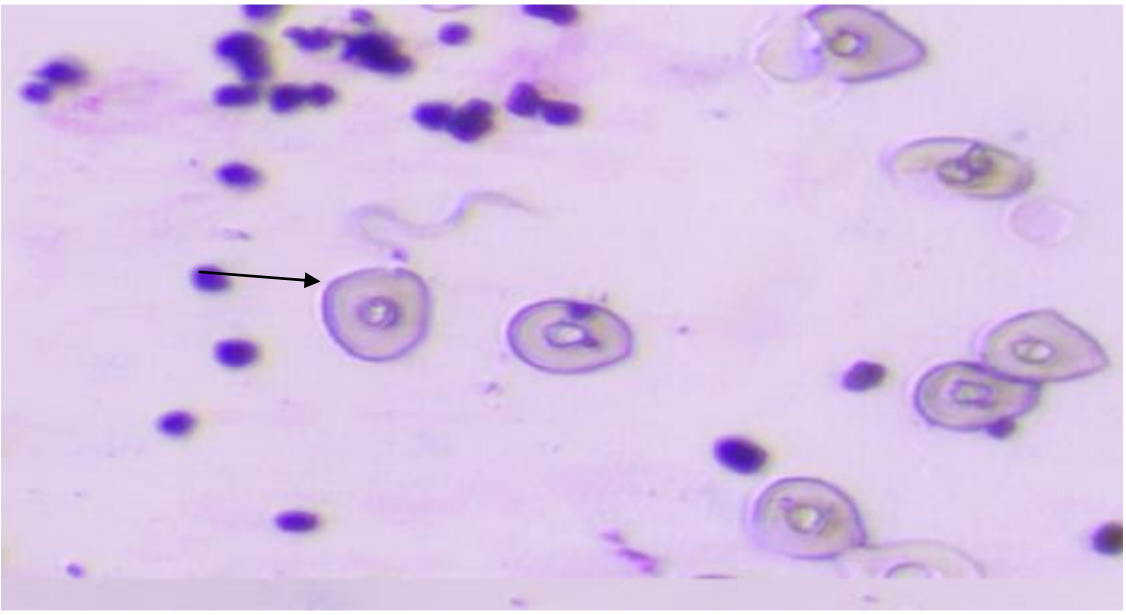


Fig. 5: Microphotographshows the trypanosomiasis induced in the outer membrane of RBC of freshwater teleost *Heteropneistes fossilis*

Table 2
Shows the percentage changes in blood parameters and growth index due to trypanosomi asis as compared with noninfected freshwater catfish *Heteropneustes fossilis*

S.N.	Parameters	Trypanosomiasis		
		Mild Infection (+)	**Moderate Infection (++)**	**Heavy Infection (+++)**
1	*Growth Factor (K Factor)*	*15.54*	*26.11*	*26.55*
2	Hb % (Haemoglobin)	10.86	24.63	31.15
3	TECx10^6/Cumm (Erythrocytes)	20.54	26.74	30.23
4	TLC /Cumm (Leucocytes)	14.01	17.28	23.36
5	MCH (Pg)	10.88	1.68	0.62

The reduced RBC count and haemoglobin levels are indicative of anaemia, a common pathological condition observed in trypanosomiasis affected fish. Additionally, the leucocytosis in infected fish may suggest an immune response to combat parasitic infection, as white blood cells play a pivotal role in defending against parasitic invasions[36]. The condition factor (K factor), which reflects fish's overall health and nutritional status, was significantly reduced in infected *H. fossilis*. *Chrysichthys nigrodigitatus* parasites and the length-weight relationship were examined by Esiest[8] in the Cross River Estuary, Itu local government area, Akwa Ibom State, Nigeria.

According to the study, there was little parasitic worm burden and little infection severity. Kurshid and Ahmad[27] demonstrated that the length of the host influenced both the prevalence and the average number of parasites.

The decline in the K factor indicates poor energy reserves, impaired feeding efficiency and a diversion of metabolic energy toward immune responses and parasite management. This reduction in the condition factor aligns with previous findings in other fish species affected by parasitic diseases[34]. Moreover, the significant changes in the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) further support the diagnosis of anemia. An increase in MCV and MCH suggests macrocytic anaemia, likely resulting from erythropoiesis stress and compensatory mechanisms activated in response to anaemia[11,40].

Alpidio et al[3] also supported the present work in their experiments in which they reported mechanical damage and metabolic harm brought on by host-parasite interactions in RBCs of sheep. The decreased hematocrit during infection is caused by the altered erythrocytes' susceptibility to mononuclear phagocytic clearance.

Conclusion

The results of the study provide evidence that trypanosomiasis significantly compromises haematological parameters and the growth factor in *Heteropneustes fossilis*. These changes affect fish physiology and have broader implications for aquaculture and fisheries management. Further studies focusing on the molecular mechanisms behind these changes and effective treatment strategies are essential to mitigate the impact of trypanosomiasis in aquaculture systems.

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