

Research Article

Alterations in haemato-biochemical indices in *Trypanosoma* sp. infected fish *Cirrhinus mrigala* (Hamilton)**Sadguru Prakash** 

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Indian major carp, *Cirrhinus mrigala*, is an important food fish in India. There is little evidence available about the effects of trypanosomiasis on the haemato-biochemical characteristic of *C. mrigala*. The present study aimed to determine the Prevalence of *Trypanosoma* in *C. mrigala* and haemato-biochemical alterations that occur in *trypanosoma*-infected *C. mrigala*. The fish samples were collected from various ponds and natural water bodies in the Balrampur district of Uttar Pradesh, India. The blood samples were collected from the caudal fin of fish to identify infection and analyse alterations in haemato-biochemical indices. The recorded data was subjected to biostatistical analysis. Prevalence of *Trypanosoma* in *C. mrigala* was 35.56%. Haemato-biochemical analysis showed that *Trypanosoma*-infected fish had significantly lower red blood corpuscles (RBC) counts ($0.62 \times 10^6 / \text{mm}^3$; $p < 0.05$) and Hb% (5.82; $p < 0.05$). The PCV% (25.8), MCHC% (24.52), serum glucose content (59.43 mg/dL), and serum protein content (20.24 mg/dL) of the infected fish were significantly lower ($p < 0.01$) than those of non-infected fish. TLC ($12.54 \times 10^3 / \text{mm}^3$), MCV (274.8 μm^3), and MCH (96.7 pg) were significantly ($P < 0.05$) higher in *Trypanosoma*-infected fish. The percentage of monocytes (7.72%), eosinophils (1.48%), neutrophils (7.32%), and triglycerides level (429.14 mg/dL) was significantly higher ($p < 0.01$) in fishes infected with *Trypanosoma*. The study highlights the physiological impact of parasites on fish health. This recommends regular monitoring of hematological and biochemical indices in aquaculture. This strategic planning can help to prevent fish mortality, ensure sustainable fisheries, and safeguard economic benefits for fish farmers and food security for society.

Keywords: Biochemistry, *Cirrhinus mrigala*, Fish parasites, Haematology, *Trypanosoma* spp..**INTRODUCTION**

Trypanosomes are haemo-monoflagellated parasitic protozoan with a free flagellum at its anterior end and are frequently encountered in the blood, lymph or cerebrospinal fluid of vertebrates (Overath *et al.*, 1999; Ralston *et al.*, 2009; Upadhyay, 2012, 2020, 2023; Upadhyay *et al.*, 2024). In fish *Trypanosoma* was first discovered in the blood of *Salmo fario* (Valentin, 1841). In

India, piscine trypanosomes are reported from several species of fish, including *Channa punctatus*, *Colisa fasciata*, *Mastacembelus armatus*, *Tor puntitor*, *Cyprinus carpio*, *Carassius carassius*, *Schizothorax curvifrons* and *Triplophysa marmorata* by various researchers (Gupta *et al.*, 2003; Gupta, 2006; Gupta *et al.*, 2006; Shahi *et al.*, 2013). Chakraborty *et al.* (2000) reported the presence of fish diseases in Karnataka and identified *Trypanosoma* in *Oreochromis mossambi-*

cus. Nandi *et al.* (2002) compiled a bibliography of 301 protozoan parasites found in Indian fishes, including over fifty *Trypanosoma* species. Several species have been documented in Uttar Pradesh, such as *T. karensis* from *Heteropneustes fossilis* (Gupta *et al.*, 2000), *Trypanosoma monomorpha* var *catlae* from *Catla catla* (Gupta *et al.*, 2000), *T. artii* from *Heteropneustes fossilis* (Gupta *et al.*, 2002), *T. piscidium* from *Colisa fasciata* (Gupta *et al.*, 2003), *T. heteropneusti* from *Heteropneustes fossilis* (Gupta *et al.*, 2006), and *T. sauli* from *Channa punctatus* (Gupta *et al.*, 2006).

These fishes are infected with monomorphic, dimorphic and pleomorphic species of *Trypanosoma*. They absorb dissolved organic matter and other substances found in blood, lymph, or cerebrospinal fluid through their entire body surface. The byproducts of the metabolism of trypanosomes can be extremely poisonous and cause their hosts to suffer from severe, frequently fatal illnesses (Lapirova and Zabolotkina, 2018). Most fish diseases may be caused by environmental pollution or parasite infestation (Hussain *et al.*, 2003). Fishes that are cultured in polluted water bodies are prone to parasitic infection. Trypanosomes spread from fish to fish through their intermediate host, leeches, during blood sucking (Hayes *et al.*, 2014; Lemos *et al.*, 2015; Ellis *et al.*, 2021).

The physiological and haemato-biochemical studies of cultured fish species are important for developing fish culture, particularly regarding identifying healthy fish. So, blood parameters can serve as indicators of fish health. Numerous trypanosomes infecting fish cause trypanosomiasis, a pathogenic disease that is dreadful for live fish. Symptoms of trypanosomiasis in fishes are anemia, leucocytosis, hypoglycemia, hypocholesterolemia etc. (Gupta and Jairajpuri, 1983). Although numerous parasitological studies have been conducted on fishes in *tarai* region of eastern U.P., most of the data on ectoparasites is mainly associated with crustacean parasites (Parmar and Prakash, 2022; Prakash and Verma, 2022), but data on piscine haemoparasites impact on the haemto-biochemical profile of infected fish are fragmented, and the problem of the influence on these protozoa on commercial species have not been studied so far. The changes associated with haemato-biochemical parameters due to various parasite infections establish a database which could be used in disease diagnosis and in guiding the implementation of treatment or preventive measures. Therefore, the present study aimed to determine how and to what extent trypanosomes cause haemato-biochemical abnormalities in *Cirrhinus mrigala* cultured in natural conditions in the lentic water bodies of Balrampur.

MATERIALS AND METHODS

In the present study, 90 live *C. mrigala* were collected from various ponds, natural lentic water bodies of

Balrampur district (27° 16' - 27°32' N and 82°03' - 82°22' E) of eastern Uttar Pradesh. For identification of *Trypanosoma* infected and non-infected *C. mrigala*, a few drops of blood from all collected fishes were taken by incising 2-3 fin rays of the caudal fin into separate precleaned glass slides and to prevent clotting, a drop of citrated salt solution was added because it does not change the motility of parasites. The freshly prepared slides were immediately examined under the light microscope for the presence of parasites, which can show active wriggling movement (Qadri, 1962). After identifying infected and non-infected fishes, the size and seasonality-based prevalence, i.e. percentage of *Trypanosoma*-infected fish, was calculated using the following formula (Margolis *et al.*, 1982):

Prevalence % in medium-size fish = Total no. of medium-size infected fish / Total no. of medium-size examined fish X 100
.....Eq. 1

Prevalence % in large-size fish = Total no. of large-size infected fish / Total no. of large-size examined fish X 100
.....Eq. 2

Seasonal prevalence % = Total no. of infected fish in a season / Total no. of fish examined in a season X 100
.....Eq. 3

The haematobiochemical indices were determined using the blood samples collected from the hearts of both the test fishes and placed into glass vials containing ethylenediamine tetraacetic acid (EDTA). Blood parameters like total erythrocytes and leucocyte counts were estimated by using a Neubauer Chamber. Blood films of trypanosome-infected and non-infected fish, *C. mrigala* were stained with Giemsa stain. The lymphocytes (monocyte, lymphocyte, neutrophil, eosinophil and basophil) were determined based on their staining quality. The Hb% and PCV% were determined by the cyanomethemoglobin method (Brown, 1980) and Wintrobe's tube method (Ramnik, 1994), while the mean cell volume (MCV) and the mean cell haemoglobin concentration (MCHC) were obtained according to the method given by Dacie and Lewis (2001). Blood glucose was analysed using an ISO-certified Glucometer. To estimate serum protein and lipid, the blood samples were centrifuged at 300 rpm for 10 minutes and the serum was collected for biochemical analysis. Protein and triglyceride were analysed by Lowry's methods (Lowry *et al.*, 1951) and Barnes and Blackstock method (Barnes and Blackstock, 1973), respectively.

Statistical analysis

The data obtained were subjected to statistical analysis using SPSS.

Animal ethical approval

Animal ethical approval was not required as the present study was based on blood sampling using a minimally invasive procedure (e.g., puncture rather than dissec-

tion). Anaesthesia was unnecessary, and the study was considered in the low-risk experiment category. The fish model *C. mrigala* used during the present study can be cultured and is not listed as an endangered or threatened species.

RESULTS AND DISCUSSION

A total of 90 freshwater fish, *C. mrigala*, were collected and examined from different water bodies in Balrampur, Uttar Pradesh. It was found that out of 90, only 32 fish were infected with *Trypanosoma* sp. The Prevalence of *Trypanosoma* sp. in *C. mrigala*, was 35.56%. The maximum prevalence was observed in medium-sized fish (42.22%) followed by larger-sized fish (28.89%) (Table 1).

The result showed that medium-sized fish become more susceptible to the pathogen than larger-sized fish because the intermediate host (leeches), is responsible for the transmission of *Trypanosoma* (Ahmed *et al.*, 1988). The finding of this study suggested that the Prevalence of *Trypanosoma* infection increased with the increasing size of fish, resulting in the decline in the growth of the fishes. In the present study, the seasonal prevalence of incidence of *Trypanosoma* in the host fish *C. mrigala* showed that the percentage of incidence was maximum in summer, followed by monsoon and winter seasons (Table 1). Parasitic protozoan *Trypanosoma* is responsible for high fish mortality (Chong, 2005). The prevalence of trypanosome infection varies from 20% to 60% in *Oreochromis niloticus*, up to 30% in *Clarias lazera* (Ahmed and Mohammed, 2011) and 23% -30.0% in *Synbranchus bengalensis* (Mahasri *et al.*, 2019) inhabiting in different water bodies. Compared to the prevalence of freshwater fish species infection, trypanosome infection among the *C. mrigala* in the present investigation seems to be similar in *Salmo salar* as reported in the appendix by Hansen (2000)

The range and average of haematological and serum biochemical parameters in both infected and non-infected fish, *C. mrigala* are shown in Table 2. The total RBC count value was significantly decreased ($P<0.05$) in infected fishes from non-infected fish by 65.16%. The haemoglobin (Hb%) content in non-infected fish, *C. mrigala* ranged between 7.6-11.8 gm% with an average 8.51gm%, whereas in infected fish ranged from 5.4 to 6.2 gm % with an average 5.82 %. The average value showed that this haemoglobin percentage was significantly reduced ($P<0.05$) in infected fish compared to non-infected fish by 31.62%. The packed cell volume (PCV) in non-infected fish, *C. mrigala* ranged between 29.2-32.2 % with an average 30.7 %, whereas in infected fish ranged from 22.8 to 28.7 % with an average 25.8 %. The average value showed that this PCV percentage significantly reduced ($P<0.01$) in infected fish compared to non-infected fish by 15.96%.

The erythrocytes count, haemoglobin concentration and PCV% were decreased among the *Trypanosoma* infected fishes, *Triplophysa marmorata*, *Carassius carassius*, *Schizothorax curvifrons*, *Abramis brama* and *Mastacembelus armatus* (Shahi *et al.*, 2013; Lapirova and Zabotina, 2018; Rauthana *et al.*, 2022) causing anaemia which was positively correlated with parasitism Shahi *et al.*, (2013) suggested that anaemia in fish is the due to continuous sucking of blood by leeches than a direct effect of the protozoan, *Trypanosoma* infection. As evident from the present study, the significant decline in the number of erythrocytes, haemoglobin % and PCV% in *Trypanosoma* infected fishes occurred as a result of the parasitic infestation that often leads to anaemia (Martins *et al.*, 2004). In the blood, trypanosomes produce protease and neuraminidase enzymes that damage the RBC membranes, resulting haemolysis (Mbaya *et al.*, 2012). The PCV was decreased significantly in all infected fishes due to the parasitization of *Trypanosoma* (Bag *et al.*, 2011) by

Table 1. Prevalence of *Trypanosoma* sp. in different season and size groups of *Cirrhinus mrigala*

Description	No. of Fish Examined	No. of fish infected	Prevalence (%)
Season	Winter Season (November-February)	4	13.33
	Summer Season (March-June)	16	53.33
	Monsoon season (July-October)	12	40.00
	Total	32	35.56
Size of fishes (cm)	Medium size (15-20cm)	19	42.22
	Large size (20-25cm)	13	28.89
	Total	32	35.56

whipping flagella into the erythrocyte membrane. Thus, erythropania in the present investigation was due to parasitic infection, not damage to the kidney's haematopoietic tissues.

The MCV was significantly increased ($P < 0.05$) in infected fish than in non-infected by 81.87%. The MCH was significantly increased ($P < 0.05$) in infected fish from non-infected by 51.22%. Mean Corpuscular Haemoglobin Concentration (MCHC) ranged between 29.29 – 33.25% with an average 31.11% in non-infected, whereas 23.14 – 26.49 with an average 24.5% in infected *C. mrigala*. The MCH was significantly increased ($P < 0.01$) in infected fish from non-infected by 51.22%. A significant decrease in MCHC with significantly increased values of MCV and MCH were observed in *Trypanosoma*-infected catfish, *Clarias batrachus* (Bag *et al.*, 2011) and in *Mastacembelus armatus* (Rauthana *et al.*, 2022).

The present investigation showed that TLC ($12.54 \pm 0.64 \times 10^3/\text{mm}^3$) and DLC (monocyte, $7.72 \pm 1.25\%$; eosinophils, $1.48 \pm 0.94\%$; neutrophils, $7.32 \pm 0.96\%$) in the *Trypanosoma*-infected *C. mrigala* significantly differed from that of the non-infected. The total Leukocyte Count (TLC) ranged between 6.95–10.15 with an average of $8.74 \times 10^3/\text{mm}^3$ in non-infected whereas from 8.15 – $14.64 \times 10^3/\text{mm}^3$ with an average of 12.54 in infected *C. mrigala*. The total TLC was significantly increased ($P < 0.05$) in infected fish from non-infected by 42.45%. Total leucocyte count increases in *Trypanosoma*-infected freshwater catfishes, *Heteropneustes fossilis*, *Clarias batrachus*, and *Mastacembelus armatus* (Gupta *et al.*, 2002; Bag *et al.*, 2011; Rauthana *et al.*, 2022).

The increased values of TLC in the infected host fish might be due to the intervention of harmful chemical substances released by haemoflagellates or a process of steady inoculation achieved by the reticuloendothelial system of the host *C. mrigala*. This toxic chemical stimulated the host's haematopoietic organ and immune system by producing antibodies and chemical substances working as defence against infection (Hansen, 2000; Lebelo *et al.*, 2001; Jarallah, 2021). The significant WBC enhancement may be due to haemolytic anaemia, which is always associated with leucocytosis (Duncan and Prasse, 1989; Małgorzata *et al.*, 2022). The increase in leucocytes count in the present investigation was the peculiar effect of anaemic condition and was dependent on trypanosome infection. The similar finding was observed by Joshi and Dabral (1981) in *Tor puntitora*, Bag *et al.* (2011) in *Clarias batrachus* (L.) and Rauthana *et al.* (2022) in *M. armatus* infected with trypanosomes.

The monocytes percentage in non-infected fish, *C. mrigala* ranged between 5.18–6.45 % with an average 5.85 %, whereas in infected fish ranged from 6.88 to 8.56 % with an average 7.72 %. The average value

showed that monocyte percentage was significantly enhanced ($P < 0.01$) in infected fish compared to non-infected fish by 31.95%. A similar finding was observed by Rauthana *et al.* (2022) in *Trypanosoma* infected *M. armatus*. Since monocytes attack and destroy pathogens that enter the body through phagocytosis, an increase in the percentage of monocytes in the present investigation showed that the immune system was using phagocytosis to fight the parasitic infection (Affandi and Tang, 2002; Mahasri *et al.*, 2019).

Lymphocyte produces antibodies against invading pathogens. The lymphocyte percentage in non-infected fish, *C. mrigala* ranged between 83.20–85.74 % with an average 84.12 %, whereas in infected fish ranged from 79.89 to 83.63 % with an average 80.69 %. The average value showed that lymphocyte percentage declined in infected fish in comparison to non-infected fish by 4.08%. Lymphocyte counts in healthy fishes ranged between 65–86% (Salasia *et al.*, 2001). Since the alterations in lymphocyte percentage between non-infected and *Trypanosoma*-infected *C. mrigala* in the present investigation were insignificant. So, it can be considered within normal range, and this variation in lymphocytes could be due to the degree of infection of *Trypanosoma* sp. in the *C. mrigala*, which was collected from different local water bodies.

The basophil percentage in non-infected fish, *C. mrigala* ranged between 2.12–2.62 % with an average 2.42 %, whereas in infected fish ranged from 2.29–2.35 % with an average 2.32 %. The average value showed that basophil percentage was decreased in infected fish in comparison to non-infected fish by 4.13%. The increased percentage of basophils may be related to parasitic infections (Santoso *et al.*, 2013).

The eosinophil percentage in non-infected fish, *C. mrigala* ranged between 1.48–1.21–1.33 % with an average 1.31 %, whereas in infected fish, ranged from 1.24–1.58 % with an average. The average value showed that eosinophil percentage was significantly increased ($P < 0.01$) in infected fish in comparison to non-infected fish by 11.49%. Eosinophil is responsible for parasite infection. The higher number of eosinophils in *Trypanosoma* infected *C. mrigala* was due to parasitic infection because of eosinophil attacks and break down parasites by producing lysozyme enzymes in phagolysosomes (Rahma *et al.* 2015; Mahasri *et al.*, 2019). Trypanosomes cause eosinophilia due to the eosinophilic chemotactic factor secreted by such protozoa (Aly *et al.*, 2005).

The neutrophil percentage in non-infected fish, *C. mrigala* ranged between 5.24–5.90 % with an average 5.42 %, whereas in infected fish ranged from 5.48 to 7.68 % with an average 7.32 %. The average value showed that neutrophil percentage was significantly increased ($P < 0.05$) in infected fish in comparison to non-infected fish by 35.06%. In the present investigation,

Table 2. Haemato-biochemical parameters of non-infected and *Trypanosoma* infected *Cirrhinus mrigala* (Mean values of 5 observations)

Haemato-biochemical Parameters	Non-infected Fish		Infected Fish	
	Range	Average	Range	Average
RBC ($\times 10^6/\text{mm}^3$)	1.70 – 2.14	1.78 ± 0.08	0.59 – 1.15	$0.62 \pm 0.14^{**}$
TLC ($\times 10^3/\text{mm}^3$)	6.95 – 10.15	8.74 ± 0.58	8.15 – 14.64	$12.54 \pm 0.64^{**}$
Hb (gm%)	7.6 – 11.8	8.51 ± 0.14	5.4– 6.2	$5.82 \pm 0.34^{**}$
PCV (%)	29.2 – 32.2	30.7 ± 1.25	22.8 – 28.7	$25.8 \pm 1.11^*$
MCV (μm^3)	128.92 – 178.12	151.1 ± 7.04	218.4 – 378.32	$274.8 \pm 31.02^{**}$
MCH (pg)	44.12 – 51.11	47.17 ± 1.83	57.77 – 142.2	$96.7 \pm 7.15^{**}$
MCHC (%)	29.29 – 33.25	31.11 ± 0.03	23.14 – 26.49	$24.52 \pm 0.02^*$
Monocyte (%)	5.18–6.45	5.85 ± 0.96	6.88–8.56	$7.72 \pm 1.25^*$
Lymphocyte (%)	83.20–85.74	84.12 ± 0.82	79.89–83.63	80.69 ± 0.84
Basophil (%)	2.12–2.62	2.42 ± 0.75	2.29–2.35	2.32 ± 0.67
Eosinophil (%)	1.21–1.33	1.31 ± 0.5	1.24–1.58	$1.48 \pm 0.94^*$
Neutrophil (%)	5.24–5.90	5.42 ± 1.24	5.84–7.68	$7.32 \pm 0.96^{**}$
Glucose (mg/dL)	64.58–68.47	66.25 ± 0.35	58.12–60.47	$59.43 \pm 0.68^*$
Total protein (mg/dL)	22.85 –23.75	23.12 ± 1.68	21.74 –22.14	$20.24 \pm 1.12^*$
Triglyceride (mg/dL)	378.16 – 418.12	408.42 ± 1.12	405.11 –4.49.21	$429.14 \pm 1.11^*$

the increased number of neutrophils might be the working mechanism of neutrophils, which is to fight bacteria or fungi (Palmer *et al.* 2016) because neutrophil specifically combats bacteria or fungi (Saad *et al.*, 2017), not due to protozoan infection (Mahasri *et al.*, 2019).

The serum glucose content in non-infected fish, *C. mrigala* ranged between 64.58 -68.47 mg/dL with an average 66.25 mg/dL, whereas in infected fish, it ranged from 58.12 -60.47 mg/dL with an average 59.43 mg/dL. The average value showed that this glucose level significantly declined ($P < 0.01$) in infected fish compared to non-infected fish by 10.29%. Trypanosomes consume blood sugar so that the carbohydrate reserve of the host becomes depleted, as reported in fish *M. armatus* infected with *Trypanosoma* showed a significant drop in total blood glucose levels ($P < 0.01$) (Rauthana *et al.*, 2022). However, Aly *et al.* (2005), Lapirova and Zabolina (2018) observed a significant increased ($P < 0.01$) in the blood glucose level in *Trypanosoma*-infected fishes, *Clarias gariepinus* and *Abramis brama*.

The total protein content in the blood of non-infected fish, *C. mrigala* ranged between 22.85 –23.75 mg/dL with an average of 23.12 mg/dL, whereas in infected fish, it ranged from 21.74 – 22.14 mg/dL with an average of 20.24 mg/dL. The average value showed that this serum protein level was significantly decreased ($P < 0.01$) in infected fish compared to non-infected fish by 12.46%. Total serum protein was decreased significantly ($P < 0.01$) in *Trypanosoma* infected fish, *M. armatus* (Rauthana *et al.*, 2022). The significant decrease in total serum protein in *Trypanosoma* infected African sharp-tooth catfish, *C. gariepinus* and *Abramis brama* could be caused by haemodilution (Osman *et al.*, 2009; Lapirova and Zabolina, 2018). However, Fudjimoto *et*

al. (2013) observed both a decrease and an insignificant increase in trypanosomes infected by seven ornamental fishes (*Leporacanthicus galaxias*, *Lasiancistrus saetiger*, *Cochliodon* sp., *Hypostomus* sp., *Pseudacanthicus spinosus*, *Ancistrus* sp. and *Rineloricaria* cf. *lanceolata*).

The serum triglyceride level in non-infected fish, *C. mrigala* ranged between 378.16–418.12 mg/dL with an average 408.42 mg/dL, whereas in infected fish, it ranged from 405.11–4.49.21 mg/dL with an average 429.14 mg/dL. The average value showed that this triglyceride level was enhanced in infected fish compared to non-infected fish by 5.07%.

Conclusion

The present study on haematological investigation showed that the infected fishes had lower total red blood cell counts, haemoglobin contents, and hematocrit values than the non-infected fishes. The numbers of neutrophils, lymphocytes, and monocytes were considerably different from those of fish that were unaffected. The trypanosomes induced extensive damage to the hemopoietic tissue of the host fish, *C. mrigala* reflects on the fish's health status. Based on alterations that occurred in haemato-biochemical parameters of *C. mrigala* infected with *Trypanosoma*, it was noted that although the prevalence of *Trypanosoma* infection was high, the intensity of infection was low. The study establishes crucial indicators of *Trypanosoma* infection by assessing blood and biochemical parameters, which can aid in early diagnosis and monitoring of parasitic diseases in aquaculture system. Thus, the haemato-biochemical response in the present investigation offers

insight into host-parasite interaction, fish health diagnostics, and potential biomarkers for early detection of parasitic infections.

Conflict of interest

The authors declare that they have no conflict of interest.

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