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ERYTHROPENIA IN PISCINE TRYPANOSOMIASIS

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ABSTRACT
Fish species may act as suitable hosts for trypanosomes. *Clarias batrachus, Channa punctatus* and *Wallago attu* were found infected with *Trpanosoma batrachi* , *T. aligaricus,* and *T. attii*, The vulnerable fishes were divided into six groups: Group I *Clarias batrachus* (IA control, IB infected), Group II *Channa punctatus* (IIA control, IIB infected) and Group III *Wallago attu* (IIIA control, IIIB infected). Studies conducted on the total erythrocyte counts (TEC) indicate a decline in this hematological parameter in all B Group fishes, maximum fall (33.19%) being recorded in *C. punctatus* infected with *T. aligaricus*. Abnormal erythrocyte cells (vacuolated and punctuated erythrocytes, macrocytes and enucleated erythrocytes showing karyorrhexis) were also encountered in infected blood. The parasites reside in the host blood and depend upon their hosts for their metabolic activities. The parasites may produce haemolytic factors and decrease the oxygen carrying capacity of the blood causing erythropenia.
KEY WORDS: Erythropenia, Red Blood Cells, *Trypanosoma*,

INTRODUCTION
Trypanosomes are haemoflagellates characterized by the presence of a free flagellum at the anterior end. They are known to cause serious diseases in man (sleeping sickness) and in domestic animals (Surra, Nagana) in the tropical countries. The pathogenic species have received great attention for the diseases they cause. However, these parasites have also aroused interest and attention of molecular biologists, geneticists and immunologists due to a number of unusual features which have made them interesting models for the study of different biological phenomena.

Trypanosomes affect many vertebrates and invertebrates and were first recognized in blood of fish and amphibia (Hegner *et al.*, 1938). Parasites in vertebrates generally require an intermediate host whereas in invertebrates they can complete their life cycle within the same host. The vectors of parasites of terrestrial hosts are blood sucking arthropods (usually insects, occasionally mites) and those of trypanosomes of aquatic forms are transmitted by leeches. Hoare and Wallace (1966) proposed the use of the terms amastigote, promastigote, opisthomastigote, epimastigote trypomastigote and choanomastigote for the various stages in the life cycle of the parasite based on the position of the flagellum and is widely accepted.

Fishes act as suitable hosts for trypanosomes. Approximately 200 species of piscine trypanosomes are on record, however host specificity of a majority of the species is not known. Species of *Trypanosoma* are parasitic in all groups of vertebrates ranging from fishes to mammals. But to what extent and how do they damage the red cells is not yet clear. The present communication describes the impact of trypanosome infection on the red blood cells (RBC) with reference to three species of freshwater fishes of Uttar Pradesh, *Clarias batrachus, Channa punctatus* and *Wallago attu.*

Host-parasite interactions
The disease caused by trypanosomes in fishes is termed as *Piscine Trypanosomiasis*. Generally the parasitaemia is low and often trypanosome detection is difficult. Fish reared and propagated in indoor ponds seldom pose a serious problem due to the lack of leech vectors. However, serious outbreaks may occur in hatcheries or outdoors ponds in the presence of leeches. Under wild situations, the actual condition of recipient fish plays an important role in determining the pathogenesis of piscine trypanosomiasis. Experimentally induced latent infections may be converted into heavy infections when the host is exposed to unfavorable conditions. The European gold fish (*Carassius auratus*) are easily infected with *T. danilewskyi* from carp (*Cyprinus carpio*) when they are under stress. Stress conditions (water temperature, feeding, nutrition and competition) influence the course of parasitaemia in fish. Perch and gold fish at low temperatures were found to have decreased trypanosomes level in their blood when transferred to high temperature, perhaps due to increased antibody production (Barrow, 1958). Too low or too high temperatures also inhibit infection (Lom, 1973a). Up to 80% mortality has been reported in gold fish experimentally infected with *T. danilewskyi* and the survivors are free from detectable infection after an average of 48 days (Lom, 1973b).

Trypanosoma and the red blood cell
Trypanosomes generally depend upon the host energy resources and the impacts of parasitism in fish are very wide ranging from physiological (Tandon and Chandra, 1977), metabolic or pathological (Lom and Dykova, 1984), biochemical (Gupta and Gupta, 1986) and asymptomatic to behavioral (Barber *et al.*, 2000) anomalies and syndromes. The erythrocytes are frequently subjected to changes in fish infected with trypanosomes.

Swammerdam was the first to observe Trypanosomes in the human erythrocytes in 1654 and named them as "*ruddy globules*". They were first accurately described in human blood in 1674 by the Dutch microbiologist, Leeuwenhoek. However, the functional significance on red cells was appreciated only when Hoppe-Seyler demonstrated that hemoglobin has the property of readily taking up and discharging oxygen (Wintrobe, 1981). Red blood cells are the principal means of delivering oxygen (O_2) to the body tissues via the blood flow through the circulatory system in vertebrates. Abnormal red blood cells are commonly reported in trypanosome infected fishes (Tandon and Joshi, 1973). Nuclear fragments, basket cells and cell casts increase in infected fish stimulating cell fragility (Joshi and Dabral, 1981). The red cells show a bizarre condition of the disintegrating nucleus. The lifespan of the cell may decrease resulting in erythropenia.

MATERIALS AND METHODS

Live fish (*Clarias batrachus*, *Channa punctatus* and *Wallago attu*) were brought to the laboratory, allowed to rest in order to recover from the stress caused by transportation and maintained in glass aquarium under proper conditions of food and aeration. Care was taken to select the experimental fish of the same size and age. Blood was collected from live fish from the caudal vein using 5-ml heparinized syringes. The sampled fish were reverted back into the aquaria. Infection was confirmed by detecting live, wriggling parasites (hanging drop preparations) or in fixed (methanol 5 mins) and stained (Giemsa + buffer pH 7.2 for 30 mins) preparations observed under 40X and oil immersion for the confirmation of species. The negative fish were treated as 'control' and the positive as 'infected'.
Total Erythrocyte Count is the number of red cells per volume of blood. The preparations for TEC in infected and non-infected blood were made by standard clinical methods using Neubauer's Haemocytometer. Hayem's fluid was used as the diluting fluid. The values are expressed in per cu mm of blood.

RESULTS

For evaluating TEC in fish due to trypanosome invasion, the following host-parasite systems were taken into consideration:

Host-Parasite systems

Parasite	Host	Host site	Group
Trypanosoma batrachi Qadri, 1962	*Clarias batrachus*	Aligarh	IA Control IB Infected
Trypanosoma aligaricus Gupta and Jairajpuri, 1982	*Channa punctatus*	Aligarh	IIA Control IIB Infected
Trypanosoma attii Gupta and Jairajpuri, 1982	*Wallago attu*	Aligarh	IIIA Control IIIB Infected

Group I.
Parasite: ***Trypanosoma batrachi* Qadri, 1962**
Host: ***Clarias batrachus***

Group II.
Parasite: ***Trypanosoma aligaricus* Gupta and Jairajpuri, 1982**
Host : ***Channa punctatus***

Clarias batrachus and *Channa punctatus* collected from Chautal pond, Aligarh were transported alive to the laboratory and processed for examination as mentioned in 'Materials and Methods'.

The blood of *Clarias batrachus* revealed the presence of *Trypanosoma batrachi* Qadri, 1962 and that of *Channa punctatus*, *Trypanosoma aligaricus* Gupta and Jairajpuri, 1982**.** TEC was lower in both the species of infected fishes as compared to the values in uninfected fish. The TEC value of 2.81 $\pm$ 0.16 X 10^6/c mm in uninfected *Clarias* declined to 2.01 $\pm$ 0.20 X 10^6/c mm in trypanosome-infected fish indicating a 28.46% fall whereas that of *Channa*, it fell from the value of 2.41 $\pm$ 0.31 X 10^6/c mm in uninfected fish to 1.61 $\pm$ 0.23 X 10^6/c mm in trypanosome-infected fish indicating a 33.19% fall.

Group III.
Parasite: ***Trypanosoma attii Gupta* and Jairajpuri, 1982**
Host: ***Wallago attu***

Wallago attu (25 - 36 cms) were collected from the freshwater ponds in and around Aligarh. They were subsequently maintained in tanks (100 X 80 X 50 cms) and the water was kept well aerated. The fish were held for one month prior to experimental infection and during this period their blood was examined for trypanosomes. The lethargic and apparently diseased fish were separated and the physically damaged or abnormal fish were discarded. Microscopic scan on the blood of 50 fish revealed active, wriggling trypanosomes identified as *Trypanosoma attii* Gupta and Jairajpuri, 1982 in 15 apparently lethargic and diseased fish at intensities ranging from 3 -15 forms / 20 minutes scan (low) to 15 - 24 forms/ 20 minutes scan (high).

TEC values fell from 2.48 $\pm$ 1.10 X 10^6 / c mm to 1.69 $\pm$ 0.90 x 10^6/ c mm in Group IIIB at day 30 p.i. The decline in TEC was 31.85% (Table 1). The flagellate-infected films also showed various abnormal erythrocyte cells [vacuolated (Fig. 1A), and punctuated erythrocytes, macrocytes, and enucleated erythrocytes showing karyorrhexis and cell death (Fig. 1B,C)].

The maximum fall in TEC was recorded in *Channa punctatus* infected with *T. aligaricus* (33.19%) amongst the three species of fishes examined followed by *W. attu* (31.85%) and *Clarias batrachus* (28.46%).

DISCUSSION

Fish infected with blood parasites indicate decreased levels of TEC. Smirnova (1970) reported low TEC levels in infected *Lota lota.* Tandon and Joshi (1973) and Joshi (1989a) observed a decline in TEC content of *Clarias batrachus* infected with *Trypanosoma*. Joshi and Tandon (1980) reported the mean values of TEC in 10 species of infected and uninfected fish. Kumar *et al.* (1984) observed that TEC, haemoglobin (Hb) and Packed Cell Volume (PCV) declined in the diseased fish, *Schizothorax plaglostomus* infected with Black Spot Disease whereas Erythrocyte Sedimentation rate (ESR) and total leukocyte count (TLC) increased. TEC values fell by 35.0% in *Nemacheilus rupicola* (Joshi, 1985). Lowered blood values after infection of trypanosomes in hill-stream fish *Garra gotyla* has also been reported (Joshi, 1989b). The anemias, or other metabolic dysfunction among higher vertebrates is due to the metabolic strains following parasite infection. Sharma and Joshi (1991) reported 21.96% fall in TEC in *Garra gotyla*. Rauthan *et al.* (1995) studied TEC in a hillstream fish, *Barilius bendelisis* recording a low value of 2.0 $\pm$ 0.15 X 10^6/c mm in trypanosome infected fish as compared to 3.0 $\pm$ 0.40 X 10^6/c mm in uninfected ones. Saha *et al*. (1997) also reported a decline in TEC levels of *Heteropneustes fossilis* infected with *T. mukundi*. Gupta *et al*. (2001) recorded the TEC content in uninfected (Group A), *Trypanosoma* infected (Group B) and a condition of *Trypanosoma* + *Trypanoplasma* infection (Group C) in *Clarias batrachus*. They recorded a maximum change (24.05% fall) in TEC of Group C as compared to other indices examined (Hb-14.48% fall; TLC 14.22% rise; PCV-8.85% fall) indicating that TEC was the most vulnerable haematological component to flagellate infection. Gupta *et al*. (2003) observed that the control values of 2.76 ± 0.04 x 10^6/c mm of blood (uninfected) decreased to 2.44 ± 0.03 x 10^6/c mm of blood (*T. piscidium*- infected) in *Colisa fasciatus* and again regarded the parasite to cause anemia in its host.

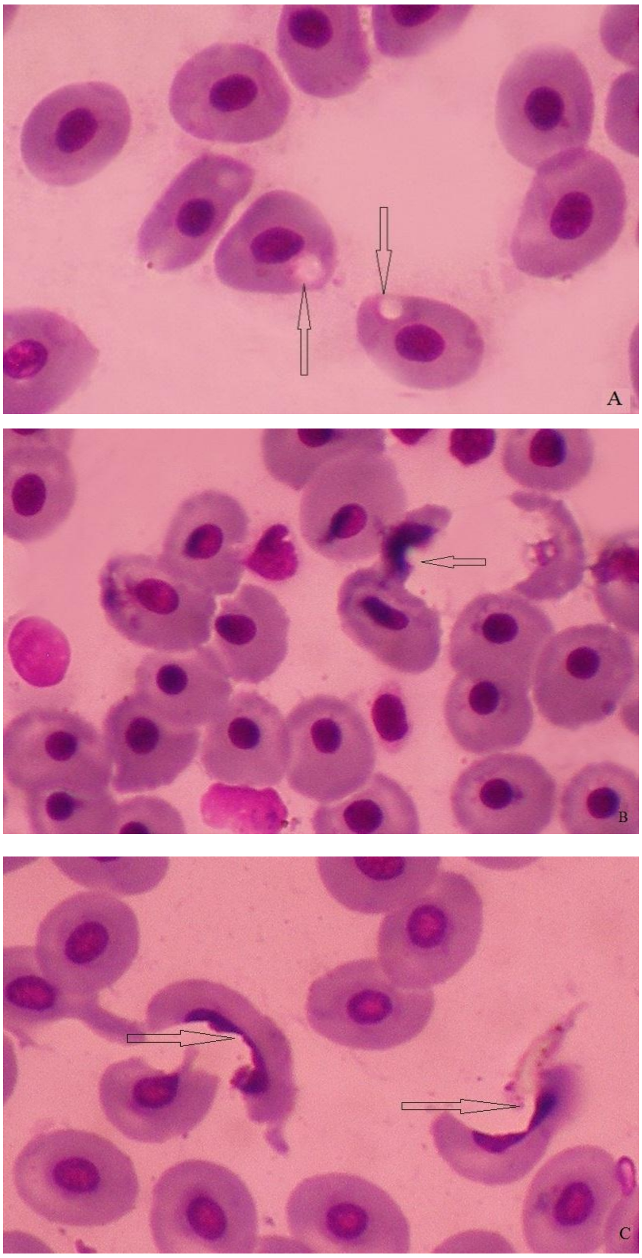
A controversy exists regarding the mechanism underlying the phenomenon. A reduction in TEC values indicates that the parasites are responsible for the destruction of erythrocytes and/or inadequate erythropoiesis by which the bone marrow is affected (Richardson and Kendall, 1957). The most arguable ones are the views of George *et al*. (1966) who assume phagocytosis of erythrocytes in the spleen of mammals greatly increased leading to anemia. Others contend that immune factors, especially autoimmune are contributory (McGhee, 1964). Phagocytosis of erythrocytes in the spleen may be insufficiently increased or the toxic factors released by the parasites (Maegraith, 1969) may play a decisive role for the ensuing anemia. Evidence also suggests that at least some trypanosomes generate soluble material which is capable of causing very slow lysis and sometimes, due to rapid and lasting type of mobility of the flagellates in the circulating blood, direct trauma to the erythrocytes may cause haemolysis (von Brand, 1973). Trypanosomes probably lower the oxygen carrying capacity of the blood, hamper the normal red cell production and produce distorted cells.

The distortion of erythrocytes may occur due to the alteration in the haemoglobin orientation and crystallization of haemoglobin. Micro and macrocytic anemia was conspicuous in infected fish suggesting that these pathological manifestations are under the influence of various physio-pathological conditions as reported in higher animals too (Wintrobe, 1981). Poikilocytosis, common in infected fishes depends on factors like physiological adaptation, activity of fish, cell size and micro and macro environment directly influencing the osmotic fragility of the cell. Factors for red cell destruction in fish infected with trypanosomes may lie within the red cell as well as in the plasma of infected animals (Yadav, 1990).

Islam and Woo (1991) postulated two factors to be responsible for causing anemia in gold fish which were correlated with trypanosome infection:

1. Secretion/excretion of haemolysin associated with acidosis and
2. Haemodilution.

Firdaus *et al*. (1994) were of the opinion that when TEC decreases, ESR rate increases and the volume of plasma also increases which indicates low plasma viscosity allowing the cells to sediment more acutely. The significant decrease in TEC even at low parasitaemia is symbolic of immediate pathological changes. Marked reduction of TEC and Hb values in high infection further accentuates the possibility of fish mortality due to acute anaemic conditions. It has been shown that TEC values also decline in *Panthera tigris* infected with trypanosomiasis (Singh *et al*., 2000). Such observations are in conformity with the findings of Tandon and Joshi (1973), Khan (1977) and Lom *et al*. (1986). The predominant or sole cause continues to be a debatable issue, but it can be almost confirmed that trypanosomes probably lower the oxygen carrying capacity of the blood. The flagellates may hamper the normal process of erythropoiesis thereby producing distorted cells. In the view of the above facts, the presence of the trypanosomes is found to be closely related to the physiology of the red blood cells, erythropenia being a frequent outcome as seen above.



Legends to figures

Figure 1. Photomicrograph of blood of Ts-infected *Wallago attu.*

A. Vacuolation of RBC's , B. Karyorrhexis of RBC, C. Cytolysis and cell death (X 1000)

Table1. Changes in Total Erythrocyte Count (TEC) in fresh water fishes infected with trypanosomes

Parasite	Host	Status of Host /Groups		TEC ($x10^6$/c mm)
Trypanosoma batrachi Qadri, 1962	*Clarias batrachus*	Control (10) Infected (10)	IA IB	2.81 ± 0.16 2.01 ± 0.20
		% Change		**(-) 28.46**
Trypanosoma aligaricus Gupta & Jairajpuri, 1982	*Channa punctatus*	Control (10) Infected (10)	IIA IIB	2.41 ± 0.31 1.61 ± 0.23
		% Change		**(-) 33.19**
Trypanosoma attii Gupta & Jairajpuri, 1982	*Wallago attu*	Control Infected	IIIA IIIB	2.48 ± 1.10 1.69 ± 0.90
		% Change		**(-) 31.85**

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