

Hematological Changes Associated with Epizootic Ulcerative Syndrome in the Asian Cichlid Fish, *Etroplus suratensis*

A. PATHIRATNE and W. RAJAPAKSHE

*Department of Zoology
University of Kelaniya
Kelaniya, Sri Lanka*

Abstract

Apparently healthy and epizootic ulcerative syndrome (EUS)-positive *Etroplus suratensis* collected from Hamilton Canal, Negombo, Sri Lanka, were subjected to a hematological examination. The hematological parameters studied were total erythrocyte count, total leucocyte count, hematocrit, hemoglobin content, mean erythrocyte volume, mean erythrocyte hemoglobin content, mean erythrocyte hemoglobin concentration and differential leucocyte count. Results indicate that severely affected fish were anemic as shown by significant reductions in total erythrocyte count, hematocrit and hemoglobin content. In addition, the differential leucocyte counts of severely affected fish indicate a significant increase in the percentage of neutrophils. The anemic condition can be attributed to the hemorrhagic lesions that resulted in blood loss in the severely affected fish. Increase in neutrophils may be a result of local inflammation and tissue damage due to severe EUS lesions.

Introduction

Epizootic ulcerative syndrome (EUS) is a serious fish disease which has been spreading across South and Southeast Asia since 1980. The disease is characterized by large hemorrhagic necrotizing ulcers extending deep into the tissues on a wide variety of wild and cultured fish species, leading most invariably to death (Roberts *et al.* 1986; Lilley *et al.* 1992). Aetiology of the outbreaks of EUS remains a mystery. Although viruses, bacteria and fungi have been isolated, prior to 1994, there has been no conclusive evidence of the involvement of any particular organism as the primary pathogen (Roberts *et al.* 1986; Roberts *et al.* 1992; Lilley *et al.* 1992; Roberts *et al.* 1993). However, more recent studies consistently associate the fungus *Aphanomyces* with the disease (Roberts *et al.* 1994; Vishwanath *et al.* 1997).

The disease was first noted in Sri Lanka in 1987, around the lower reaches of the Kelani River. The disease spread very rapidly within a number of waterbodies, affecting over 20 species of freshwater and brackishwater food fishes in the South-Western Zone (Costa and Wijeyaratne 1989). Since then, the disease has recurred annually, affecting a variety of fish species in a number of waterbodies in the Dry and Wet zones. The Pearl Spot, *Etroplus suratensis* (family:Cichlidae) is one of the food fish affected by EUS in Sri

Lanka (Pathiratne *et al.* 1994). This species is found in both freshwater and brackishwater habitats in India and Sri Lanka and is suitable for aquaculture (Costa 1983).

Hematological studies have been employed as aids in assessing the health of fish exposed to various diseases (Mulcahy 1975; Barham *et al.* 1980; Nair and Nair 1983; Hoffmann and Lommel 1984; Evenberg *et al.* 1986; Lowe-Jinde 1986). However, only a few studies have been conducted on the hematological profile of fish affected with EUS. In the present investigation, selected hematological parameters of the circulating blood of healthy *E. suratensis* were studied and compared with those of EUS-positive fish to evaluate the effects, if any, of EUS on the general hematological profile of this species.

Materials and Methods

Fish

E. suratensis of both sexes were collected from Hamilton Canal, Negombo, Sri Lanka, during an outbreak of EUS in that area in 1992-93. The fish were first examined for clinical signs of EUS or the presence of external parasites. Fish infested with the ectoparasitic copepod *Ergasilus ceylonensis* were eliminated. Fish affected with EUS had hemorrhagic fins and/or hemorrhagic lesions along the sides of the body. Apparently healthy and EUS-positive fish were brought to the laboratory in separate containers. EUS-positive fish were divided into two groups based on the severity of the lesions. Fish with slight hemorrhagic areas of scale loss were considered as mildly affected fish. Fish with hemorrhagic/ulcerative muscular lesions were considered as severely affected fish. Diseased and healthy fish were maintained separately in glass aquaria filled with water collected from their natural habitats prior to blood sampling. Apparently healthy *E. suratensis* were also collected from the same habitat 3-4 months after the EUS outbreak for blood sampling.

Blood sampling and hematology

The blood samples were taken by puncturing the caudal vessels of the fish. The blood was used immediately for hematological studies. Total erythrocyte and total leucocyte counts were made using modified Shaw's solution for dilution and improved Neubauer ruling hemocytometer for counting (Hesser 1960). Hematocrit values were obtained using heparinized micro-hematocrit tubes and centrifuging at 5,000 rpm for 5 min in a hematocrit centrifuge. Hemoglobin concentration was determined by the cyanohemoglobin method using diagnostic kits from Sigma Diagnostics, USA. Hematological indices, namely, the volume of a single erythrocyte (MEV), the hemoglobin content per single erythrocyte (MEH), and the mean hemoglobin concentration (MEHC) were estimated as described by Houston (1990). Blood smears, at two slides per fish, were prepared from fresh blood, air-dried and fixed in methanol and stained with Leishman-Giemsa stain or Sudan Black B (Houston 1990). In several visual fields at 1000x, all blood cells were identified as described by Hibiya (1982) and Houston (1990). For the differential leucocyte count, a total

of 100 leucocytes was observed from each stained smear and the relative abundance of different leucocytes was recorded.

Statistical analysis

Data are presented as mean $\pm$ SD of a number of fish per group. Hematological parameters were compared using one-way analysis of variance (ANOVA) and the differences between the mean values of the hematological parameters were compared using Scheffe's test (Zar 1984). The accepted level of significance was $P < 0.05$.

Results

In the present study, a total of 33 apparently healthy fish (73-110 g body weight) were sampled. Statistical analysis showed that the hematological profile of healthy fish collected during the EUS outbreak was not significantly different from those of the fish collected in the non-EUS period. In addition, no significant differences in hematological parameters were found between males and females. Therefore, individual hematological parameters of all healthy fish (irrespective of sex) were pooled in order to calculate the baseline values of the selected erythrocyte and leucocyte parameters for the apparently healthy *E. suratensis* collected during the study (Table 1).

Four types of leucocytes, namely, thrombocytes, lymphocytes, neutrophils and monocytes, were identified in the circulating blood of *E. suratensis* (Fig. 1). Majority of the leucocytes were thrombocytes. A thrombocyte was a spindle-, circular- or oval-shaped cell with a compact deep blue staining nucleus and a narrow pale blue staining cytoplasm. A lymphocyte was a circular or slightly oval shaped cell with a rounded or slightly oval nucleus. Almost the entire cell was occupied by the nucleus which was stained purple, surrounded by a narrow ring of cytoplasm stained blue with Leishman-Giemsa stain. The only

Table 1. Hematological parameters of the circulating blood of healthy *Etroplus suratensis* in the Hamilton canal, Sri Lanka.

Parameter	n	Mean	SD
Erythrocytes ($10^6 \cdot \text{mm}^{-3}$)	33	1.12	0.21
Hematocrit (%)	32	30.9	5.1
Hemoglobin ($\text{g} \cdot \text{dl}^{-1}$)	29	10.8	3.1
MEV (nm^3)	32	275	46
MEH (μg)	29	9.6	2.8
MEHC ($\text{g} \cdot \text{dl}^{-1}$)	29	34.9	6.2
Leucocytes ($10^4 \cdot \text{mm}^{-3}$)	4	4.51	0.19
Differential Leucocyte Count (%)	18		
Thrombocytes		57.5	10.6
Lymphocytes		28.3	9.6
Neutrophils		13.8	2.3
Monocytes		0.4	0.2

MEV - mean erythrocyte volume, MEH - mean erythrocyte hemoglobin, MEHC - mean erythrocyte hemoglobin concentration.

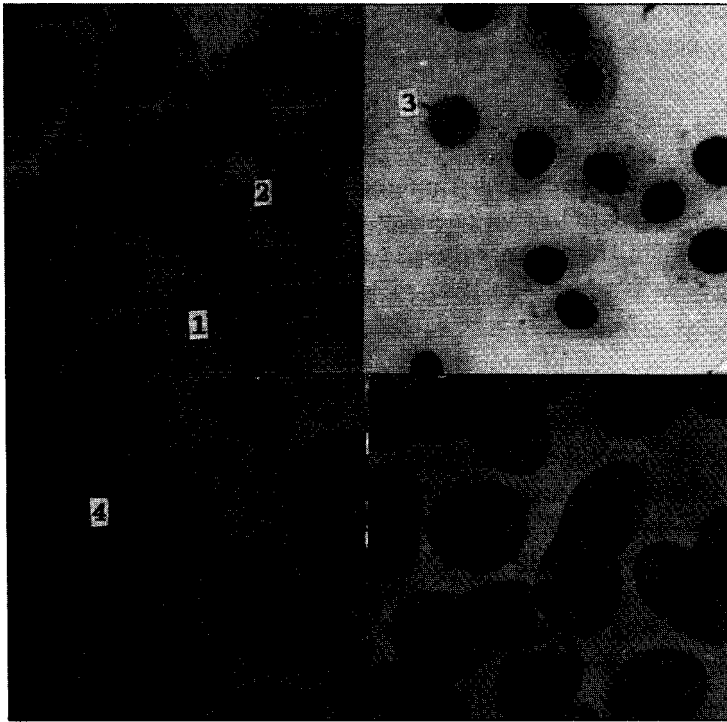


Fig. 1. Blood smear of *E. suratensis* (x 1000, Leishman-Giemsa stain) 1. erythrocyte; 2. thrombocyte; 3. lymphocyte; 4. neutrophil; 5. monocyte.

granulocyte seen in the circulating blood was the neutrophil. The cells were rounded or slightly oval in shape. Two forms of neutrophils were seen: immature and mature neutrophils. Immature neutrophils contained slightly indented or round nuclei which stained deep purple and light blue staining cytoplasm filled with granules. Mature neutrophils contained a bluish pink staining, lobulated nuclei and light blue or pink staining cytoplasm. Monocytes contained oval nuclei which stained bluish purple to deep blue with the Leishman-Giemsa stain and light blue staining cytoplasm occasionally containing cytoplasmic vacuoles. Monocyte-like cells were rarely seen in the blood of these fish.

EUS-affected fish sampled during the study period were divided into two groups based on the degree of the EUS lesions. The hematological parameters of these two groups were analyzed separately. Mildly affected fish (69-98 g, n=14) had slightly hemorrhagic areas with surface erosion of the skin. On the other hand, severely affected fish (75-116 g, n=11) had hemorrhagic necrotic muscular lesions on the body (Fig. 2). Comparison of erythrocyte parameters of healthy, mildly affected and severely affected fish are presented in Figure 3. The results show that the primary erythrocyte indices, namely, mean erythrocyte counts, hematocrit levels and hemoglobin concentration, were significantly lower in fish with severe lesions than in the mildly affected fish or healthy fish. However, derived erythrocyte indices, namely, the mean erythrocyte volume, mean erythrocyte hemoglobin level and mean erythrocyte hemoglobin concentration of the severely affected fish were not significantly different from those of the mildly affected fish or healthy fish. No significant differences were found between the mildly affected and healthy fish with regards to these erythrocyte parameters.

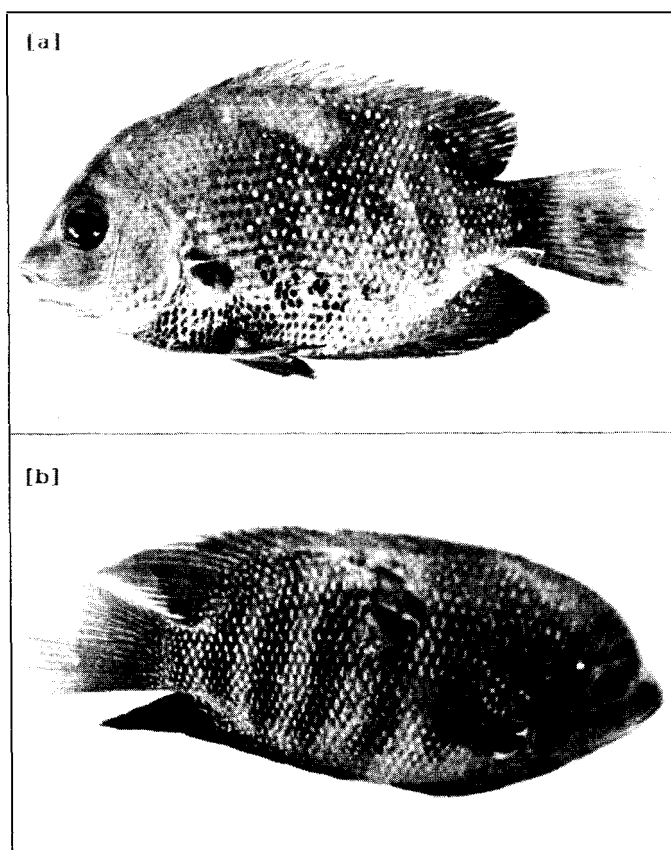


Fig. 2. (a) mildly affected *E. suratensis*; (b) *E. suratensis* with a severe EUS lesion.

Total leucocyte counts of the severely-affected fish were significantly higher than that in the healthy or mildly-affected fish (Fig. 4). Figure 5 presents a comparison of differential leucocyte counts of healthy fish and EUS-affected fish. Changes in the distribution of individual leucocytes in the severely affected fish were detected. There was a significant increase in the percentage of neutrophils when compared with the healthy fish and mildly affected fish. Percentage of lymphocytes present in the blood of severely affected fish was found to be lower than that in healthy fish. No significant changes in the levels of thrombocytes or monocytes were observed. Leucocyte parameters in the mildly affected fish were not found to be significantly different from those in the healthy fish.

Discussion

In the present study, the hematological profile of apparently healthy *E. suratensis* sampled during the EUS outbreak was comparable to that of fish collected during the non-EUS period. In addition, early stages of EUS had no significant effect on the hematological parameters studied, despite the occurrence of small hemorrhagic areas in these fish. However, advanced stages of EUS resulted in alterations of the general hematological profile.

The primary erythrocytic indices, namely, total erythrocyte count, haematocrit and haemoglobin concentration, were significantly reduced in

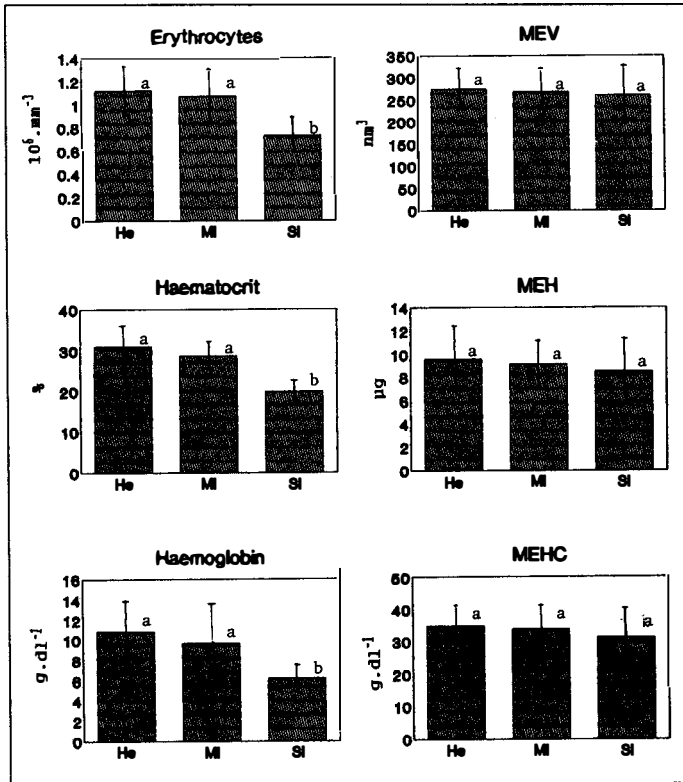


Fig. 3. Erythrocyte parameters in the blood of healthy and EUS-affected *E. suratensis*. He - healthy; Mi - mildly affected; Si - severely affected. Bars represent mean $\pm$ SD. Means with the same letter in a graph are not significantly different. $P \leq 0.05$.

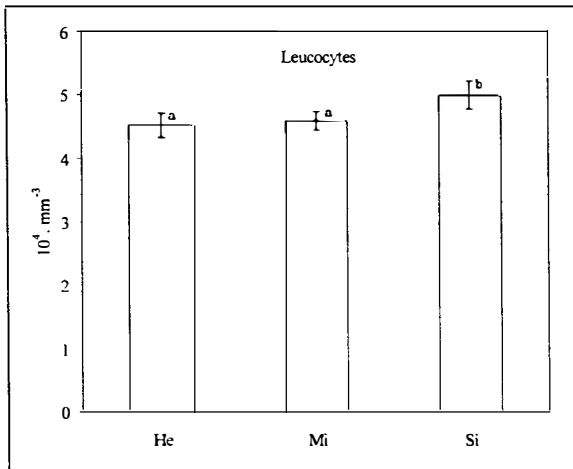


Fig. 4. Total leucocyte counts in the blood of healthy and EUS-affected *E. suratensis* (He = healthy, Mi = mildly affected, Si = severely affected). Bars represent mean $\pm$ SE. Means with the same letter are not significantly different ($P \leq 0.05$).

severely affected *E. suratensis*, leading to anemia. Anemia could be attributed to increased destruction or loss of erythrocytes and/or suppression of erythropoiesis. It may also be due to hemodilution caused by disturbed osmoregulation (Wedemeyer *et al.* 1990). If the erythrocyte destruction rate is increased without suppression of erythropoiesis, it may cause an increase in the proportion of immature and smaller erythrocytes. This should be reflected by a decline in the derived erythrocyte indices, MEV, MEH and MEHC, because younger and immature cells are smaller and contains less hemoglobin. However, derived erythrocytic indices did not change significantly in severely affected fish in the

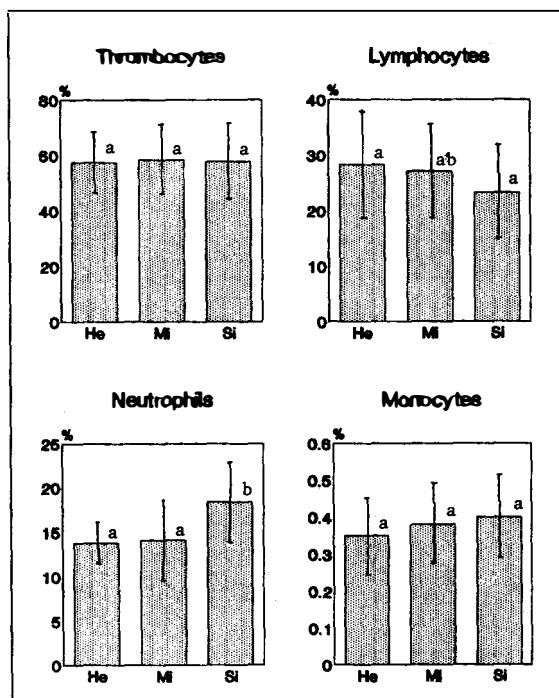


Fig. 5. Differential leucocyte counts in the blood of healthy and EUS-affected *E. suratensis*. He - healthy; Mi - mildly affected; Si - severely affected. Bars represent mean $\pm$ SD. Means with the same letter in a graph are not significantly different. $P \leq 0.05$.

present study. Therefore, the observed reductions in the primary erythrocyte indices in the severely affected fish may not be due to the increased proportion of immature erythrocytes. One reason for the anemic condition may be hemodilution which seems to be brought about by loss of body fluids from advanced hemorrhagic/necrotic lesions in severely affected fish. However, suppression of erythropoiesis in the severely affected fish cannot be ruled out. Decreased oxygen carrying capacity of the blood due to anemic condition may be a contributing factor in producing mortalities observed in *E. suratensis* with severe EUS lesions.

Anemia observed in severely affected *E. suratensis* is consistent with the previous report on EUS-affected snakeheads, *Ophicephalus striatus*. Decreased hemoglobin concentrations and hematocrit levels have been observed in *O. striatus* exhibiting advanced stages of EUS lesions collected from Laguna de Bay, Philippines. These have been attributed to hemodilution (Cruz-Lacierda and Shariff 1994). In Thailand, EUS-affected snakeheads collected during the 1984 outbreak showed significantly lower levels of hemoglobin as a result of extra- and intra-vascular destruction of erythrocytes (Tangtrongpiros *et al.* 1985).

Total leucocyte counts of the severely-affected fish indicate leucocytosis. Percentage distribution of different types of leucocytes in severely affected *E. suratensis* showed significant changes from the normal distribution pattern. The percentage of neutrophils was found to have increased significantly along with a marginal decrease in the percentage of lymphocytes. However, the percentages of thrombocytes and monocytes were unchanged. In fish, neutrophils may be involved in the cellular immune response and lymphocytes have been

regarded as immunocompetent cells involved in humoral immune response (Ellis *et al.* 1976). Numerous reports have described heterophil (neutrophil) migration to injury sites, such as those resulting from bacterial infection, parasitic infection and mechanical injury, in fish (Thorpe and Roberts 1972; Roberts *et al.* 1973; Jay and Jones 1973). Neutrophils have also been observed to be capable of phagocytic activity (Finn and Nielson 1971). The increased percentages of neutrophils in the circulating blood of *E. suratensis* with severe EUS lesions may be related to their cellular immune function as a response to the local inflammation and increasing damage to the tissues of these fish. The observed marginal decrease in lymphocytes may be associated with decreased humoral immune response in fish in the advanced stages of EUS.

In conclusion, advanced stages of EUS caused significant reductions in total erythrocyte count, hematocrit level and hemoglobin concentration and a significant increase in the percentage of neutrophils in the circulating blood of *E. suratensis*. Early stages of EUS had no significant effect on general hematological profile in these fish. More hematological studies on other species of EUS-affected fish are needed to characterize the hematological changes associated with EUS.

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