Haematology of experimental *Trypanosoma brucei rhodesiense* infection in vervet monkeys

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SUMMARY

Haematological aberrations associated with human infective trypanosomes were investigated in the vervet monkey model of the Rhodesian sleeping sickness. Four monkeys were infected intravenously with 10^4 *Trypanosoma brucei rhodesiense* and monitored for changes in the blood profile using a haematological analyser. A chronic infection lasting between 48 and 112 days was observed. Microcytic hypochromic anaemia, which was characterized by a decline in packed cell volume (PCV), red blood cell (RBC) numbers, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCH) developed at an early stage, and persisted throughout the infection. The mean platelet counts declined significantly from 3 x 10^5/μl (day 0 post infection) to 6.8 x 10^4/μl (day 7 post infection) and remained low in all the animals. However, the mean platelets volume rose during the course of the infection. An initial decline in total white blood cell (WBC) counts occurred between day 0 and 7 (3.1 x 10^6/μl) and remained low up to day 35 post infection (3.5 x 10^6/μl). This was followed by an increase in WBC counts, principally associated with increased lymphocyte numbers. It is concluded that microcytic hypochromic anaemia, thrombocytopaenia and an initial leucocytopaenia are the most important haematological changes associated with a chronic infection of *T.b. rhodesiense* infection in vervet monkeys.

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Introduction

African trypanosomosis is characterized by haematological changes, which drastically influence the pathogenesis of the disease. Although many studies on this phenomenon have been conducted in the past most of them have focussed on livestock trypanosomosis with only a few on human infections [1, 2]. The disease is associated with a rapid decline in red blood cell (RBC) counts, haemoglobin (Hb) concentration and packed cell volume (PCV) and pallor in mucous membranes in the infected hosts, confirming that anaemia is a critical feature in the pathogenesis of African trypanosomosis. In livestock, the anaemia has been typed as either normocytic normochromic, or macrocytic normochromic [1]. Thus, the ability of the erythropoietic system to continue to produce normal cells from the stem cell precursors is quite remarkable. Conversely, a number of studies on the anaemia caused by the human infective *T.b. rhodesiense* and *T.b. gambiense* in rodents have shown that it ranges between macrocytic normochromic to microcytic hypochromic anaemia [1]. The difference in the type of anaemia could be attributed to a number of factors, including the stage of disease, pathogenicity of trypanosomes, and host species. Although the use of red cell distribution width (RDW) has revolutionized the study of diseases affecting the erythropoetic system, similar work has not been carried out on human African trypanosomosis (HAT) [2]. There have been inconsistent reports on the pattern of leucocyte changes in African trypanosomosis. In livestock, most workers report a distinct leucocytopaenia, which correlates with neutrocytopaenia and lymphocytopaenia [1, 3]. However, leucocytosis was reported in *T. brucei* infections in deer mice and rabbits [4]. Thrombocytopenia is a significant feature of trypanosomosis, which has been observed in a number of studies [1, 5]. However, changes in morphology of the platelets during trypanosome infection have not been described. Serial changes in platelets volume can be a useful adjunct to the other blood parameters when determining the progression of a disease [6]. The vervet (*Chlorocebus aethiops*)

model of sleeping sickness provides a good opportunity to understand the pathogenesis of human infective *T.b. rhodesiense* on an animal species that is phylogenetically close to humans [7]. This model has been used to describe the various aspects of the disease, but only scanty information on haematology is available. Haematological information describing various aspects of the disease using this model has been scanty. Thus the current study was designed to evaluate the sequential haematological changes in vervet monkeys infected with *T.b. rhodesiense*.

Materials and Methods

Ethical review

All protocols and procedures used in the current study were reviewed and approved by the Trypanosomiasis Research Centre (TRC) Institutional Animal Care and Use Committee.

Animals

Four vervet monkeys (*Chlorocebus aethiops*) of both sexes (2 females and 2 males), weighing between 2.6-3.7 kgs were used. The monkeys caught from the wild were subjected to a 90-days quarantine period, in which they were screened for evidence of diseases including zoonotics, simian immunodeficiency virus, and helminthes. During this period, they were accustomed to handling and staying in squeeze-back steel cages. Prior to the study, the vervets were transferred to experimental wards and allowed to settle for another two weeks. They were fed twice daily with commercial monkey pellets, green maize, carrots, tomatoes, and bananas. Water was provided *ad libitum*. They were maintained at ambient temperatures of between 20 and 25^{o}C.

Trypanosomes

Trypanosoma brucei rhodesiense stabilate KETRI 3741, which was used in this study, was cloned from *T.b. rhodesiense* KETRI 2537. The latter was a derivative of EATRO 1989 that was isolated in Uganda from a human patient, by direct inoculation of the patient's blood and lymph node fluid into a monkey, and later cryo-preserved [8].

Experimental design

The animals were infected by intravenous injection with approximately 10^4 trypanosomes in one ml of phosphate saline glucose (PSG). Before and during the course of infection, a daily clinical evaluation was carried out. The parasitaemia levels were estimated using the rapid matching method described by Herbert and Lumsden [9]. The animals were anaesthetized at weekly intervals with Ketamine Hydrochloride (Rotexmedica, Trittau, Germany) at a dosage 10 mg/kg body weight and Diazepam (May and Baker, U.K.) at a dosage of 1 mg/kg body weight with weighing, and detailed examination undertaken. 1 ml of cerebrospinal fluid (CSF) was obtained by lumbar puncture and white cell count determined using the haemocytometer method. Ethylenediaminetetraacetic acid (EDTA) blood was collected by venipuncture of femoral vein. Detailed haematological analyses were conducted using an automated haematology analyser (Coulter A^{c}.T diff, Beckman coulter, Miami, USA). The packed cell volume (PCV) was determined using the standard micro-haematocrit method [1]. Data was analysed using the Statsview® statistical program. Significance of differences was determined by ANOVA and t-test using Statview for Windows Version 5.0.1 (SAS Institute Inc, 1995-1998, Cary, NC). $P<0.05$ was considered significant.

Results

Parasitaemia

The pre-patent period was 3 days. This was followed by a rise in parasitaemia to a peak at 6 days post infection (DPI). The parasitaemia dropped to undetectable levels (below antilog 5.4) between 9 and 10 DPI, followed by a second parasitaemia wave. Thereafter, the parasitaemia remained high, characterized by only minor fluctuations.

Clinical signs

All the animals developed classical symptoms of trypanosomosis, which included fever, raised hair coat, increased respiratory and pulse rates, pallor of mucous membranes, enlarged spleen and lymph nodes, increased aggressiveness and loss of weight. The 4 monkeys were euthanised *at extremis* on 48, 49, 70, and 113 DPI, respectively. Signs of late stage disease including hind leg paralysis, sleepiness, lethargy, and substantial increase (>20 cells/µl) in the cerebrospinal fluid white blood cell counts were observed in the vervet which died at 113 DPI.

Haematology

RBC count and haemoglobin (Hb) concentration (Fig. 1)

The infection was characterized by a decrease in RBC and Hb values such that by day 70 PI both values had significantly dropped by 45% ($p = 0.0002$) and 30% ($p<0.0001$) respectively. The rate of drop was lower in the animals with a more chronic infection.

Haematocrit (Fig.1)

The packed cell volume (PCV) (provided by the microhaematocrit method) and haematocrit (provided

by the haematology analyser) values from the single samples differed, with PCV values being always slightly (2-3 %) higher. There was a 40% drop in haematocrit between day 0 and 70 PI (p=0.0002).

Other red cell indices (Fig. 2 and 3)

The mean corpuscular volume (MCV) levels declined significantly (p=0.0287) between 0 and 63 DPI (Fig. 2) and this was followed by a slight increase, with fluctuations between day 70 and 84 PI. Thereafter, the MCV values declined significantly and were lowest at 98 DPI (p=0.0346). The red cell distribution width (RDW) increased significantly during the course of infection and being 21% (p<0.0001) at 49 DPI and 23.1% (p<0.0001) at 112 DPI. However, there was a decline between day 56 and 84 post infection. The mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values also decreased during the course of infection (Fig. 3). The most significant drops for MCH and MCHC was at *91 DPI (p=0.0016) and 98 DPI (p<0.0001), respectively*

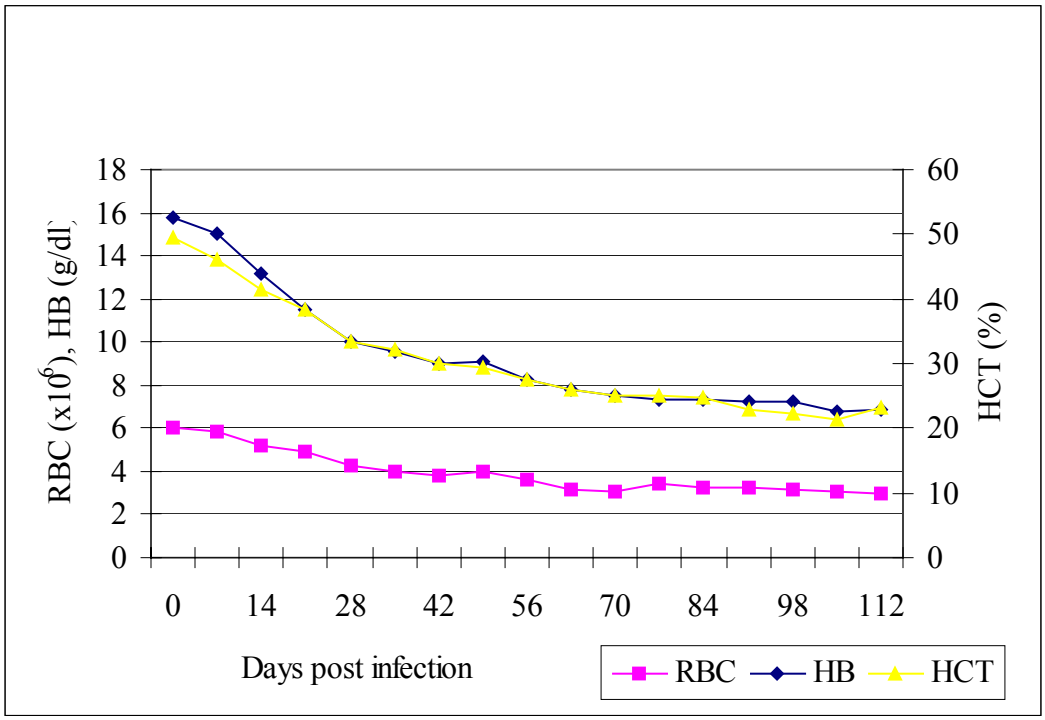
Platelets (Fig. 4)

The platelet counts declined significantly (p<0.0001) by day 7 PI. The platelets level remained low, rarely going above $1x10^5$ cells/µl, throughout the infection. In contrast, the volume of the platelets (MPV) rose and remained elevated during the infection, being most significant at 49 DPI (p<0.0001).

Leucocytes (Fig. 5)

There was a significant (p=0.0293) fall in total WBC count between day 0 and 7 PI, where after it remained low until 35 DPI. This was followed by a rise in WBC counts up to when the animals were euthanised. The difference between total WBC values at day 0 and 112 was not significant (p=0.7244). The changes in WBC were mainly due to lymphocyte counts, although between days 98 and 112 PI; the increase could also be attributed to both granulocytes and monocytes.

Fig 1. Mean RBC, HB and HCT changes in monkeys infected with *T.b. rhodesiense* KETRI 3741



Discussion

Infection of the monkeys with *T.b. rhodesiense* caused a disease that was acute in two monkeys and chronic in two other monkeys. A variation in pathogenicity of *T.b. rhodesiense* in human patients and animal models has been observed in other studies and is related to the relative innate immunity of different hosts [7, 8]. The present study illustrates the complex haematological changes associated with trypanosomosis. Previous studies using this model reported the occurrence of anaemia and leucocytopaenia without going in to details [8]. That the occurrence of anaemia in this model is one of the significant contributing factors that curtail the longevity of the disease course in the monkeys was evident in this study. The total RBC counts, Hb, and haematocrit, declined progressively, with the decline following a sigmoid pattern. The rate of decline was faster in the acutely affected monkeys, reflecting the inability of the erythropoiesis to overcome the effects of the trypanosomes. The pallor observed in the mucous membrane coincided with the decline in these values.

Red cell indices i.e., MCV, MCH, MCHC, and RDW are used to determine the type of anaemia [2]. Microcytic hypochromic type of anaemia was observed in the early stages of the disease and was characterized by dramatic decline in MCV and a slight decline in MCH and MCHC. However, as the disease progressed, the MCV values increased although they were characterised by fluctuations. The consistent increase in RDW in the present study implies that there was an increase in variation of red cells released by the bone marrow as the disease progressed, although the decline in RDW between days 56-84 PI, could indicate a temporary recovery. Microcytic hypochromic anaemia has also been reported in rabbits infected with *T.b. gambiense* [4]. However, in the latter an initial macrocytosis was observed [4]. The microcytic hypochromic type of anaemia has previously been associated with iron deficiency [10]. It is possible that during *T.b. rhodesiense* infection, failure of iron incorporation into red cell precursors even in presence of adequate iron storage will precipitate the occurrence of this type of anaemia [11]. Inefficient recovery of iron from the phagocytosed RBC can also lead to an iron "deficiency" status in the body [11]. In such a case, it will be imperative to consider evaluating level of iron stores in the body.

The significant fall in platelets counts soon after trypanosome infection in this study has also been reported by other investigators [1, 12]. In contrast, the volume of the platelets (MPV) rose significantly and remained elevated during the whole infection period. An increase in MPV is associated with an increased growth of megarkaryocytes in response to thrombopoetic stress especially where there is peripheral destruction of platelets [13]. In this study, the low platelet count combined with increasing platelets volume could be indicative of hyper-destruction of platelets by toxic products emanating from the trypanosomes [6]. Low platelet counts could also be due to other factors which include: pooling of blood in the spleen, removal of platelets by mononuclear phagocytic system and increased 'consumption' of platelets by disseminated intravascular coagulation reaction which have been widely reported in trypanosomosis infections [12]. In studies on white blood cells in trypanosomosis, there are reports of distinct leucocytopaenia following infection [1,14].

In the current study, low leucocyte counts were observed between day 7 and 35 PI, followed by a consistent rise thereafter. As in other studies, the pattern of white blood cells strongly coincided with that of lymphocytes, showing that the latter plays active role in immunopathogenesis of trypanosomosis. The rise in lymphocyte counts after 35 DPI can be regarded as a good prognostic sign, although its effectiveness in limiting disease pathogenicity is doubtful. The neutrocytopenia observed during the course of infection is thought to increase susceptibility of infected animals to concurrent infections [1]. The occurrence of leucopaenia has been attributed to factors such as leucophagocytosis as a result of trypanosomal antigen coating of leucocytes and depression of leucocyte production [15]. In other trypanosomosis studies, leucocytosis has been observed in rabbits and trypanotolerant breeds of animals [4, 16]. Thus, the leucocyte response in trypanosomosis is mainly determined by the stage of disease, trypanosome species, and host involved.

The significant fall in both platelets and leucocytes by 7 DPI coincided with the first peak in parasitaemia, showing that the drop could be related to increased levels of biological products generated by the trypanosomes. The association of peak parasitaemia and other parameters, including fever with IFN-gamma production, have been reported in this monkey model [17]. It would be important to examine the effect IFN-gamma and other cytokines have on the haematopoetic system.

Fig 2. Mean MCV and RDW of monkeys infected with *T.b. rhodesiense* KETRI 3741

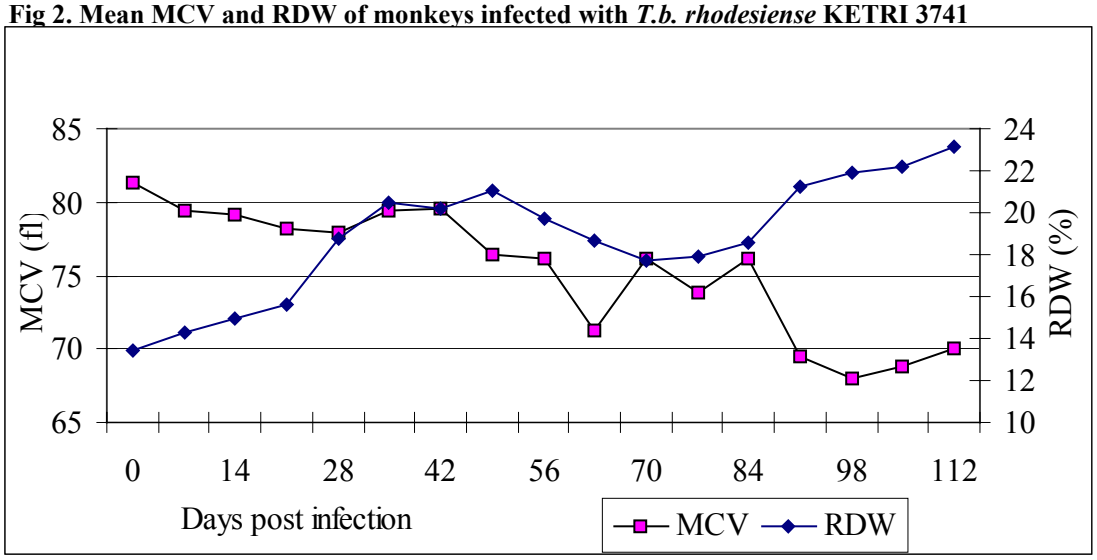


Fig. 3. Mean MCH and MCHC in vervet monkeys infected with *T.b. rhodesiense* KETRI 3741

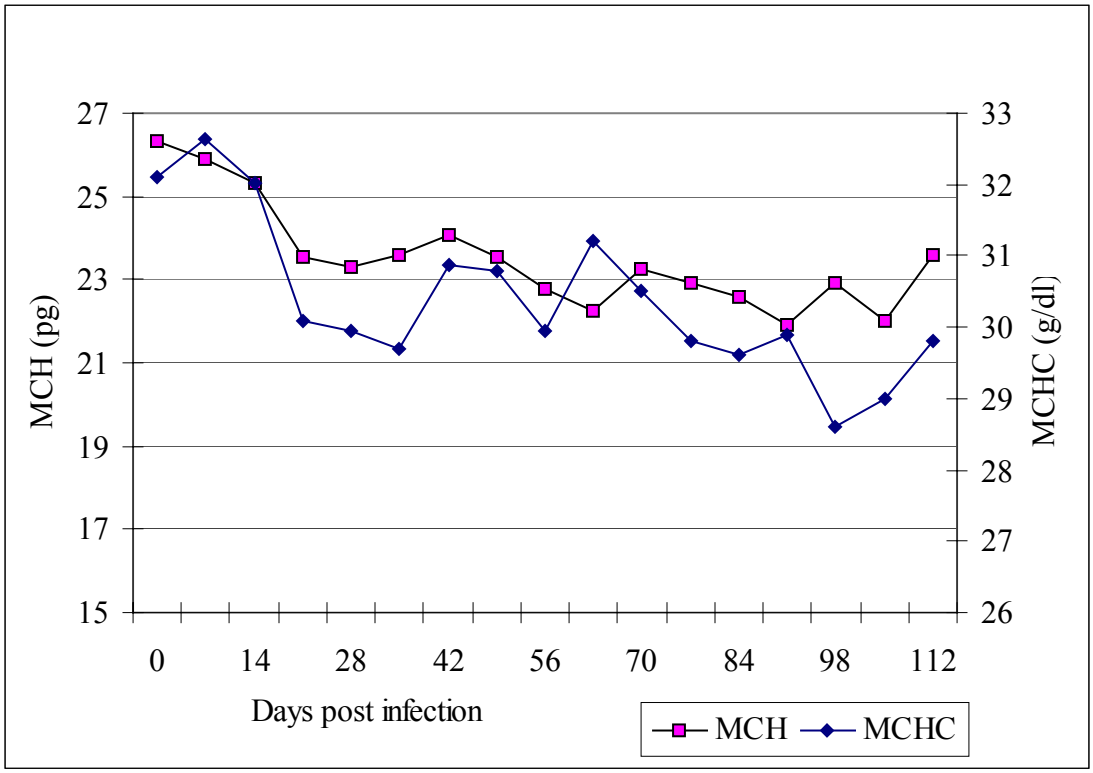


Fig 4. Platelets changes in monkeys infected with *T.b. rhodesiense* KETRI 3741

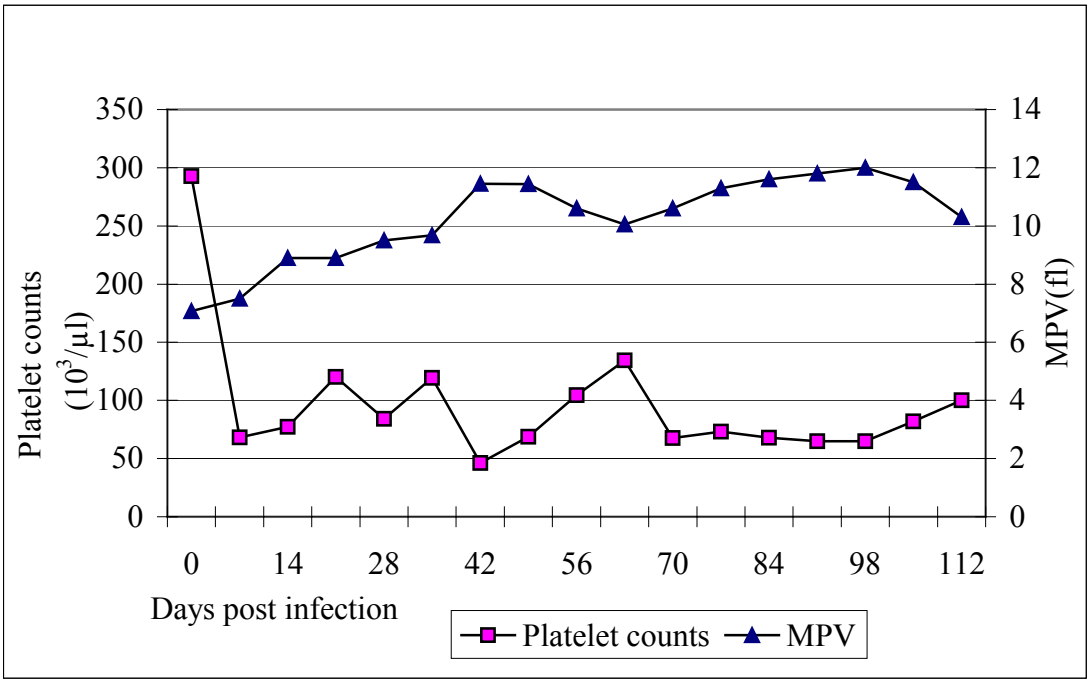
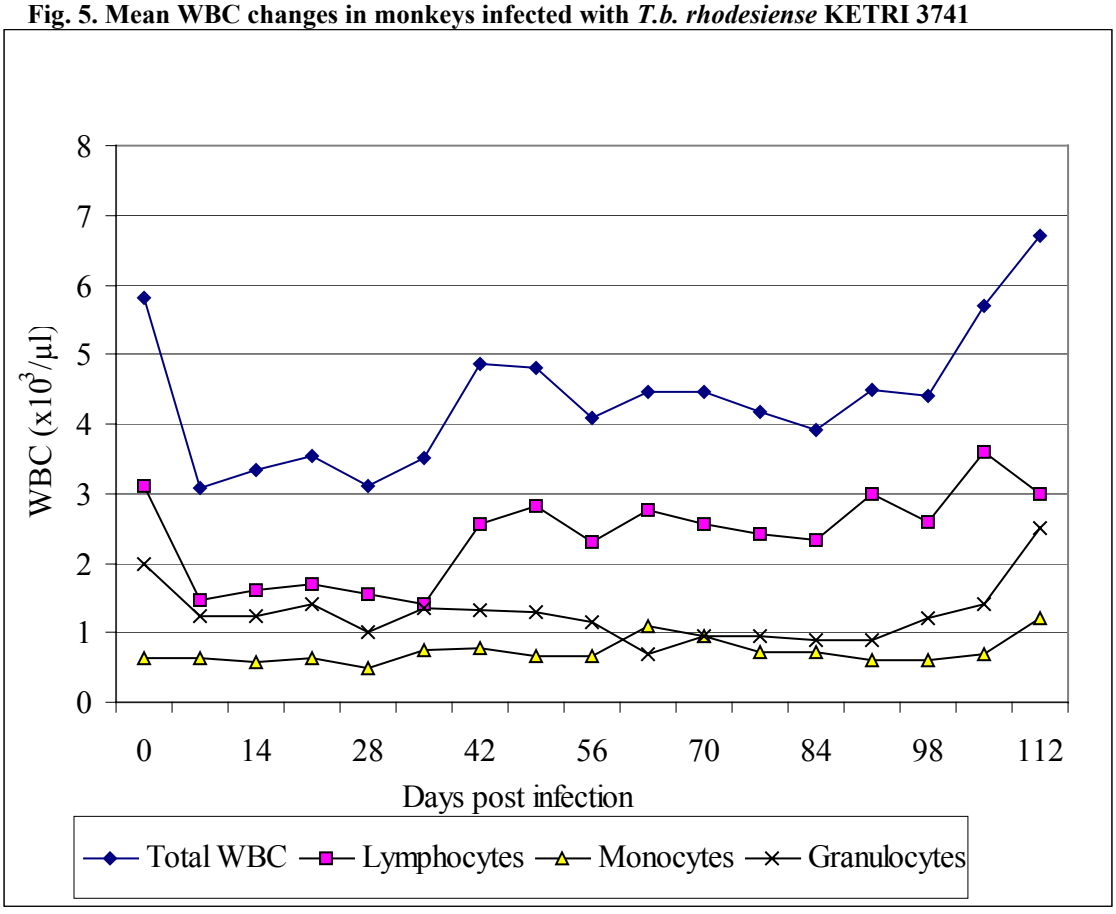


Fig. 5. Mean WBC changes in monkeys infected with *T.b. rhodesiense* KETRI 3741



Conclusion

The current study has shown that a *T.b. rhodesiense* infection in the vervet monkey model of HAT leads to development of severe microcytic hypochromic anaemia, thrombocytopaenia and leucocytopaenia, although the latter improves as the disease progresses. To our knowledge, this is the first time that RDW and MPV have been used to describe changes in size and volume of RBC and platelets respectively.

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