



Renal Integrity Probably Determines Tolerance to Infection with *Trypanosoma congolense* in Rats and Natural Hosts

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Authors' contributions

This work was carried out in collaboration between all authors. Author JNA designed the study, wrote the protocol and interpreted the data. Author ETA anchored the field study, gathered the initial data and performed preliminary data analysis. Authors JNA and COE managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The research was carried out to determine the role of renal function in susceptibility to anemia in rats infected with *Trypanosoma congolense*.

Study Design: The study utilized a total of twenty-four (24) male albino rats, divided into two groups of eight (8) albino rats as control group and 16 albino rats to serve as infected group, inoculated intraperitoneally with *T. congolense* (1×10^3 parasites).

Place and Duration of Study: The study was carried out in the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

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Methodology: The study involved determination of parasitaemia, post infection derangements in hematology and serum urea and creatinine levels of rats.

Results: *T. congolense* precipitated an acute, severe and fatal infection in rats characterized by, anemia, and renal dysfunction, leading to death. At the end of 2 weeks post infection (PI), anemia characterized by drop in the packed cell volume (PCV), was found to be significantly higher in rats infected with *T. congolense*, compared to the rats in the control group. The PCV of control rats fluctuated within normal range.

Serum biochemical changes included elevation in levels of blood urea nitrogen (BUN) and creatinine. The anemia was severer in rats with higher increases in BUN and creatinine levels.

Conclusion: The study points to early development of renal pathology and subnormal erythropoietin production as some of the important factors behind dyserythropoiesis and crippling effect of anemia in *T. congolense* infections. Early renal pathology may be an important antigenic property that determines severity of anemia and tolerance in *Trypanosoma congolense* infections.

Keywords: Renal pathology; anemia; *Trypanosoma congolense*; rats.

1. INTRODUCTION

Trypanosomes cause severe anemia in both humans and animals, the severity of which is dependent on many factors including specie of infecting parasite [1,2]. Ability to resist anemia has been identified as one of the hallmarks of trypanotolerance [3]. Trypanosomes have been divided into two groups namely, the hematic or intravascular groups made up of *Trypanosoma vivax* and *T. congolense* and, the humoral or extravascular group which is made up of the *T. brucei* sub-group. This sub-group is in turn made up of the animal infective *T. brucei brucei*, and human infective *T. b. gambiense* and *T. b. rhodesiense* [4]. Earlier finding of extravascular development of *T. congolense* suggests, it interphases between these two groups [5]. Trypanosomosis due to *T. congolense* is endemic in several parts of sub-Saharan Africa including Nigeria [6,7]. As a member of the hematic group, the parasite causes primary lesions in the blood, blood vessels, lymphoid system as against those in the humoral group which produces lower parasitaemia and cause primary lesions in solid tissues [4,8]. The most important hematological changes of the disease include anemia, leukopenia and thrombocytopenia [1]. Trypanosomes may also cause some biochemical changes in blood of both laboratory and domestic animals as well as that of humans [9].

The mechanism of anemia in trypanosomosis is complex and multifactorial in origin [3], some of which had been described by Anosa [1]. These include hemolysis, hemorrhage and dyshaematopoiesis. *T. congolense* causes infection leading to severe anemia in cattle, sheep, goats, pigs and horses. Nok and Balogun

[10] demonstrated the presence of sialidases in *T. congolense* infected mice and reported its roles in the pathogenesis of anemia in African trypanosomiasis through cleavage of sialic acid from the erythrocyte surfaces of infected animals with subsequent erythrophagocytosis.

Dyserythropoiesis, a component of dyshaematopoiesis is a phenomenon in which the haemopoietic organs are unable to produce enough erythrocytes to match the rate of erythrocyte loss [11,12]. Although the liver and kidneys play critical roles in erythropoiesis and their roles in biosynthesis of erythropoietin are expected to be compromised in the pathology of these organs arising from trypanosome infections, there has not been sufficient investigation on their roles in the timing of onset of dyserythropoiesis. This has impacted negatively on management of anemia, a critical clinical feature of trypanosomosis and success of chemotherapy.

The aim of this research was to evaluate the role of renal pathology in anemic grades and possible determination of tolerance in *T. congolense* infected rat model. This is to also provide additional data that would help in the success of early stage chemotherapy of trypanosomiasis in animals.

2. MATERIALS AND METHODS

A total of twenty four (24) male albino rats weighing an average of 200 ± 50 g were used for this study. The animals were obtained from the Small Animal House of Department of Biological Science, Ahmadu Bello University, Zaria, and then, separated randomly into three cages of eight rats each, where they were kept for two

weeks before the start of the experiment. The animals were housed under standard conditions of temperature; humidity and 12 hour light (7.00 am - 7.00 pm). The cages were constantly cleaned in order to prevent the animals from contracting disease. They were fed with standard commercial rat cubes and given water *ad libitum*. The animals were divided into two experimental groups as follows:

- Group One: This consisted of eight (8) albino rats. They were treated with normal saline and served as the control group.
- Group Two: This consisted of 16 albino rats. They were infected intraperitoneally with *T. congolense* (1×10^3 parasites) obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Vom – Jos, Nigeria. The parasite was characterized by Centre for Biotechnology, Ahmadu Bello University, Zaria. Parasitaemia was monitored by wet mount preparations of blood obtained from animal tail and viewing under light microscope at x40 magnification.

Commercial heparinized capillary tubes were $\frac{3}{4}$ filled with blood collected from the tail vein, sealed on one end with hot flame and centrifuged for five minutes in a microhaematocrit centrifuge at 7,000 rpm. The packed cell volume (PCV) was read off the microhaematocrit reader as described [13].

At the peak of parasitaemia, 11-15 days post infection (PI), the animals were given painless chemical euthanasia and blood collected for hemogram and biochemical analysis. Blood for hemogram was collected into commercial sample bottles containing EDTA while blood for biochemical analysis was collected into commercial sample bottles without EDTA.

The blood collected for biochemical analysis was kept at room temperature for 3-4 hours after which it was centrifuged with the use of clinical centrifuge and serum separated as described by Sood [13]. The serum samples were preserved in the freezer at -20°C until analysis was done.

The erythrocyte values were enumerated using the Neubauer haemocytometer using standard procedures as described [13].

Serum biochemical parameters, creatinine and blood urea nitrogen (BUN) were analyzed using

Kodak DT60 auto-analyzer (Eastman Kodak Co, New York).

2.1 Data Analysis

The data collected was statistically analyzed to test the significant differences in various groups using, t- test, Microsoft Excel 2010. In all cases values of $P < 0.05$ were considered significant.

3. RESULTS

Parasites were first detected in the tail blood of rats infected with *T. congolense*, 5 to 7 days post infection. At the end of the second week, the other rats were given painless chemical euthanasia for necropsy. *T. congolense* infected group showed a slight increase in daily PCV from pre-infection value of $42.1 \pm 0.9\%$ to $44.1 \pm 1.0\%$ by the 4th day but thereafter dropped progressively to $33.2 \pm 1.0\%$ on day 15 ($P < 0.05$., Fig. 1). There was little or no decrease in the mean daily PCV of the rats in the control group from day 0 to day 15.

There was a significantly lower RBC counts ($P < 0.05$) of the infected group compared to that of the control group (Fig. 2). The total WBC counts of the infected group was however only slightly lower than that of the control group. Absolute differential WBC counts of infected and control rats were as shown in Table 1.

Table 1. Summary of changes in absolute differential WBC ($\times 10^9/L$ of blood) of control and infected rats at 15days PI

	Control rats N=8	Infected rats N=16
Neutrophils ($\times 10^9/L$)	1.3 ± 0.1	0.8 ± 0.0
Lymphocytes ($\times 10^9/L$)	6.6 ± 1.0	6.2 ± 0.9
Monocytes ($\times 10^9/L$)	0.1 ± 0.0	0.3 ± 0.0
Eosinophils ($\times 10^9/L$)	0.0 ± 0.0	0.1 ± 0.0

The BUN concentration of the *T. congolense* infected group was about three and a half times higher than that of the control group (Fig. 3) while that of serum creatinine levels was about three times higher than that of the control group (Fig. 4).

The effect of kidney function on anemia grades in *T. congolense* infected rats is as shown in Table 2. The urea and creatinine concentrations of the rats with PCV of less than 30% were

higher when compared to those of rats having PCV higher than 30%.

4. DISCUSSION

The strain of *T. congolense* used in this study showed marked pathogenicity in albino rats with parasitaemia developing five to seven days post infection (PI), an observation consistent with previous findings in *T. congolense* infection of mice and rats [10,14] respectively in which *T.*

congolense produced acute disease from parasitaemia which developed within a few days and peaked 2 to 3 weeks P.I. *T. congolense* infected albino rats exhibited more severe hematological changes here and were characterized by marked drop in the PCV and RBC counts. The susceptibility to anemia in *T. congolense* infection also did not differ from the pattern previously observed in *T. congolense* [10, 14] infections.

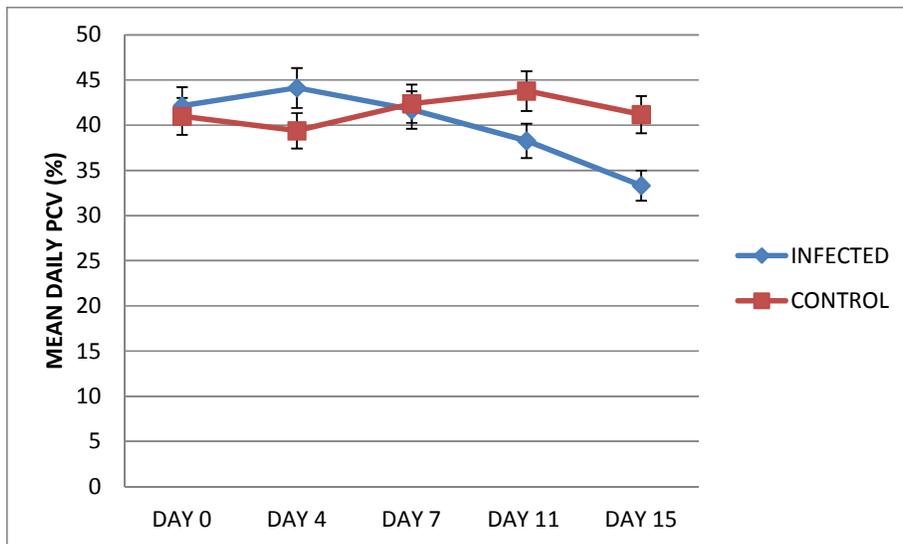


Fig. 1. Packed cell volume (%) of control and infected rats

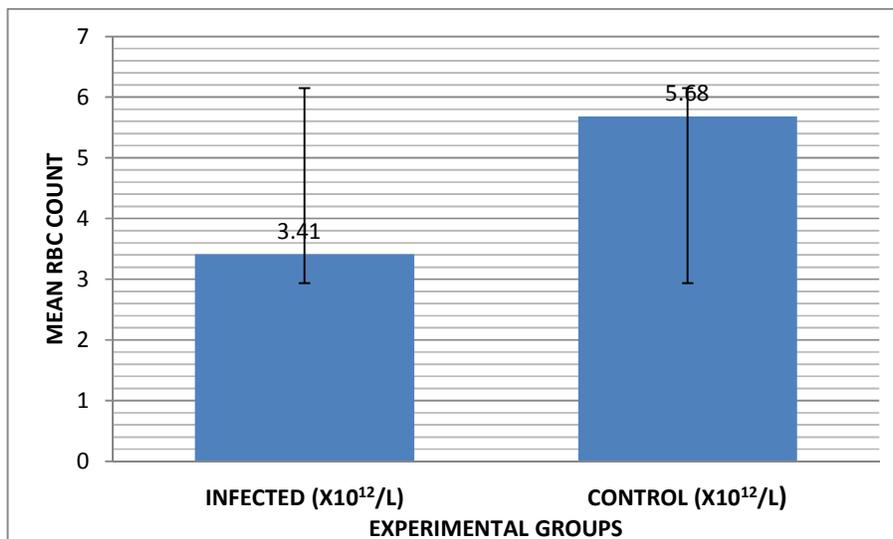


Fig. 2. Red blood cell count ($\times 10^{12}/L$) of control and infected rats

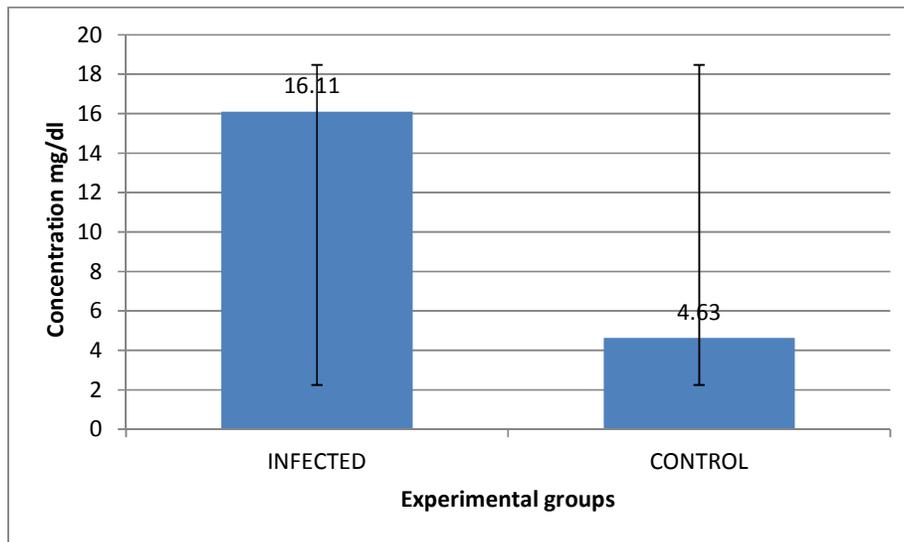


Fig. 3. Urea concentration (mg/dl) of control and infected rats

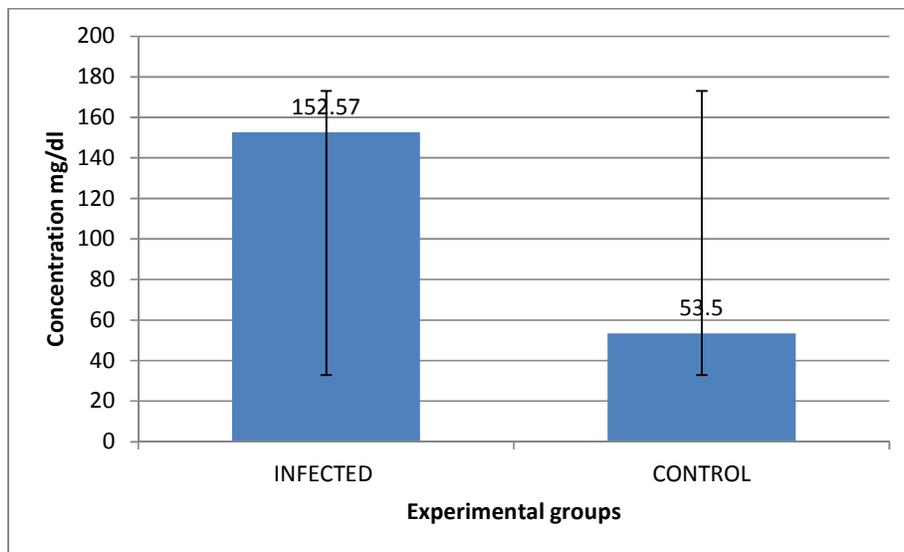


Fig. 4. Creatinine concentration (mg/dl) of control and infected rats

Table 2. Effect of kidney function on anemic grades in *T. congolense* infected rats

Anemic grade	Blood urea nitrogen (mg/dl)	Creatinine concentration (mg/dl)
PCV ≤ 30%	16.7 ± 7.4	156 ± 70.7
PCV > 30%	15.9 ± 4.8	151.2 ± 46.3

This was characterized by a drop in PCV and RBC counts which was mild to severe in nature. Factors involved in anemia in all stages of the *T. congolense* infection had been identified which

include, intravascular hemolysis, phagocytosis [1] and oxidative damage as a result of reduced glutathione on the membrane of the red cells. Leukocyte values of the rats were also determined to provide additional hematological values of the rats. Leucocytopenia was also a general feature in *T. congolense* infected rats. This was characterized by lymphopenia, neutropenia, eosinopenia and monocytosis which is consistent with features of pancytopenia inherent in trypanosomiasis [1]. The fall in lymphocyte numbers suggests that *T. congolense* infection caused marked antigenic

stimulation leading to accelerated transformation of lymphocytes to plasma cells and transferred lymphocyte resulting to lymphopenia. Similarly, marked depression of precursor cells and marked phagocytosis of neutrophil precursors in the bone marrow and spleen may have been responsible for the fall in neutrophil numbers in the *T. congolense* infected rats.

The biochemical parameters indicated here pointed to kidney damage in the *T. congolense* infected albino rats [9]. Increases in serum creatinine and urea concentrations observed here presumably arose from impaired glomerular filtration in association with trypanosome induced renal pathology. Whereas, creatinine usually passes through the glomerular filtrate without reabsorption, any impairments in glomerular filtration increases its concentration in circulation. Increase in serum urea concentration had however been associated with not only kidney diseases such as glomerulonephritis and urinary tract obstruction but also with excessive protein catabolism in association with severe toxic and febrile conditions [9]. Fever and glomerulonephritis are consistent pathological features of African Trypanosomiasis [4]. Since the serum creatinine and urea concentrations of the *T. congolense* rats were remarkably higher than those of control rats, it is safe to surmise that there was renal pathology sufficient to have caused impaired erythropoietin biosynthesis.

The erythrocyte values of infected rats were grouped into two groups, those with a PCV < 30% and those with PCV>30%. The mean blood urea nitrogen and creatinine values were higher in the group of rats with PCV< 30% when compared with group of rats with PCV>30%. Although these findings by themselves do not conclusively suggest that the drop in PCV values in the *T. congolense* infected rats was due to a deficiency of the hormone, erythropoietin, they give credence to the assumption that subnormal erythropoietin production arising from renal pathology in *Trypanosoma congolense* infections of the rats played roles in severity of anemia and determination of trypanotolerance. These findings collaborate recent findings in the early phase of *T. brucei* infection in pigs [15].

5. CONCLUSION

In conclusion, the strain of *T. congolense* produced acute and fatal disease in the albino rats leading to death from anemia which was severer in rats with higher increase in serum

urea and creatinine levels. This suggests that early renal pathology may be an important antigenic property that determines severity of anemia in *Trypanosoma congolense* infections. These findings may find application in natural trypanosome infection of man. Since the kidney is known to play prominent roles in erythropoietin biosynthesis and erythropoiesis, further investigations are needed to establish its roles in trypanotolerance and the place of administration of exogenous erythropoietin as an option in reducing economic losses due to anemia in African trypanosomiasis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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