Effects of trypanosomosis on hemogram and some biochemical parameters of guinea pigs experimentally infected with *Trypanosoma brucei brucei* in Maiduguri, Nigeria

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Publication history: Received on 15 January 2019; revised on 25 March 2019; accepted on 02 April 2019

Article DOI: https://doi.org/10.30574/gscbps.2019.7.1.0007

Abstract

The study was designed to evaluate the effect of trypanosomosis on hemogram and some biochemical parameters of guinea pigs. Guinea pigs of both sexes weighing (5-10kg) were divided into six groups (A, B, C, D, E and F) with five guinea pigs in each group. At day zero, to establish the baseline data, all the animals in each of the six groups were bled for haematology and serum biochemistry and also clinical parameters (rectal temperature, respiratory rate, pulse rate and heart beats) were recorded while general body condition and physical signs were also evaluated. Groups A, B and C were intraperitoneally (IP) inoculated with 1×10^6 dose of *Trypanosoma brucei brucei* contained in 0.5 ml of blood. Thereafter, blood samples were collected every other four (4) days for evaluation of hematology and serum electrolytes through the experimental period. Group D, E and F was uninfected control. All the infected groups (A, B, and C) had a pre-patent period of 16 days with similar levels of parasitaemia of 45.7±3.38 across the groups. The observed clinical signs among the infected groups (A, B and C) were pyrexia, pale feet, snout, pinnae and mucous membrane, anaemia, dullness, emaciation and loss of weight. In group A, a mean parasitaemia of 2.8 ± 0.84 occurred by day 16 post-infection which continued to rise significantly without abating (p<0.05) to a peak count of 120.2 ± 5.48 by day 40 post infection. Similar findings was noticed across the groups. In groups D, E and F, their respective pre-infection RBC values of 6.20 ± 1.24, 6.24 ± 1.24 and 6.18 ± 1.24 remained fairly constant (p>0.05). Dose of Veriben® administered at the dose rate of 7.0 mg/kg and 3.5 mg/kg have the abilities of curbing the state of anaemia, immunosuppression, and serum electrolytes levels in trypanosome-infected guinea pigs placed on a dose dependent manner.

Keywords: Hemogram; Biochemical parameters; Trypanosomosis; Guinea pigs

1. Introduction

Trypanosomosis is one of the most important zoonotic disease commonly found in Africa regions and South America [1]. African trypanosomosis is caused by a protozoan parasite belonging to the genus *Trypanosoma*, and is transmitted by tsetse flies to the final host. The disease is characterised by high morbidity and mortality of infected livestock. Animal trypanosomosis has been estimated to cost Africa about US$ 4.5 billion per year [2]. Trypanosomosis affect almost all vertebrates particularly man and livestock. But wild animals such as Bovidae and suidae, act as asymptomatic carriers.

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[3]. Biological transmission (cyclic) of these parasites by tsetse fly (Glossina), is rampant in tsetse belt zone of Nigeria [3, 4]. While arthropod vectors, haematophagus, of the family Tabanidae, Hippoboscidiae, Stomoxynae Haematopta, lypeosia, and Chrysops species are used for the mechanical transmission of the parasite in the tropics [5, 6]. Tsetse flies are efficient transmitters of trypanosomes especially Trypanosoma vivax which develop in their mouth [7]. Transplacental transmission of trypanosomes has been reported in cattle [8]. Trypanosoma vivax, T. congolense and T. brucet have been reported to cause Nagana in cattle, while T. evansi caused surra in camels (Camelus dromedarius) [9]. Trypanosoma brucei gambiens and T. brucei rhodesiens are responsible for human sleeping sickness in East and West Africa countries respectively, while T. cruzi transmitted by triatomid bugs (Triatonia Magista) is responsible for causing chagas disease in humans in South America [10]. The trypanosomes group of T. brucei, T. b. gambiens, T. b. rhodesiens and T. evansi are more of tissue invading (humoral) parasites whereas, T. congolense, T. vivax and T. cruzi are restricted as hemoparasites (blood circulation parasites) or (haemical) [11, 12]. Animal trypanosomosis is lethal if left untreated. It causes severe losses in livestock industries as a result of poor growth, weight loss, and low milk yield, poor capacity to work, infertility and abortion have been reported in low levels of infection [3]. Control of the disease therefore is significant through hematological and biochemical evaluation that can lead to a reliable diagnosis that can guarantee optimistic treatment that will restore production in endemic areas and also boost livestock production industries.

2. Material and methods

2.1. Source of Trypanosoma brucei brucei

The trypanosoma parasites used for the study was “Federe” strain of Trypanosoma brucei brucei, which was obtained from NITOR (Nigerian Institute of Trypanosome and Onchocerciasis) Kaduna State, Nigeria. The organism was isolated from an outbreak of bovine trypanosomosis in Nassarawa State of Nigeria. It was identified based on its morphology and negative blood inhibition and infectivity test and was stabilized by four passage in rats before storage in liquid nitrogen (LN). Four donor rats were used to multiply the parasites and transported by road from Kaduna to the Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria. The parasites were then maintained in Albino rats by serial passage until used.

2.2. Experimental animals

Total of Thirty (30) apparently healthy Guinea pigs of both sexes and of different ages were used in the study. The animals were purchased from breeders in Plateau State. On arrival, they were kept in clean and well ventilated cages in the Large Animal Veterinary Clinic, Faculty of Veterinary Medicine, university of Maiduguri, Nigeria. They were routinely screened for ecto, haemo and endo parasites using standard methods [5]. The Guinea pigs were housed in a suitable locally made wire mesh cages with sawdust as bedding, fed with varieties of vegetables and commercial growers feed (Vital Feeds, PLC, Nigeria), and water provided ad libitum. They were allowed to acclimatize to laboratory condition for two weeks prior to commencement of the experiment. The experimental procedure was in accordance with regulations of the Ethical Committee of the Faculty of Veterinary Medicine, University of Maiduguri.

2.3. Experimental infection

Four (4) adult albino rats were used as donors of T. brucei brucei, the rats were purchased from NITOR, Kaduna State. They were screened for internal and external parasites using standard method as described by [5]. They were inoculated intra peritoneally with 0.5 ml of T. brucei brucei parasiteemic blood with multiple parasites. At 4 days post inoculation, parasitemia was established and became obvious. The donor rats were bled via the tail vein into a petri dish, the blood was diluted with phosphate buffered saline glucose (PBSG) (pH 7.4). Each Guinea pig in groups A, B and C were inoculated intraperitoneally with 0.5 ml of blood containing $1.0 \times 10^6$ Trypanosoma brucei brucei as quantified using serial dilution as reported by Herbert and Lumsden [13].

2.4. Experimental design

Thirty Guinea pigs were randomly divided into six groups with five Guinea pigs in group (A, B, C, D, E and F). At day zero, all the animals were bled for haematology and serum biochemistry to establish a baseline data. Thereafter, physical condition and clinical parameters of all the animals were evaluated (rectal temperature, respiratory rate, pulse rate and heart beats) and body weights respectively. Groups A, B and C were inoculated with 0.5 ml of blood containing $1.0 \times 10^6$ Trypanosoma brucei brucei intraperitoneally (IP). Blood sample were collected for haematology and serum electrolytes profiles at interval of 4 days from day zero until the end of the experiment. Group A was infected with 0.5 ml of blood containing $1.0 \times 10^6$ Trypanosoma brucei brucei (untreated control), Group B was infected with 0.5 ml of blood containing $1.0 \times 10^6$ Trypanosoma brucei brucei (treated with Diaminazene diaceturate Veriben® at day 28 post...
infection at the dose rate of 7.0 mg/kg body weight. Group C was infected with 0.5 ml of blood containing $1.0 \times 10^6$ *Trypanosoma brucei brucei* (treated with Diaminazene diaceturate (Veriben®) at day 28 post infection at the dose rate of 3.5 mg/kg body weight. While Group D and Group E were uninfected/untreated (control) and uninfected treated with diaminazene diaceturate (Veriben®) at day 28 (p.i) at the dose rate of 7.0 mg/kg body weight respectively. Group F was uninfected (treated with diaminazene diaceturate (Veriben®) at day 28 post infection at the dose rate of 3.5 mg/kg body weight.

### 2.5. Post infection evaluation of guinea pigs

All the Guinea pigs were observed daily for the manifestation of clinical signs of trypanosomosis, which include morbidity and mortality. Meanwhile, detection of parasitaemia was done every 4 days post day zero and the degree of parasitaemia was projected by the rapid matching technique as described by Herbert and Lumsden [13].

### 2.6. Blood collection

Blood for haematological and serum electrolytes examination were aseptically collected at 4 days interval preliminary from day 0 to day 64 post infection. The Guinea pigs were bled using 2 ml syringe through cardiac puncture 0.5 ml and 1 ml of the blood was transferred into tubes containing anticoagulants (EDTA) and plain for hematological indices and sera for biochemical profiles respectively. Hematological and biochemical parameters were determine as described Henry et al [14] and Tietz [15].

### 2.7. Statistical analysis

Data generated were expressed as mean ± standard deviation (SD) using Two-way analysis of variance was used to compare the data between groups and value $p < 0.05$ was considered significant (Graph Pad. Instat, 2009).

### 3. Results

The prepatent period in both groups was the same form 4 - 8 days post infection. Following patency, parasitaemia fluctuated considerably in both groups with a higher recorded and least in group A and B respectively as presented in figure 1.

![Figure 1](image)

**Figure 1** Effect of diminazene diaceturate (Veriben®) on the mean parasite counts (x103/µL) of Guinea pigs (C. porcellus) experimentally infected with *T. brucei brucei* and their controls

There was a significant decrease in PCV values of both infected groups compared with their controls (Fig 2). The decline in PCV values began with the arrival of trypanosomes in circulation of all the infected groups with different degrees of anaemia as shown in figure 2.
Figure 2 Effect of diminazene diaceturate (Veriben®) on the mean packed cell volume (%) of Guinea pigs (C. porcellus) experimentally infected with T. brucei brucei and their controls

The value of RBC declined significantly (p<0.05) in all the infected groups following parasitaemia without reduction up to day 40 post infection in group A as presented in figure 3.

Figure 3 Effect of diminazene diaceturate (Veriben®) on the mean red blood cell counts (x106/mm3) of Guinea pigs (C. porcellus) experimentally infected with T. brucei brucei and their controls

Group A, Hb value significantly decreased following parasitaemia hence, the Hb value continued to declined significantly (p<0.05) without abating up to day 40 post infection as documented in figure 4.

Figure 4 Effect of diminazene diaceturate (Veriben®) on the mean haemoglobin concentration (g/dl) of Guinea pigs (C. porcellus) experimentally infected with T. brucei brucei and their controls

From day 16 (pi) when parasitaemia became patent in all the infected groups, the WBC value continuous to declined significantly particularly prominent in group A and B as shown in figure 5.
The differential leucocytes counts of all the infected groups declined following the establishment of parasitaemia by day 16 post infection, hence the value continue to declined significantly (p<0.05) in group A, B and C as indicated in figure 6, 7 and 9. While there was significant decreased in platelets count in all the infected groups following parasitaemia appearance in day 16 post infection in group A and B as shown in figure 8.
Figure 8 Effect of diminazene diaceturate (Veriben®) on the mean platelet counts ($x^3$/mm$^3$) of Guinea pigs ($C. porcellus$) experimentally infected with $T. brucei brucei$ and their controls.

In group A, the values of MCV decreased significantly ($p<0.05$) following establishment of parasitaemia by day 16 post infection similar findings was observed on the mean corpuscular haemoglobin MCH of the Guinea pigs ($C. porcellus$) as presented in (Fig 10, 11 and 12).

Figure 9 Effect of Diminazene diaceturate (Veriben®) on the mean absolute lymphocyte counts ($x^3$/mm$^3$) of Guinea pigs ($C. porcellus$) experimentally infected with $T. brucei brucei$ and their controls.

Figure 10 Effect of diminazene diaceturate (Veriben®) on the mean corpuscular volume (fl) of Guinea pigs ($C. porcellus$) experimentally infected with $T. brucei brucei$ and their controls.
Figure 11 Effect of diminazene diaceturate (Veriben®) on the mean corpuscular haemoglobin (pg) of Guinea pigs (C. porcellus) experimentally infected with T. brucei brucei and their controls.

The mean chloride ion concentrations of the Guinea pigs (C. porcellus) continually decreased following establishment of parasitaemia by day 16 post infection in all the treated groups at different days, similar findings was noticed on the mean bicarbonate ion concentrations of the Guinea pigs (C. porcellus) at day 16 post infection and mean serum sodium levels of Guinea pigs (C. porcellus) the serum sodium levels decreased significantly following establishment of parasitaemia by day 16 post infection in all the treated groups as show in (Fig 13, 14 and 15).

Figure 12 Effect of diminazene diaceturate (Veriben®) on the mean corpuscular haemoglobin concentration of Guinea pigs (C. porcellus) experimentally infected with T. brucei brucei and their controls.

Figure 13 Effect of diminazene diaceturate (Veriben®) on the mean serum chloride ion concentration (mmol/L) of Guinea pigs (C. porcellus) experimentally infected with T. brucei brucei and their controls.
Figure 14 Effect of diminazene diaceturate (Veriben®) on the mean serum bicarbonate ion levels (mmol/L) of Guinea pigs (C. porcellus) experimentally infected with T. brucei brucei and their controls.

The mean serum calcium ion concentration, mean serum potassium levels and magnesium ion concentration of Guinea pigs (C. porcellus) experimentally infected with T. brucei brucei were continually decreased following establishment of parasitaemia by day 16 post infection in all the treated groups at different days interval as presented in (Fig 16, 17 and 18).

Figure 15 Effect of diminazene diaceturate (Veriben®) on the mean serum sodium levels (mmol/L) of Guinea pigs (C. porcellus) experimentally infected with T. brucei brucei and their controls.

Figure 16 Effect of diminazene diaceturate (Veriben®) on the mean serum potassium levels (mmol/L) of Guinea pigs (C. porcellus) experimentally infected with T. brucei brucei and their controls.
Figure 17 Effect of diminazene diaceturate (Veriben®) on the mean serum calcium ion concentrations (mmol/L) of Guinea pigs (C. porcellus) experimentally infected with T. brucei brucei and their controls

Figure 18 Effect of diminazene diaceturate (Veriben®) on the mean serum magnesium ion concentrations (mmol/L) of Guinea pigs (C. porcellus) experimentally infected with T. brucei brucei and their controls

4. Discussion

The main clinical signs observed following experimental infection were respiratory distress, anemia, raised hair coat, dullness, anorexia and emaciation, this agreed with findings of several author reported in several species of laboratory animals and livestock. After the manifestation of clinical signs, the infected Guinea pigs shows parasitaemia by day 16 post infection. This is contrary to the findings of Umar et al [16] who observed a prepatent period of 4 days in rats infected with Trypanosoma brucei brucei. Infected Guinea pigs, started losing weight two weeks post infection. This may be associated with anorexia observed earlier, this was similar to the findings reported by Krinstensson et al [17] and Nyakundi et al [18] in rats infected with T. brucei brucei and rats infected with T. brucei gambiense [19] and also similar findings in rabbits infected with T. brucei brucei [20].

Despite the sudden changed in red cell parameters by decreased values of (PCV, RBC, Hb) during bouts of parasitaemia but gradually increased during the period of low and no parasitaemia. This tallies with the report of Yusuf et al. [21] who observed a significant decrease in PCV, Hb, RBC and platelet count in rats infected with T. b. brucei. This showed an inverse relationship between parasitaemia and anaemia in most of the infected animals [22, 23]. Anaemia is a consistent feature of trypanosome infections associated by oxidative stress that inflict damage to erythrocytes membrane and associate components. Equally, reactive oxygen radicals generated during trypanosomosis can attack erythrocyte membranes, which may induces oxidative stress that can trigger haemolysis [24]. It was also observed in the current study that only the group of Guinea pigs infected with T. b. brucei and treated with 3.5 mg/kg diminazene diaceturate (Veriben®) developed relapse parasitaemia at day 36 post treatment. Relapse in trypanosomosis has been linked to the parasites moving into areas of the body that are not accessible to the drugs like the brain [25]. If infection is allowed to
continue for some time before treatment then it is extremely difficult to obtain a permanent cure [25, 26, and 27]. Also Waitumbi et al. [26] reported relapse parasitaemia in rabbit infected with *T. brucei brucei* following treatment with 25 mg/kg of diaminazene diaceturate. The difference with the current study in the occurred relapse might be attributed to difference species of animal used and the drug dosage. The haematological results of the present study agreed with earlier studies reported by [28, 11 and 29]. The low PCV observed in the infected group may be linked to acute hemolysis that occur due to the progressive nature of the infection. Previous studies have shown that infection with trypanosomes resulted in increased susceptibility of red blood cell membrane to oxidative damage probably due to depletion or reduction of glutathione on the surface of the red blood cell [11, 30, 31 and 32]. Severity of anaemia has been related to reflect the intensity and length of parasitaemia. Several reports [33, 34, 29 and 35] have also ascribed acute anaemia in trypanosomosis to proliferating parasites. The low leucocytes (WBC, lymphocytes, and neutrophils) and platelet counts observed in the infected group may be attributed to the immunosuppressive actions of trypanosome infection [36, 37]. The major function of platelets is to activate blood clotting mechanism and prevent loss of blood [29]. In this study, the low platelet count observed in the infected untreated groups may indicate destruction of platelets by toxic products emanating from the trypanosomes [38]. Low platelet counts may also be attributed to other factors like pooling of blood into the spleen, removal of platelets by mononuclear phagocytic system and increased consumption of platelets by disseminated intravascular coagulation reaction in trypanosome infection [39]. However, leukocytosis, lymphopenia and neutrophilia reported, may be due to trypanosomosis and these conditions usually occur as a result of wear and tear syndrome associated with animal immune system caused by the ever changing variable surface glycoprotein of the infecting trypanosomes [36]. The lymphopenia encountered among the infected Guinea pigs may probably be due to an increased demand on the system for lymphocytes, which is a common requirement in both immune and inflammatory responses in trypanosomosis [11]. Inefficient recovery of iron from phagocytized RBCs is known to cause iron deficiency in the body [39]. Igbokwe [40] and Mbaya et al. [41] reported that dyserythropoiesis is associated with animal trypanosomosis, and this may be attributed to the decreased MCV that was observed in the present study among the infected untreated groups.

The *T. brucei brucei* infected Guinea pigs were observed to be associated with marked reduction in serum sodium and chloride ion levels, and this might have been due to renal tubular damage of the kidneys.

The decreased level of serum potassium observed in the current study was probably due to dehydration associated with tissue hypoxia [15]. Reduction in bicarbonate ion (HCO₃⁻) levels may be probably due to acidosis. The reduction may also be due to decreased alveolar ventilation and tissues hypoxia similar findings was reported by Ogunsanmi et al. [42] in sheep infected by trypanosome.

The low bicarbonate levels can also be attributed to the massive leakages of some electrolytes from cells and tissues damage. However, the intermittent increase, low level and subsequent return of these electrolytes to pre-infection levels suggest the efficacy of the therapies, otherwise it might be as a result of massive cell and tissue damage at the terminal phase of this single infection. The decrease in the levels of calcium that was observed in this study agrees with the findings reported in cattle infected with *T. congolense* [43] and sheep infected with *T. brucei brucei* [42]. This is said to be due to the deficiency in the parathyroid hormone as a consequence of the destruction of the parathyroid glands or a decrease in serum carriers, which in this case happens to be albumin. The drop in the level of serum magnesium concentration noted among the *T. brucei brucei* infected Guinea pigs observed in this study does not tally with the findings of Sow et al. [44] among donkeys in Burkina Faso and that of Chaudhary and Iqbal [45] among camels in Pakistan. This may be as a result of the difference in the species of animals used. The drop in magnesium concentration in blood observed in this study might be due to lowered dietary intake due to the infections. Biochemical evaluation of the body fluids give an indication of the functional state of the various body organs and biochemical changes in body fluids that result from infections depend on the species of the parasite and its virulence [46].

5. Conclusion

It is clearly understood the observed clinical signs among the infected groups (A, B and C) were pyrexia, pale feet, snout, pinnae and mucous membrane, anaemia, dullness, emaciation and loss of weight. In group A, a mean parasitaemia of 2.8 ± 0.84 occurred by day 16 post-infection post infection which continued to rise significantly without abating (p<0.05) to a peak count of 120.2 ± 5.48 by day 40 post infection. Which is absent in non-infected groups and that dose of Veriben® administered at the dose rate of 7.0 mg/kg and 3.5 mg/kg have the abilities of curbing the state of anaemia, immunosuppression, and serum electrolytes levels in trypanosome-infected guinea pigs placed on a dose dependent manner.
Compliance with ethical standards

Acknowledgments

The team of authors acknowledged the contribution of department of veterinary medicine, faculty of veterinary medicine and ethical department on animal welfare university of Maiduguri for the success of this research.

Disclosure of conflict of interest

All the authors agreed on the content of the manuscript. There is no any conflict of interest exist.

Statement of ethical approval

Ethical committee on animal Welfare University of Maiduguri, Borno State, Nigeria has given a full approval to go ahead with the research.

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How to cite this article