HOSTED BY

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Parasitological research doi: 10.1016/S2222-1808(16)61082-4

Comparative parasitaemia and haematology of mice, rats and rabbits experimentally infected with *Trypanosoma brucei brucei* and their responses to diminazene diaceturate (Veriben®) therapy

Amina Ibrahim[1], Albert Wulari Mbaya[2], Maduka Boniface Anene[3], Joshua Luka[2*]

[1]*Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Nigeria*

[2]*Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Nigeria*

[3]*Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria*

ARTICLE INFO

Article history:
Received 25 May 2016
Received in revised form 1 Jun, 2nd revised form 2 Jun 2016
Accepted 12 Jun 2016
Available online 24 Jun 2016

Keywords:
Parasitaemia
Haematology
Mice
Rats
Rabbits
Diminazene diaceturate

ABSTRACT

Objective: To compare parasitaemia and haematological changes of *Trypanosoma brucei brucei* infection in mice, rats and rabbits and their chemotherapeutic responses to diminazene diaceturate (Veriben®) therapy under the same experimental conditions.

Methods: A total of 20 adult BALB/c mice, 20 Wister albino rats and 20 New Zealand rabbits of both sexes were used for this study. Each rodent group was divided into four groups (A, B, C and D) of five animals each. Animals in Groups A and C were individually infected with 0.5 mL of blood from donor rats containing 1.5×10^6 *Trypanosoma brucei brucei*, while Groups B and D remained uninfected. Experimental animals were treated thus [Group A (infected and untreated control), Group B (uninfected and untreated control), Group C (infected and treated) and Group D (uninfected and treated)]. All treatments commenced 12 days after infection. Parasitaemia and haematological parameters were determined every four days till the end of the experiment.

Results: The prepatent period for mice and rats was 4 days while that of rabbits was 8 days. Observed clinical signs consisted of pallor of mucous membranes, lethargy and starry hair coat observed mainly among mice and rats, while the rabbits had mainly mild pallor of the ocular mucous membranes. Parasitaemia in Groups A rose significantly by Day 20 post-infection (*p.i.*) in the mice, Day 24 in the rats and Day 28 in rabbits. By these respective days, all the infected untreated animals in Group A died of the infection. However in Group C, parasitaemia declined significantly ($P < 0.05$) among mice, rats and rabbits and were completely eliminated from peripheral circulation by Days 20, 20 and 24 (*p.i.*) respectively, with no death. An inverse relationship was observed between parasitaemia and the haematological parameters for all the species of the rodents. As parasitaemia increased, the packed cell volume, red blood cell count, haemoglobin concentrations and white blood cell counts of mice, rats and the rabbits experienced a significant decline ($P < 0.05$) in Group A, leading to anaemia. These decline in haematological parameters were however the most severe in rats and mice than in rabbits.

Conclusions: Mice and rats are better animal models for studying the progression of the disease than rabbits and diminazene diaceturate (Veriben®) is capable of eliminating parasitaemia and modulating haematological changes in mice, rats and rabbits.

1. Introduction

Trypanosomosis is a disease of man and, domestic and wild animals[1-7]. It is characterized by fever, anaemia, loss of condition, reduced productivity and high mortality[8]. *Trypanosoma brucei gambiense* affects man in 24 countries of Central and West Africa, while *Trypanosoma brucei rhodesiense* affects man in 13 countries of Eastern and Southern Africa[2]. *Trypanosoma cruzi* affects man in South America[9]. The animal trypanosomes include *Trypanosoma vivax*, *Trypanosoma brucei brucei* (*T. b. brucei*), *Trypanosoma congolense* (*T. congolense*), *Trypanosoma simiae*, *Trypanosoma equiperdum* and *Trypanosoma evansi*, each of which affects one or more species of animals[2]. Laboratory animals are readily infected by *T. b. brucei* and *T. congolense*[10]. The pathology and the effect of trypanosomosis on haematology of infected hosts have been

*Corresponding author: Dr. Joshua Luka, Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Nigeria.
Tel: +234 08030407578
E-mail: joshuadiriki@yahoo.com

All experimental procedures involving animals were conducted in accordance to international ethics for the use of animals for biomedical research and approved by the Faculty of Veterinary Medicine Ethics Committee.

The journal implements double-blind peer review practiced by specially invited international editorial board members.

widely reported by several workers[11-15].

Control of trypanosomosis is aimed at destroying the parasite in the host with chemotherapeutic agents such as diminazene aceturate or through medicinal plants[16-21].

So far, little has been achieved in terms of understanding the variations in prepatent period, course of infection, and the haematology of the disease in various laboratory animal species especially as it relates to *T. b. brucei*. Furthermore, diminazene aceturate (Berenil) is currently no longer available in the market. It is against this backdrop that this study was conducted with a view of identifying the most suitable animal host model for *T. b. brucei* and the effect of diminazene diaceturate (Veriben®) on the most recent and readily available aromatic diamidines on the parasitaemias and haematological changes that might ensue in the course of the infection in mice, rats and rabbits under the same experimental conditions.

2. Materials and methods

2.1. Experimental animals

A total of 20 adult BALB/C mice, 20 Wister albino rats and 20 New Zealand rabbits of both sexes were used for this study. They were obtained from the National Veterinary Research Institute, Vom, Nigeria. They were routinely screened for blood, intestinal and external arthropod parasites using standard criteria. All animals were rescreened for parasites every two days for 30 days for relapse of parasitaemia. The animals were fed with pelleted commercial feed (Vital Feeds LTD, Jos, Nigeria) while water was provided *ad libitum*. They were allowed to acclimatize to their new environment for 14 days before the commencement of experiment. All experimental procedures involving animals were conducted in accordance with international ethics for the use of animals for biomedical research and approved by the Faculty of Veterinary Medicine Ethics Committee[22].

2.2. Source of Trypanosomes

T. b. brucei, Federe strain used for this study was obtained from the Nigeria Institute for Trypanosomosis and Onchocerciasis Research in Jos, Nigeria. The organism was first isolated from N'dama and Muturu cattle in 2006. It was identified as *T. b. brucei* based on morphology and negative blood inhibition and infectivity test and stabilized by four passages in rats before storage in liquid nitrogen. They were passaged twice in donor rats. Tail blood from the donor rats was diluted with phosphate buffered saline, pH 7.2. The mice, rats and rabbits were infected intraperitoneally with 0.5 mL of infected blood from the donor rats containing 1.5×10^6 *T. b. brucei*. The initial detection of parasitaemia was conducted by the wet mount and haematocrit buffy-coat methods, while the degree of parasitaemia was estimated by the rapid matching method[23,24].

2.3. Experimental design

The mice, rats and rabbits were weighed and randomly separated into four groups (A, B, C and D) of five each. The groups were infected and treated as follows, Group A: infected and untreated control; Group B: uninfected and untreated control; Group C: infected and treated intraperitoneally with a single standard dose of diminazine diaceturate (Veriben, B12 LA® Ceva, Sante Animale-La Balastiere, France) at 3.5 mg/kg body weight by Day 12 post-infection, while Group D served as uninfected and treated intraperitoneally with a single standard dose of diminazine diaceturate (Veriben®) at 3.5 mg/kg body weight by Day 12 post-infection.

2.4. Monitoring of physical changes and determination of parasitaemia and haematological parameters

Physical changes such as conditions of the mucous membranes, hair coat and activeness were monitored daily. Parasite counts were estimated every four days using the rapid matching technique of Herbert and Lumsden, while blood was collected every four days via tail vein[24]. Packed cell volume (PCV), red blood cell (RBC) counts, haemoglobin (Hb) concentrations and white blood cell (WBC) counts were determined using standard methods[25].

3. Results

3.1. Comparative changes in parasitaemia ($\times 10^3/\mu L$) of mice, rats and rabbits infected with T. b. brucei and treated with diminazine diaceturate (Veriben®) and their controls

The mean parasitaemia ($\times 10^3/\mu L$) of mice, rats and rabbits infected with *T. b. brucei* and treated with Veriben® and their controls are presented in Figure 1A–C respectively. In mice and rats, parasitaemia became patent by Day 4 post infection (*p.i.*) while in rabbits, it was by Day 8 (*p.i.*). The clinical signs observed were pallor of mucous membranes, lethargy and starry hair coat, which were observed mainly among mice and rats, while rabbits had mainly mild pallor of the ocular mucous membranes. The parasitaemia in the infected control (Group A) reached their respective peaks in mice, rats and

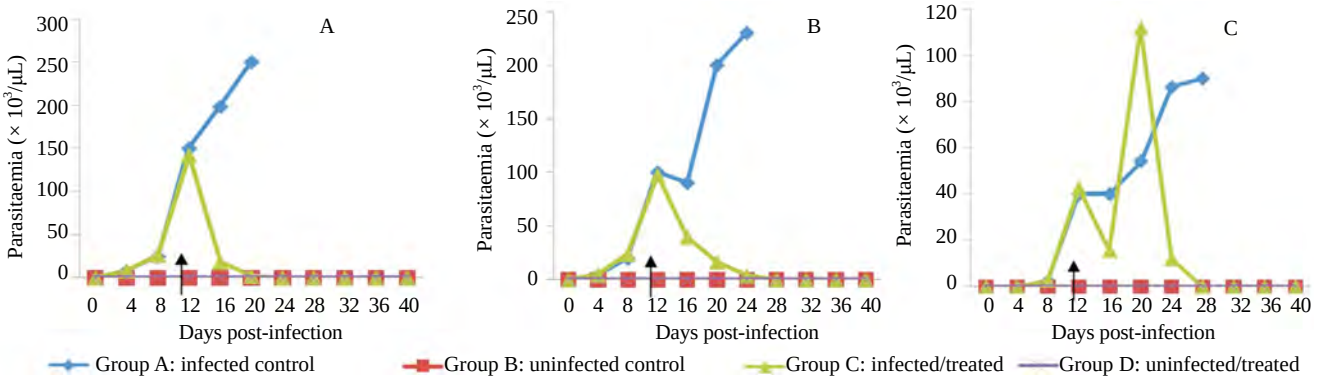

Figure 1. Comparative changes in parasitaemia ($\times 10^3/\mu L$) of mice (A), rats (B) and rabbits (C) infected with *T. b. brucei* and treated with Veriben® and controls (day of treatment, arrowed).

rabbits on Days 20, 24 and 28 of the infection, when all the rodents (n = 5) died of the infection. No death or parasitaemia was recorded in Groups B and D. In Group C, mice, rats and rabbits showed peak parasitaemia by Days 16, 12 and 12 respectively. This however declined significantly (P < 0.05) by Days 20, 24 and 20 in mice, rats and rabbits respectively. Complete disappearance of parasites from peripheral circulation was observed by Day 24 (*p.i.*) or Day 8 post-treatment (*p.t.*) in mice, Day 28 (*p.i.*) or by Day 12 (*p.t.*) in rats and Day 24 (*p.i.*) or by Day 12 (*p.t.*) in rabbits.

3.2. Comparative changes in PCV (%) of mice, rats and rabbits infected with T. b. brucei and treated with diminazine diaceturate (Veriben®) and their controls

The mean PCV (%) of mice, rats and rabbits infected with *T. b. brucei* and treated with Veriben® and their controls were presented in Figure 2A–C respectively. In all rodents, PCV of the infected control (Group A) declined significantly (P < 0.05) by Day 20 in mice and rats and Day 28 in rabbits, when all the rodents in the groups died. However, in Groups B and D, the values remained fairly constant (P > 0.05) throughout the study. In Group C, the pre-infection PCV in mice and rats (44.20% ± 0.83% and 42.80% ± 0.82% respectively) declined significantly (P < 0.05) following the establishment of parasitaemia by Day 4 (*p.i.*). Following treatment by Day 12 (*p.i.*), the values rose (P < 0.05) to their pre-infection values by Days 20 and 29 (*p.t.*) in mice and rats respectively. No mortality was recorded in either of the rodent groups. In Group C of the rabbits, the pre-infection value declined significantly (P < 0.05) from 50.00% ± 0.88% to 20.00% ± 0.56% by Day 12 (*p.i.*) when parasitaemia became patent. However, following treatment by Day 12 (*p.i.*), the value rose (P < 0.05) to its pre-infection value by Day 40 (*p.i.*) or by Day 29 (*p.t.*). Similarly, no mortality was recorded in this group.

3.3. Comparative changes in RBC counts of mice, rats and rabbits infected with T. b. brucei and treated with diminazine diaceturate (Veriben®) and their controls

The mean RBC ($\times 10^6$/mm^3) counts of mice, rats and rabbits infected with *T. b. brucei* and treated with Veriben® and their controls were presented in Figure 3A–C. In Group A, mean pre-infection RBC counts declined significantly (P < 0.05) by Days 20 and 24 (*p.i.*) in mice and rats respectively, while in rabbits, the pre-infection value declined significantly (P < 0.05) from (8.20 ± 0.36) × 10^6/mm^3 in Day 8 (*p.i.*) to (2.20 ± 0.17) × 10^6/mm^3 by Day 28 (*p.i.*). However, in Groups B and D, these values remained fairly constant (P > 0.05). Meanwhile, in Group C, the RBC of the infected mice, rats and rabbits, respectively declined significantly (P < 0.05) from (3.80 ± 0.24) × 10^6/mm^3 by Day 12 (*p.i.*), (2.80 ± 0.21) × 10^6/mm^3 by Day 12 (*p.i.*) and (5.00 ± 0.28) × 10^6/mm^3 by Day 12 (*p.i.*) to their respective pre-infection values. Following treatment, the values rose significantly (P < 0.05) again to their respective pre-infection states by Day 36 (*p.i.*) or 24 (*p.t.*) in mice, by Day 40 (*p.i.*) or Day 28 (*p.t.*) in rats and by Day 32 (*p.i.*) or Day 20 (*p.t.*) in rabbits.

3.4. Changes in Hb (g/dL) concentration of mice, rats and rabbits infected with T. b. brucei and treated with diminazine diaceturate (Veriben®) and their controls

The mean Hb concentrations (g/dL) of mice, rats and rabbits infected with *T. b. brucei* and treated with Veriben® and their

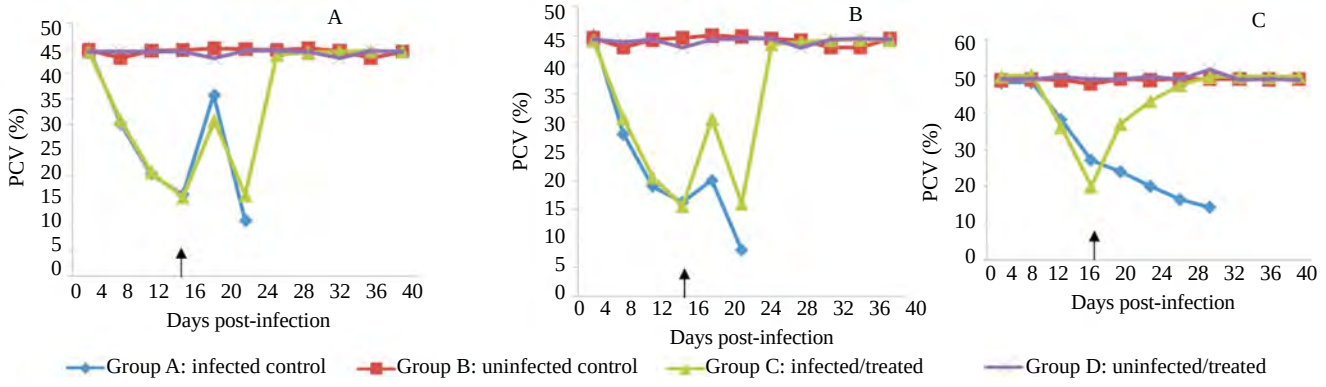

Figure 2. Comparative changes in PCV (%) of mice (A), rats (B) and rabbits (C) infected with *T. b. brucei* and treated with Veriben® and their controls (day of treatment, arrowed).

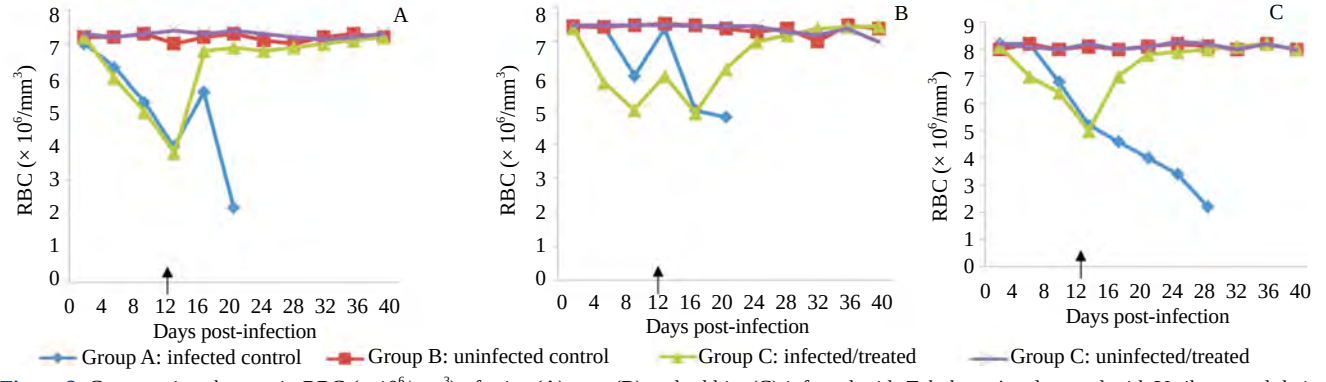

Figure 3. Comparative changes in RBC ($\times 10^6$/mm^3) of mice (A), rats (B) and rabbits (C) infected with *T. b. brucei* and treated with Veriben® and their controls (day of treatment, arrowed).

controls were presented in Figure 4A–C. In all rodents (Group A), the pre-infection values declined significantly ($P < 0.05$) by Day 20 (*p.i.*) in mice, Day 24 (*p.i.*) in rats and Day 28 in rabbits. In Groups B and C, Hb concentrations for all rodents remained fairly constant ($P > 0.05$) throughout the study and ranged between (14.20 ± 0.47) g/dL and (14.80 ± 0.43) g/dL in mice, rats and rabbits. Similarly, the values declined significantly ($P < 0.05$) to (7.30 ± 0.34) g/dL by Day 12 (*p.i.*) in mice, (11.40 ± 0.42) g/dL by Day 12 (*p.i.*) in rats and (8.20 ± 0.36) g/dL by Day 28 (*p.i.*) among infected rabbits. These values increased significantly ($P < 0.05$) to their pre-infection states by Day 40 (*p.i.*) or by Day 28 (*p.t.*) in mice, by Day 32 (*p.i.*) or by Day 20 (*p.t.*) and by Day 40 (*p.i.*) or by Day 28 (*p.t.*) in rabbits.

3.5. Changes in WBC counts of mice, rats and rabbits infected with T. b. brucei and treated with diminazine diaceturate (Veriben®) and their controls

The mean WBC ($\times 10^3/mm^3$) changes of mice, rats and rabbits infected with *T. b. brucei* and treated with 3.5 mg/kg of Veriben® and their controls were presented in Figure 5A–C. In mice, rats and rabbits of Group A, the pre-infection values declined significantly ($P < 0.05$) by Days 20, 24 and 28 (*p.i.*). Among the uninfected control (Group B) and uninfected but treated with 3.5 mg/kg of Veriben® (Group D), the WBC values ranged between (14.00 ± 0.47) $\times 10^3/mm^3$ and (14.30 ± 0.47) $\times 10^3/mm^3$ which remained fairly constant throughout the study ($P > 0.05$). In Group C, pre-infection values for mice, rats and rabbits declined significantly ($P < 0.05$) by Day 12 (*p.i.*). These values however rose back significantly ($P < 0.05$) by Day 40 (*p.i.*) or by Day 28 (*p.t.*) in all rodents.

4. Discussion

In this study, typical clinical signs of parasitaemia such as pallor of mucous membranes, lethargy and starry hair coat were observed mainly among mice and rats while the rabbits had mainly mild pallor of the ocular mucous membranes. This contrasts to the works of Anosa[26] and Losos and Ikede[27] that reported severe clinical signs due to *T. b. brucei* infection in rabbits. This might probably be associated with strain variation and individual infectivity or host susceptibility. Successive waves of parasitaemia were recorded among the infected mice, rats and rabbits. However, parasitaemia was low among the rabbits as compared to mice and rats. Successive waves of parasitaemia are known features of trypanosomosis commonly caused by antigenic variation[18,28]. The ability of host to limit the peak and number of each wave of parasitaemia is however dependent on whether the infection is acute, sub-acute or chronic[10]. And this may explain the reason why parasitaemia in the rabbits is appreciated but declined following treatment by Day 12 (*p.i.*) and by Day 16 (*p.i.*) as it maintained a gradual increase and declined again on its own accord. The drug diminazene diaceturate (Veriben®) was very effective in modulating parasitaemia and the haematological changes in all the different rodent species to their pre-infection values was proved to be effective against *T. congolense* infection in a German Shephard dog[29]. This trypanocide is one of the most recent aromatic diamidines and has a similar mechanism of action as the former diminazene aceturate (Berenil®). Like Berenil®, it distorts the helical structure of trypanososme DNA and has a higher therapeutic index and an added advantage being marketed in combination with vitamin B12, vitamin 2a and antipyrine which has the ability to eliminate pyrexia within 1 h[30]. This study, where a standard dose of the inoculum was administered and uniform pre-patent periods were

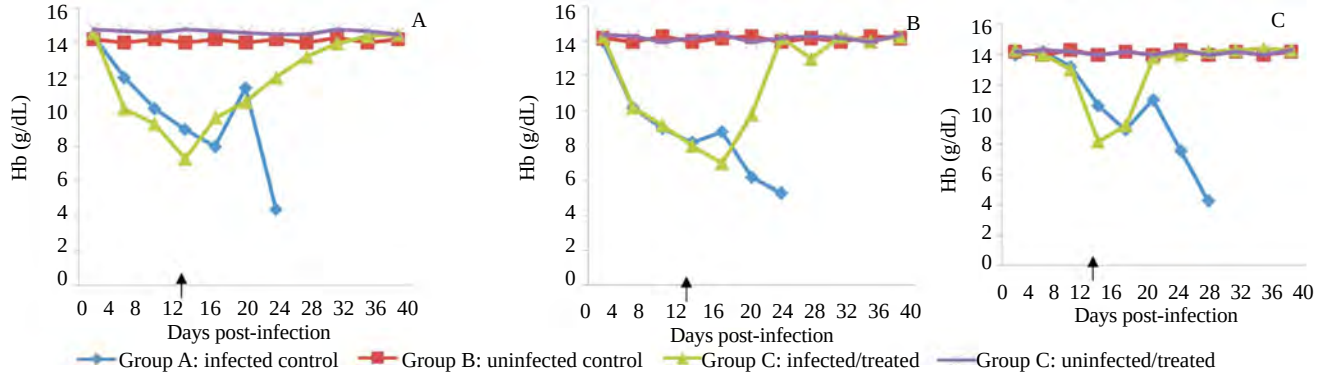

Figure 4. Comparative changes in Hb (g/dL) of mice (A), rats (B) and rabbits (C) infected with *T. b. brucei* and treated with Veriben® and their controls (day of treatment, arrowed).

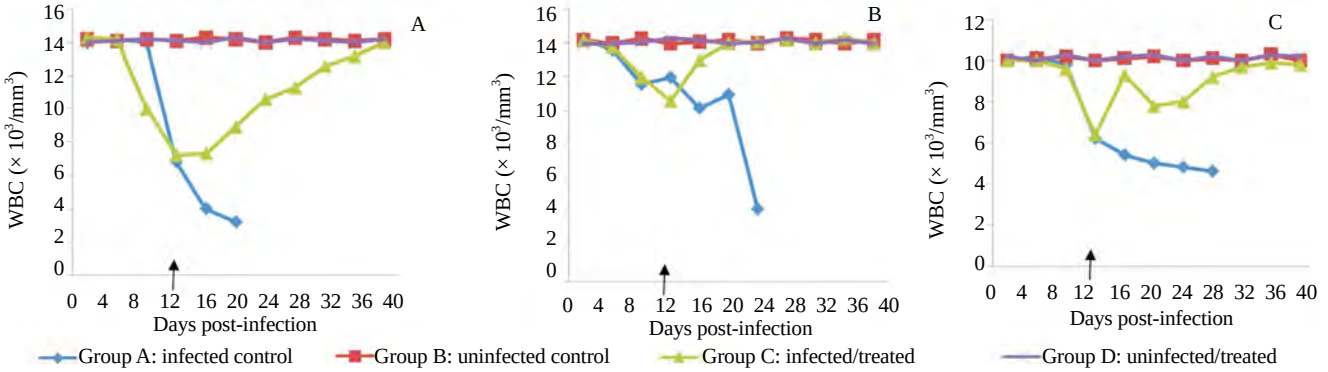

Figure 5. Comparative Changes in WBC ($\times 10^3/mm^3$) of mice (A), rats (B) and rabbits (C) infected with *T. b. brucei* and treated with Veriben® and their controls (day of treatment, arrowed).

encountered in all infected groups, means that the initial parasite replication rates were similar irrespective of the rodent species or susceptibility. These observations have been reported in *T. b. brucei* infection of dogs, red fronted gazelles (*Gazella rufifrons*), giant rats (*Cricetomys gambianus*) and in *Trypanosoma brucei gambiense* infection in vervet monkeys (*Cercopithecus aethiops*) and baboons (*Papio anubis*)[3-7,28,31,32].

In this study, the infected mice, rats and rabbits showed a significant decline in mean PCV, RBC and Hb parameters. The decline in these parameters which started at the onset of parasitaemia was indicative of anaemia which was less prominent among rabbits as compared to the other rodents. However, variation in prepatent period among the different rodent species showed that the prepatent period was shorter (4 days) in mice and rats and longer (8 days) in rabbits. Similarly, the severity of the anaemia was less prominent among rabbits than among mice and rats. This agrees with several reports that the prepatent period in mice and rats was 4 days and the anemia which is often haemolytic in nature began at the 1st wave of parasitaemia[11,26,28,33]. Similarly, the prepatent period of 8 days observed among the rabbits is in consonance with the reports of Anosa[26]. However, haemolytic anaemia and prepatent periods in most cases often depend on the species of trypanosomes involved and the root of inoculation of the parasite.

The expanded and active mononuclear phagocytic system has been a major player in haemolytic anaemia in trypanosomosis through erythrophagocytosis which develops soon after infection and continued thereafter, in the various phases of the disease[6,11,26,33,34]. The anaemia encountered among the different rodent species might have been associated with activation of the mononuclear phagocytic system due to increased demand on the system to remove dead RBCs, tissue cells, trypanosomes, antigen-antibody complexes and to participate in immune responses[28]. The fact that the red cell parameters (PCV, RBC, Hb) decreased sharply during bouts of parasitaemia but maintained a gradual increase during the periods of low parasitaemia in the three animal species, showed an inverse relationship between parasitaemia and anaemia[28,33,35,36]. The infected mice, rats and rabbits also exhibited leukopenia which was indicative of immunosuppression. Leucopenia after an initial period of leucocytosis is a common finding in *T. b. brucei* infection in rats and rabbits and other hosts[18,20,37-39].

It was therefore concluded that the experimental *T. b. brucei* infection among the mice, rats and rabbits caused various parasitaemia and hematological changes which were reversed by treatment with diminazene diaceturate (Veriben®). Secondly, mice and rats were better animal models for studying the progression of the experimental *T. b. brucei* than rabbits.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors wish to acknowledge the efforts of Mr. Bitrus Wampana of the Department of Veterinary Physiology, Pharmacology and Biochemistry and Mallam Samaila Gadaka of Veterinary Pathology Department, who both helped with the haematological analysis. The management of the University of Maiduguri is highly appreciated for making available the laboratory reagents and equipment used in the study. This study was supported by University of Maiduguri under its staff development/training in respect of the first author for the period 2010–2014.

References

[1] Fajinmi AO, Faleke OO, Magaji AA, Daneji AI, Gweba M. Presence of trypanosome species and determination of anaemia in trade cattle at Sokoto abattoir, Nigeria. *Res J Parasitol* 2011; **6**: 31-42.

[2] World Health Organization. Trypanosomiasis, human African (sleeping sickness). Geneva: World Health Organization; 2016. [Online] Available from: http://www.who.int/mediacentre/factsheets/fs259/en/ [Accessed on 18th May, 2016]

[3] Abenga JN, Anosa VO. Clinical studies on experimental *Gambian trypanosomosis* in vervet monkeys. *Veternarski Arhiv* 2006; **76**(1): 11-8.

[4] Mbaya AW, Aliyu MM, Nwosu CO, Ibrahim UI. Effect of DL-α-difluoromethylornithine on biochemical changes in baboons (*Papio anubis*) experimentally infected with *Trypanosoma brucei gambiense*. *Niger Vet J* 2009; **31**(1): 34-44.

[5] Mbaya AW, Aliyu MM, Nwosu CO, Taiwo VO, Ibrahim UI. Effects of melarsamine hydrochloride (Cymelarsan) and diminazene aceturate (Berenil) on the pathology of experimental *Trypanosoma brucei* infection in red fronted gazelles (*Gazella rufifrons*). *Vet Parasitol* 2009; **163**(1-2): 140-3.

[6] Mbaya AW, Nwosu CO, Kumshe HA. Genital lesions in male red fronted gazelles (*Gazella rufifrons*) experimentally infected with *Trypanosoma brucei* and the effect of melarsamine hydrochloride (Cymelarsan®) and diminazene aceturate (Berenil®) in its treatment. *Theriogenology* 2011; **76**(4): 721-8.

[7] Mbaya AW, Kumshe HA, Geidam AY, Wiam I. Effect of diminazene aceturate (Berenil®) on the pathology of experimental *Trypanosoma brucei* infection in African giant rats (*Cricetomys gambianus*). *Niger Vet J* 2011; **32**(3): 192-8.

[8] Yakubu DP, Dawet A, Olaleye NA. Effects of vitamin E and selenium on some blood parameters of *Trypanosoma brucei brucei* infected rats. *Br J Appl Sci Technol* 2014; **4**(7): 1100-8.

[9] Petersen C, Grinnage-Pulley TL. Trypanosomiasis. In: Kahn CM, Line S, editors. *The Merck veterinary manual*. 10th ed. New Jersey: Merck and Co; 2010.

[10] Soulsby EJL. *Helminths, arthropods and protozoa of domesticated*

animals. London: Bailliere Tindall; 1982, p. 46-9.

[11] Mbaya AW, Kumshe HA, Nwosu CO. The mechanisms of anaemia in trypanosomosis: a review. Croatia: In Tech. Publishers; 2012, p. 270-82.

[12] Abenga JN. A comparative pathology of *Trypanosoma brucei* infections. *Glob Adv Res J Med Med Sci* 2014; **3**(12): 390-9.

[13] Nwoha RIO. A review on trypanosomosis in dogs and cats. *Afr J Biotechnol* 2013; **12**(46): 6432-42.

[14] Osuagwuh UI, Okore OO. Comparative haematological changes in West African dwarf goats experimentally infected with *Trypanosoma vivax* and *Trypanosoma brucei*: changes caused by treatment with diminazene aceturate (Diminaze®). *Glob Vet* 2014; **13**(3): 378-84.

[15] Ukpai OM, Nwabuko OP. Effects on haematological parameters and pathology of internal organs of *Trypanosoma brucei brucei* infected albino rats. *Niger J Biotechnol* 2014; **27**: 8-13.

[16] Adenike SF, Stephen AO. Changes in haematological indices and protein concentrations in *Trypanosoma brucei* infected rats treated with homidium chloride and diminazene aceturate. *EXCLI J* 2010; **9**: 39-45.

[17] Mbaya AW, Kumshe HA, Alaka OO, Kyari F. Effect of polyethylene glycol on single and combined topical application of diminazene aceturate (Berenil®) and metronidazole (Metrodine®) in *Trypanosoma brucei*- and *T. congolense*-infected mice. *Comp Clin Pathol* 2014; **23**: 397-405.

[18] Mbaya AW, Nwosu CO, Onyeyili PA. Toxicity and anti-trypanosomal effects of ethanolic extract of *Butyrospermum paradoxum* (Sapotaceae) stem bark in rats infected with *Trypanosoma brucei* and *Trypanosoma congolense*. *J Ethnopharmacol* 2007; **111**: 526-30.

[19] Mbaya AW, Ahmed MI, Adamu M, Gyang SN. Toxicity and combined therapeutic activity of artemether and lumefantrine in *Trypanosoma-brucei*-infected rats. *Niger J Parasitol* 2009; **30**(1): 43-7.

[20] Mbaya AW, Ibrahim UI, God OT, Ladi S. Toxicity and potential anti-trypanosomal activity of ethanolic extract of *Azadirachta indica* (Maliacea) stem bark: an *in vivo* and *in vitro* approach using *Trypanosoma brucei*. *J Ethnopharmacol* 2010; **128**: 495-500.

[21] Ibrahim MA, Mohammed A, Isah MB, Aliyu AB. Anti-trypanosomal activity of African medicinal plants: A review update. *J Ethnopharmacol* 2014; **154**: 26-54.

[22] Council for International Organization of Medical Science. International guiding principles for biomedical research involving animals. Geneva: Council for International Organization of Medical Sciences; 2012. [Online] Available from: http://www.cioms.ch/images/stories/CIOMS/IGP2012.pdf [Accessed on 18th November, 2015]

[23] OIE. Manual of diagnostic tests and vaccines for terrestrial animals 2016. Paris: OIE; 2016. [Online] Available from: http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/ [Accessed on 15th May, 2016]

[24] Herbert WJ, Lumsden WH. *Trypanosoma brucei*: a rapid "matching" method for estimating the host's parasitaemia. *Exp Parasitol* 1976; **40**: 427-31.

[25] Weiss DJ, Wardrop KJ. *Schalm's veterinary haematology*. 6th ed. Iowa: Wiley-Blackwell; 2010.

[26] Anosa VO. Haematological and biochemical changes in human and animal trypanosomiasis. Part I. *Rev Elev Med Vet Pays Trop* 1988; **41**(1): 65-78.

[27] Losos GJ, Ikede BO. Review of pathology of diseases in domestic and laboratory animals caused by *Trypanosoma congolense*, *T. vivax*, *T. brucei*, *T. rhodesiense* and *T. gambiense*. *Vet Pathol* 1972; **9**(1): 1-79.

[28] Nwosu CO, Ikeme MM. Parasitaemia and clinical manifestations in *Trypanosoma brucei* infected dogs. *Rev Elev Med Vet Pays Trop* 1992; **45**: 273-7.

[29] Kimeli P, Kipyegon AN, Mwangi WE, Mande JD. A case of *Trypanosoma congolense* savannah type infection and its management in a dog. *J Adv Vet Anim Res* 2014; **1**(4): 232-4.

[30] Onyeyili PA, Egwu GO. Chemotherapy of African trypanosomiasis: a historical perspective. *Protozool Abstr* 1995; **19**: 229-41.

[31] Mbaya AW, Aliyu MM, Nwosu CO, Ibrahim UI. Effect of Berenil® or Cymelarsan® on the alteration of biochemical parameters in red-fronted gazelles (*Gazella rufifrons*) experimentally infected with *Trypanosoma brucei*. *Rev Elev Med Vet Pays Trop* 2008; **61**(3-4): 169-75.

[32] Mbaya AW, Aliyu MM, Ibrahim UI. The clinico-pathology and mechanisms of trypanosomosis in captive and free-living wild animals: a review. *Vet Res Commun* 2009; **33**: 793-809.

[33] Igbokwe IO. Nutrition in the pathogenesis of African trypanosomiasis. *Protozool Abstr* 1995; **19**: 797-807.

[34] Bezie M, Girma M, Dagnachew S, Tadesse D, Tadesse G. African trypanosomes: virulence factors, pathogenicity and host responses. *J Vet Adv* 2014; **4**(11): 732-45.

[35] Mbaya AW, Aliyu MM, Nwosu CO, Egbe-Nwiyi T. The relationship between parasitaemia and anaemia in a concurrent *Trypanosoma brucei* and *Haemonchus contortus* infection in red fronted gazelles (*Gazella rufifrons*). *Vet Arhiv* 2009; **79**(5): 451-60.

[36] Osuagwuh U. Haematological response of West African dwarf bucks experimentally infected with *Trypanosoma brucei* and treatment with diaminazine aceturate (Diminaze®). *World J Life Sci Med Res* 2014; **3**(3): 88-93.

[37] Takeet MI, Fagbemi BO, Olaniyi MO, Takeet OVA. Comparative study on hematological and plasma biochemical responses of rabbits to experimental single and mixed infections of *Trypanosoma brucei* and *Trypanosoma congolense*. *Niger Vet J* 2012; **33**(2): 462-74.

[38] Eghianruwa KI, Anika SM. Effects of DMSO on diminazene efficacy in experimental murine *T. brucei* infection. *Int J Anim Vet Adv* 2012; **4**(2): 93-8.

[39] Dagnachew S, Bezie M, Terefe G, Abebe G, Barry JD, Goddeeris BM. Comparative clinico-haematological analysis in young Zebu cattle experimentally infected with *Trypanosoma vivax* isolates from tsetse infested and non-tsetse infested areas of Northwest Ethiopia. *Acta Vet Scand* 2015; **57**: 24.