Trypanosoma brucei brucei is more pathogenic in rats compared to mice, making rats a better candidate for the relevant research studies

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ABSTRACT

Trypanosomosis is an economically important disease that has raised great and diverse kinds of research using different types of animals. Hence, this study is aimed at determining the better laboratory animal between the Swiss albino mice and Wistar albino rats in Trypanosoma brucei brucei studies. This study assessed the pathogenesis of T. b. brucei in Swiss albino mice and Wistar albino rats by probing the level of parasitemia, mean temperature, mean weight, hematological and histopathological parameters caused by the hemoprotozoan. Twenty laboratory animals, of mice (10) and rats (10) were grouped in two (control (5) and infected (5)), with the infected group inoculated with the blood protozoan intra-peritoneally. Trypanosoma b. brucei was detected in the blood of both laboratory animals on day one post-infection, with all the infected animals dying between day seven and eight post-infection. The protozoan exerted a significant (p < 0.05) effect on the mean temperature, mean weight, and hematological parameters of the infected animals. Pathological effects of T. b. brucei infection were seen in the liver and lungs of mice, and the liver, lungs, kidney and spleen of rats. The pathogenesis of T. b. brucei was more severe in rats compared to mice based on the studied parameters. These findings showed that rats are better candidates for T. b. brucei studies.

Keywords
parasitemia; pathogenesis; Swiss albino mice; Trypanosoma brucei brucei;
Wistar albino rats

Abbreviations
Hgb: Hemoglobin
PCV: Packed cell volume
RBC: Red blood cell
SEM: Standard error of the mean
T.: Trypanosoma
T. b.: Trypanosoma brucei

TWBC: Total White blood cell count

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Introduction

Trypanosomosis is one of the world’s most important diseases of humans and animals that is caused by flagellated blood protozoans that belong to the family Trypanosomatidae [1,2]. Trypanosomosis is generally characterized by high levels of parasitemia, severe anemia, cellular infiltrations, marked changes in the lymphoid system, progressive emaciation, and often death [1,3]. A number of Trypanosoma species are known to cause the general trypanosomosis in animals and human, these include but not limited to T. brucei, T. congolense, T. vivax, T. avium, T. suis, T. melophagium, T. melophagium, T. cervi, T. musculi and T. cruzi [1,4,5].

Trypanosoma brucei is a model for trypanosome studies and one of the most important Trypanosoma species [4]. Trypanosoma brucei (T. b.) has several subspecies including T. b. brucei, T. b. gambiensae, T. b. rhodesiense, T. b. evansi and T. b. equiperdum, with T. b. gambiensae and T. b. rhodesiense been the causative agent for human African trypanosomiasis (HAT) [1,4,5]. Trypanosoma b. brucei is one of the Trypanosoma species responsible for causing nagana also known as African animal trypanosomosis (AAT) in a wide variety of animals [6]. It is a vector-borne Trypanosoma species that is being transmitted by tsetse flies (Glossina species) and it inhabits the blood plasma, intercellular tissues, and body cavity fluid of an infected animal, thereby causing anemia and tissue damage [3,7]. African animal trypanosomosis results in either an acute, subacute or chronic disease and it constitutes a serious threat characterized by intermittent fever, anemia, occasional diarrhea leading to a severe reduction in productivity, loss of weight, decreased milk yield, reduction in carcase quality and capacity for work, and even death due to the failure of animals to utilize available food efficiently [8,9]. Trypanosoma brucei is known to cause pathological lesions in the liver, spleen, kidney, lung, and hearts of domestic and wild animals [1].

Various studies on T. b. brucei have been conducted using laboratory mice [10,11] and albino rats [2,9] with satisfactory levels of results. This body of evidence is aimed at determining the more suitable laboratory animal between laboratory mice and albino rats for T. b. brucei studies by assessing the level of parasitemia, mean temperature, mean weight, and hematological and histopathological parameters between the two animal subjects when infected with T. b. brucei.

Results

After infection, Trypanosoma mean parasitemia and standard error of the mean (SEM) was determined. Parasitemia was seen on the first day post-infection in both mice and rats with the parasitemia of mice higher than that of rats but the difference was not significant (p > 0.05). The level of parasitemia increased progressively in both laboratory animals with parasitemia peaking on day 7 in mice and day 8 in rats. The difference in the total parasitemia level was not significant (p > 0.05) between the laboratory animals (Fig. 1).

There was no significant difference (p > 0.05) in the mean body temperature (°C) between the control and the infected mice until day 4 post-infection, while that of rats showed significant difference (p < 0.05) by day 3 post-infection (Figs. 2A and 2B). The mean weight of infected mice decreased progressively post-infection with the difference being significant (p < 0.05) by day 6 post-infection, while the reduction in the mean weight of rats was significant (p < 0.05) by day 3 post-infection (Figs. 2C and 2D).
Trypanosoma b. brucei is more pathogenic in rats than mice in terms of body temperature, weight, packed cell volume, and hemoglobin concentration.

**Figure 2.**
Mean body temperature (°C) of mice (A) and rats (B); mean weight (grams) of mice (C) and rats (D) infected with Trypanosoma b. brucei. Each column represents the mean ± SEM. Different alphabets (a,b) in each day post-infection represent statistical significance at p < 0.05.

**Figure 3.**
Mean packed cell volume (%) of mice (A) and rats (B); mean hemoglobin concentration (g/dl) of mice (C) and rats (D) infected with Trypanosoma b. brucei. Each column represents the mean ± SEM. Different alphabets (a,b) in each day post-infection represent statistical significance at p < 0.05.
Trypanosoma b. brucei is more pathogenic in rats than mice

Figure 4.
Mean red blood cell count (×10^{12}/L) of mice (A) and rats (B); mean total white blood cell count (×10^9/L) of mice (C) and rats (D) infected with Trypanosoma b. brucei. Each column represents the mean ± SEM. Different alphabets (a,b) in each day post-infection represents statistical significance at \( p < 0.05 \).

Table 1.
Kaplan-Meier mean survival time of Trypanosoma b. brucei infection in mice and rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice infected with T. brucei</td>
<td>4.7^a</td>
</tr>
<tr>
<td>Mice (control)</td>
<td>8.0</td>
</tr>
<tr>
<td>Rats infected with T. brucei</td>
<td>5.4^a</td>
</tr>
<tr>
<td>Rats (control)</td>
<td>8.0</td>
</tr>
</tbody>
</table>

^a = significant at \( p < 0.01 \) within each animal species.

trol group (Fig. 4A). A similar finding was observed in rats with a significant difference (\( p < 0.05 \)) seen from day 3 post-infection (Fig. 4 B). The mean total white blood (TWBC) count of the infected mice was initially significantly lower (\( p < 0.05 \)) than that of the control group until day 4 post-infection, between days 5 and 6, the TWBC count becomes significantly higher in infected compared to the control (\( p < 0.05 \)), while by day 7 it was not significantly different (\( p > 0.05 \)) (Fig. 4 C). Fig. 4 D shows the mean TWBC count between the control and infected rats. The mean TWBC count was consistently lower in the infected group compared to the control group until day 6 post-infection when it reversed. The difference was significant (\( p < 0.05 \)) from day 2-7 post-infection.

The histopathological sections of the livers of the infected groups of mice revealed livers with congested central veins and perivascular cuffing (Fig. 5A), while that of rats showed hypertrophy of the hepatic cords with obstruction of the sinusoids (Fig. 6A). The histopathological sections of the lung of the infected mice showed congestion in the blood vessels, with airways edema (Figs. 5B), and expansion of the interstitial, with emphysema in infected rats (6B). Significant histopathological changes were observed in the kidney sections of T. b. brucei-infected rats compared to the uninfected rats, and this was characterized by congested intertubular space (Fig. 6C). Histopathological sections of the spleen of infected rats showed depletion of the lymphoid follicles (Fig. 6D). There were no histopathological lesions seen in the kidney and spleen of infected mice.

Discussion

The establishment of parasitemia in all the infected mice and rats is in line with previous studies done

Trypanosoma b. brucei is more pathogenic in rats than mice
Trypanosoma b. brucei is more pathogenic in rats than mice. The detection of the protozoan in the peripheral blood immediately after infection (first day post-infection) was investigated in studies by Udensi and Fagbenro-Beyioku [10] and Ademola and Odeniran [13] who detected T. b. brucei in the blood of mice 2 days and 9 days post-infection, respectively. In studies conducted by Egbe-Nwiyi et al. [2] and Habila et al. [3] the presence of the hemoprotozoan in the blood of albino rats was confirmed 7 days and 4 days post-infection, respectively. Factors such as the strains and virulence of the isolates (T. b. brucei), the immune status, nutritional requirement, and degree of susceptibility of the hosts to the hemoprotozoan may have resulted in the varied onset of the parasitemia. The progressive nature of the parasitemia level in both mice and rats attests to the invasive nature of the protozoan.

We observed that all the mice died by the 7th day post-infection, with rats all dying by the 8th day. Similarly, Udensi and Fagbenro-Beyioku [10] reported a 100% mortality with a mean survival time of 5 ± 1 in...
rats infected with *T. b. brucei*. Death due to *T. b. brucei* infection is associated with congestive heart failure which occurs as a result of anemia and myocarditis in infected hosts [1].

*Trypanosoma b. brucei* is associated with pyrexia in infected animals [1,14], this supports the increase in mean body temperature that was observed in our study.

Loss of appetite (anorexia) is a vital clinical sign associated with *T. b. brucei* infection in animals, and this ultimately leads to weight loss and emaciation [15] which was observed among the infected mice and rats in our study. Ademola and Odeniran [1] reported a significant weight loss among mice infected with *T. b. brucei* compared to uninfected mice, while Habila et al. [3] observed a similar finding among rats infected with the hemoparasite compared to those not infected.

The significant decrease in PCV, Hgb concentration, and RBC count we observed in both infected Swiss albino mice and Wistar albino rats suggest the presence of blood loss resulting to anemia, which is associated with an increase in the level of parasitemia of *T. b. brucei* infection. The release of hemolytic factors into the infected host's blood by the dead trypanosomes destroys the red blood cells, thereby leading to a reduction in PCV, Hgb, and RBC count [16,17]. Notably, a significant decrease in these parameters was first observed in rats compared to mice. Our observation is consistent with previous reports by Nwoha and Omamegne [7], Ukpai and Nwabuko [9], and Ademola and Odeniran [13].

The increase in TWBC count in the latter days of the infection in both the infected mice and rats indicates the presence of advanced infection. In response to this infection, the body employs its immune arsenal to fight the invading *T. b. brucei* and this process of immune response will boost the production of a high number of WBC [9,18], which is similar to our findings.

The histopathological lesions seen in the tissues and organs are a result of *Trypanosoma b. brucei* infection. *Trypanosoma b. brucei* infection in animals is known to cause immunoproliferative disorder of B-lymphocytes and plasma cells. Disorder in these cells may either directly or indirectly be responsible for the impaired functions of various organs [1,19].

The effect of *Trypanosoma b. brucei* infection was more severe in rats compared to mice, and the histopathological lesions were seen more in the organs of rats than the organs of mice. This showed that rats have more overlapping clinical and pathological data that are seen in *T. b. brucei* infection in animals, compared to that of mice. The suitability of an animal model for infectious disease studies is dependent on how well the animal model shows a sufficient amount of overlapping data that are seen in the humans biological (physiological, pathological, and pharmacological) information matrix [20]. In a similar study, Muchiri et al. [21] reported that *Trypanosoma brucei rhodesiense* caused more prominent clinical and pathological lesions in *Mastomys natalensis* rats compared to Swiss white mice, and concluded that *Mastomys natalensis* was a suitable model for studying the pathophysiology of human African trypanosomiasis.

*Trypanosoma b. brucei* induced noticeable pathogenesis in both Swiss albino mice and Wistar albino rats. There was an earlier significant increase in the mean body temperature and weight loss, and an earlier significant decrease in PCV, Hgb concentration, and RBC count in the infected rats compared to infected mice. There was an earlier significant increase in the TWBC count in infected mice compared to infected rats. The pathogenesis of *T. b. brucei* was manifested more in the organs of rats (liver, lungs, kidney, and spleen) compared to the organs of mice (liver and lungs). These findings, therefore, showed that *T. b. brucei* is more pathogenic in rats compared to mice, making rats a better candidate for *T. b. brucei* studies.

### Materials & Methods

#### Experimental animals and grouping

Ten (10) male and female Swiss albino mice, 7–8 weeks of age and weighing between 15 and 18 grams, and 10 Wistar albino rats (of both sexes, 9–11 weeks of age and weighing between 127 and 131 grams) were acquired from the faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The mice and rats were housed in plastic cages covered with wire mesh and allowed to acclimatize for 2 weeks before the onset of the experiment. The animals were maintained on commercial grower’s poultry feed, maize bran, and groundnut cake, compounded in a ratio appropriate for the type of laboratory animals. Water was provided ad libitum. The animals (mice and rats) were divided into two groups of five animals each (Group A = the control; and group B = the infected).

#### Source and infection of *T. b. brucei*

The *T. b. brucei* used for the study was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria. It was originally isolated from a natural infection in cattle, in Kaduna State. It was confirmed to be *T. b. brucei* using features described by Taylor et al. [1]. The *T. b. brucei* was inoculated into Wistar albino rats and transported to the Protozoology Research Laboratory, Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, for subsequent studies.

For the purpose of infections, infected blood with *T. b. brucei* was obtained from infected donor rats. The parasite populations were counted and diluted in phosphate buffer saline. The laboratory animals were inoculated intraperitoneally with 0.20 ml of *T. b. brucei*-infected blood estimated to be 1×10⁶ parasites/ ml using the hemocytometer.

*Trypanosoma b. brucei* is more pathogenic in rats than mice
**Parasitemia, body temperature, body weight and survival assessment**

The infected Swiss albino mice and Wistar albino rats were screened for trypanosomes at 24 h interval post-infection using the wet blood mount technique as described by Taylor et al. [1]. A drop of blood was placed on a clean glass slide, and a cover glass slip was placed over it. The quantity of blood was just insufficient to fill the whole space under the cover glass slip when this was pressed down gently. The film was examined under 400X magnification of a light microscope (Olympus®, Japan). A field was chosen in which the cells are evenly distributed. The rapid matching counting method as described by Herbert and Lumsden [22], was used to assess the number of T. b. brucei. Briefly, the microscopic appearance of a wet film, that has more than one T. b. brucei parasite was matched with one of a series of eight pictures of microscopic fields in the chart and table for estimating trypanosome parasitemias of Herbert and Lumsden [22]. The value in the box of the corresponding charts and the tables was used as the logarithm of the number of T. b. brucei per ml. The rectal temperature for each experimental animal was assessed daily at 7.00am for 5 days, using the digital thermometer. A portable weighing scale (Atom Digital Precision A-110C, China) was used to determine the weights of the animals at 72 h interval during the course of infection. Survival was determined by daily inspection post-infection with the trypanosome and Kaplan-Meier survival curve was used in calculating the mean survival time.

**Hematological analysis**

Blood sample for hematological studies was collected from the median canthus of the eye of each infected and non-infected mice and rats. Hematological analysis was carried out using standard protocols [23]. The total white blood cell (TWBC) count was determined by using a coulter counter (Cyan Hemocytometer, Belgium) as described by Cheesbrough [24].

**Histopathological examination**

Tissue samples collected from the liver, lungs, kidney and spleen were preserved in 10% buffered neutral formalin (BNF). After 48 hours of fixation, the tissue samples were processed (washed in 50% and 70% alcohol), embedded in paraffin wax and sectioned at 5 microns using a microtome. The sections were mounted on clean grease-free glass slides and stained with Hematoxylin and Eosin (H&E) stains as described by Luna [25]. The stained slides were examined microscopically using the 40X objective (400X magnification). The tissue samples were assessed using standard protocols. Histopathological lesions were observed, recorded and photo-micrographed with the aid of a digital camera (AmScope MT300 3.1MP, made in The United States of America).

**Statistical analysis**

Statistical analysis was conducted using the One-way Analysis of variance (ANOVA). Tukey’s multiple comparison test was used as Post-Hoc Test. The test was used to measure the differences in the various parameters within the different groups. Statistical significance was set at 5% (p < 0.05). GraphPad Prism Version 5.0 for Windows (GraphPad, San Diego, CA, USA) was used for the statistical analysis. The mean survival time was calculated using the Kaplan-Meier survival curve.

**Ethics approval**

The study protocol was approved by the Research and Ethical Committee of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. International, national, and/or institutional guidelines for the humane use and handling of laboratory animals were adequately followed.

**Authors’ Contributions**

KH, IAL, and SA conceived and planned the experiments. KH, IAL, and SA carried out the experiments. KH and IAL contributed to sample preparation. KH, SDO and SAA contributed to the interpretation of the results. SDO took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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**Competing Interests**

The authors declare that there is no conflict of interest.

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