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A Study on the Effect of Monomorphic *Trypanosoma brucei rhodesiense*, (Eatro 3 ET at 1.2) on Experimentally Infected Goats and Swiss White Mice

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Abstract

A study was done to assess the effect of *Trypanosome brucei rhodesiense* on experimentally infected goats and mice to see the effect of the study parasite and to determine the minimum dose that should be used to establish chronic infection. Three goats, identified with number as 908, 713, and 907, received 10^6, 5×10^4, and 10^3, parasite in dose volume of 0.5 ml intravenously, respectively. All infected goat showed clinical sign of trypanosomosis. Number 908 was died on 55th day post infection with PCV of 24%. A total of 11 Swiss white mice were also infected to study the effect of the study parasite. A total of 6 mice were infected in the first round (firsts batch), with 1×10^3 parasites in 0.3 ml dose volume, ip. All mice died before the 5th day post infection. There was no significant change in PCV and body weight almost in all infected mice during the course of infection. A total of 5 mice were infected in the second round (second batch), with 1×10^3 parasites in 0.2 ml dose volume, ip. Mice number 2 died after 72 hours post infection and its parasitaemia was 5.01×10^8. Mice number 1 and 4 were euthanized after 72 hrs and 3 and 5 after 93 hrs with parasitaemia of 2.51×10^8, each. There was statistical significant change in PCV during the course of the infection. The post mortem examination done on these mice reveals no visible inflammatory or pathological changes on examined tissues and organs (lung, heart, liver, spleen, kidney and intestinal organ) The survival period for the second batch of mice was also less than one week. This study indicated that Swiss white mice are more affected by the study parasite than goats.

Keywords: Parasitaemia; Packed cell volume; *Trypanosome brucie rhodensiese*

Abbreviations: AAU: Addis Ababa University; EATRO: East Africa Trypanosome Research Organization; EDTA: Ethylene Diaminetetra Acetic Acid; FVM: Faculty of Veterinary Medicine; HAT: Human African Trypanosomosis; i.p: Intraperitoneally; IV: Intravenous; ml: Milliliter; No: Nitric Oxide; PCV: Packed Cell Volume; PSG: Phosphate Saline Glucose Solution; RBC: Red Blood Cells; VSG: Variant Surface Glycoprotein; WBC: White Blood Cells

Introduction

Trypanosomosis is a complex disease that affects both human and animal [1]. The disease is characterized by intermittent fever, anaemia, lymphadenopathy, splenomegaly and cachexia often followed by death in untreated cases [2]. Trypanosomosis occurs in large areas of Africa, Latin America, the Middle East and Asia [2]. In sub-Saharan Africa this disease have an enormous impact on human health and livestock health which can be mirrored as huge annual economic loss and with the smilingly eternal poverty [1,3]. Trypanosomosis is caused by the protozoan parasite which is classified under the sub-kingdom of protozoa, phylum Sarcomastigophora, order Kinetoplastida, family Trypanosomatidae and genus *Trypanosoma*. This genus has two groups, Stercoraria and Salivaria [4]. Stercoraria contain genera in which the trypanosome completes its development in the hindgut and transmission is by fecal contamination. The species in Stercoraria include *T. cruzi* that causes Chagas disease in South America. The

salivarian group completes development in the salivary glands, and transmission is by inoculation of metacyclics with the saliva. The main genera in this group are: *Duttonella* (species: *Trypanosoma vivax*, and *T. uniforme*; Nannomonas (species: *T. congolense* and *T. simiae*); *Pycnomonas* (species: *T. suis*) and *Trypanozoon* (species and subspecies: *T. brucei brucei*, *T. b.rhodesiense*, *T. b. gambiense*, *T. evansi* and *T. equiperdum*) *Duttonella*, *Nannomonas*, *Pycnomonas* and some species in *Trypanozoon* (*T. brucei brucei*, *T. evansi* and *T. equiperdum*) cause disease in animals [4]. Mechanically transmitted trypanosomes such as *T. evansi*, *T. vivax* and *T. equiperdum* cause major production losses in the respective hosts. The distribution of African trypanosomosis in domestic animals and human coincides with the known distribution of the tsetse fly vector [2,5].

Closely related *T. brucei* subspecies *T. b. rhodesiense* and *T. b. gambiense* cause a disease known as Human African trypanosomiasis (HAT) or sleeping sickness and it is an illness endemic to sub-Saharan Africa [6]. In East and South Africa HAT is caused by *Trypanosoma brucei rhodesiense* and in West and Central Africa by *Trypanosoma brucei gambiense* [6,7]. Both *Trypanosomes* are transmitted to human hosts by bites of infected tsetse flies which are found only in Africa. *Trypanosomes* that cause disease in animals are not infective to humans due to their sensitivity to human serum that hinders their survival in man. However, in individuals lacking the lytic factor, trypanosome infection is possible. Indeed a case of human trypanosomosis caused by *T. evansi* has recently been reported in India [8]. On the Contrary, there are reports that show that trypanosomes that cause HAT can be found in domestic ruminants [9]. Despite the

carrier role of these animals to the human infective trypanosomes, however, if these parasites cause pathology in such animals is not well documented. Recently, a laboratory strain of *T. b. rhodesiense* was kindly provided by Professor Phillipe Buscher (Institute of Tropical Medicine (ITG), Antwerp, Belgium) to postgraduate program launched in collaboration between Addis Ababa University, College of Veterinary Medicine and Agriculture and Belgium Catholic University of Leuven. It was seen advantageous to test the effect of this parasite on small ruminants to generate additional data for understanding the impact of the human infective trypanosome on goats and mice.

Therefore the objectives of this study were to determine the reasonable dose that should be used to establish chronic infection on goats for future intended research work; to observe the effect of *Trypanosoma brucei rhodesiense* on experimentally infected goats and to assess the effect of this parasite on Swiss White mice which have been and are used in most trypanosomosis research work in Ethiopia.

Materials and Methods

Study area

The current study was conducted at the Addis Ababa University, College of Veterinary Medicine and Agriculture, Debre Zeit (Bishoftu) It is located at 90 N latitude and 400 E longitudes at an altitude of 1850 meters above sea level in the central high lands of Ethiopia [10]. Debre Zeit is located 47 kilometers south of Addis Ababa. The area has an annual rainfall of 866 mm of which 84% is in the long rainy season from June to September. The dry season extended from October to February. The mean annual maximum and minimum temperature are 260c and 140c respectively, with mean relative humidity level of 61.3% [10].

Study animals

Mice: Eight to 10 weeks of male Swiss white mice, weighing 30-35 gram, was obtained from the breeding colony of the National Veterinary Institute, Debre Zeit and Ethiopia Health and Nutrition Research Institute, Addis Ababa. Mice were kept under commercial pellet with *ad libitum* water, at the school of veterinary medicine, Debre Zeit under fly proof room. During the experiment infected and non-infected mice were kept on separate cage and each mouse was marked at the base of its tail for identification.

Goat: Three female goats, Arsi-Bale type, aged between one and two years and weigh between 17 to 20 kg were purchased from local market in Debre Zeit. All goats were dewormed up on arrival and kept under isolated fly proof net during the entire period of trial. They were allowed to acclimatize to the environment and fed, roughage, wheat barn, and maize for about six weeks. Water was provided *ad libitum*. During this period goats were treated with diminazine diaceturate (Rangtryps®) (7 mg/kg) and examined two times, at different period, for trypanosome with woo test. Each goat was identified with numbers tied to their neck during the experiment period.

Parasite: The study parasite, *Trypanosoma brucei rhodesiense* (EATRO3 clone Et at 1.2), was kindly provided by Professor Philip Bucher (Institute of Tropical Medicine (ITG), Antwerp, Belgium) The parasite was monomorphic population; populations consist of 'long slender' forms only. It was provided as cryostabilate and maintained in liquid nitrogen.

Chemicals and reagents

Phosphate saline glucose solution (PSG): Stock phosphate buffered saline glucose solution was prepared. Na_2HPO_4 (anhydrous) (13.48 g), $NaH_2PO_4.2H_2O$ (0.78 g) and NaCl (4.25 g) were measured and distilled water was added to make it 1 liter. Finally, glucose was added (1%).

Cryopreservant (14% glycerol): A 14% glycerol was prepared by diluting it with PSG. During preparation of stabilate 14% glycerol, equal in volume of infected blood, was added to cryovials, mixed gently with pipette, and vortexed for few seconds (4-5) before suspending it in vapour phase of liquid nitrogen. It was then checked for viability of trypanosome before immersed in liquid nitrogen for long term storage.

Drugs

2.36 g of diminazine (Rangtryps) containing 1.05 g diminazine diaceturate, sufficient for 300 kg body weight was used to treat goats before and after infection. It was prepared by dissolving it in 15 ml of distilled water, according to the manufacturer (CIPLA LTD, Mumbai, India).

Dilution factor

Based on the number of parasite estimated using the rapid matching technique [11] a dilution factor was calculated to prepare the required number of parasite in 0.5 ml (goat inoculation) and 0.3 (mice inoculation) ml dose volume. The dilution factor was calculated with [Dilution factor = {(Count/ml) x (dose volume)}/ dose required].

Experiment Set Up

Propagation of parasite and cryostabilate preparation

Four mice, obtained from Ethiopia Health and Nutrition Research Institute, were treated with 0.002 ml dexamethasone (ampoule containing dexamethasone sodium phosphate: 4 mg/ml) per day for six consecutive days. Mice then inoculated with varying volume of the stabilate. Whole blood was collected from ether anesthetized mice by cardiac puncture and placed immediately in EDTA coated vacutainer tube. Parasitemia was estimated before blood was collected [11]. Collected blood was dispensed on cryovial and mixed with equal volume of 14% glycerol. It was vortexed for few seconds before hanged on vapour phase of liquid nitrogen for about one hour. Viability of the parasite was checked before it was immersed in liquid nitrogen for long term storage.

Goat and mice inoculation

Three goats identified as #908, #713, and #907 are inoculated intravenous with 1×10^6, 5×10^4, and 1×10^3 parasites, respectively, in 0.5 ml of dose volume. Blood collected from mouse with parasitaemia of 1.2×10^8 was used to infect these goats.

Mice inoculation

A total of 11 mice were inoculated with the parasite in different batch. In the first round (first batch), a total of six mice was inoculated intraperitoneally with 1×10^3 parasites in dose volume of 0.3 ml and parameters were measured every other day. Parasite used to infect this batch of mice was directly collected humanely from mice via cardiac

puncture. In the second round (second batch), a total of five mice was inoculated with slightly more than 1×10^3 parasites in dose volume of 0.2 ml and parameters were measured in several hours interval. Parasites used to inoculate this batch of mice were used directly from stabilate that has been stored in liquid nitrogen. Mice used for this trial were obtained from Ethiopia Health and Nutrition Research Institute.

Parasite detection and measuring packed cell volume

The blood concentration technique of microhematocrit centrifugation (mHCT) or sometimes known as capillary tube centrifugation technique or the Woo test [12,13] was used to diagnose the parasite [12]. In brief, capillary tubes were filled three-quarters full with blood, collected with EDTA coated vacutainer tube. The end was sealed with plasticine and it was centrifuged for 5 minute at 13,000 g. The capillary tube was then mounted in a special holder and observed at magnification of 100X. Wet blood film and stained smear from buffy coat was also used in some cases.

Estimation of parasitaemia

A rapid matching technique, developed by Herbert and Lumsden [11], was used to estimate parasitaemia. Briefly, a wet film of the blood of infected mouse was made under a 7×22 mm cover glass. The quantity of blood placed on glass slide was just insufficient to fill the whole space under the cover glass when it was pressed down gently. The film was examined under 400X magnification and a field is chosen in which the cells are evenly distributed (single layer of RBC) Parasite counted per field (to a maximum of 20 fields) was computed to estimated number of parasite per milliliter of blood, as indicated on chart [11]. However, when there was no parasite after examining 20 fields, counting of parasite was done using the woo test [13] to appreciate the parasitaemia wave during the course of infection.

Differential leukocyte count

Differential leukocyte count was done after thin blood smear was stained in diff quick staining solution. The smear was examined under 1000X magnification. The counting was done according to Meander (called battlement) system [14], which ensures that areas of the slides were examined and a total of 100 white blood cells were counted and grouped as their type.

Euthanizing of animals and voiding of dead animals

Infected goats were euthanized humanely. First high dose of ketamine was administered intravenously. After the animal was slept, it was euthanized with concentrated salt solution of potassium chloride, administered intracardialy. Except those mice which died from infection, the rest mice were euthanized humanely using ether. All dead animals were disposed by incineration.

Data collection and analysis

Goats were observed for appetite, alertness, changes in body condition, changes in eye's mucous membrane, and changes in the regional lymph nodes every other day during the course of infection. Rectal temperature, packed cell volume, and parasite load was recorded every other day. Woo test, wet blood film and stained smear made from buffy coat were used to diagnose parasite. Day of inoculation was recorded as day zero. Data was entered on Excel 2007

spread work sheet and descriptive statistics was done using SPSS version 15. Graph was made using Excel 2007.

Results

Goat

Goats have been observed for two months after infection. The infection went well for two months except for Goat #908 which was found died on day55 post infection due to complication. Goat #908 and #713 was positive on day 4 and #907 on day 5, post infection. Packed cell volume (PCV %) of goats before infection were 27%, 27% and 25% for #908, #713 and #907 respectively. Except on very few cases, the parasitaemia of all infected goats was less than 2.5×10^5 parasites per ml of blood. As a result of this, estimation of parasitaemia for each examined day was done by counting what was observed on woo test. And data obtained from this counting was used to depict the graphs indicated below. When the number of parasite was more than 250 under one field of vision during counting, it was difficult to count all. Thus the number 300 was picked just to simplify the pictorial representation of the graph. All goats show depression and enlargement of regional lymph node during the course of infection. There was also intermittent fever and in appetite during the study period. After 4-5 weeks, post infection, the mucus membrane of the eye indicated sign of anemic. Body condition of infected goats gets worse as the disease progress.

During the course of infection PCV of goat #908 was gradually decrease from 29% to 24% in 55 days, post infection (Figure 1). The mean PCV value during the course of infection was 26.4%. During the course of infection high rectal temperature was observed on day 32 and day 55 (40.5°C on both days). The highest parasite burden, on woo test, was observed on day 39 and 55, post infection. The rectal temperature on these days was, 40 and 40.5°C, respectively. The parasitic wave during the course of infection is depicted on Figure 1.
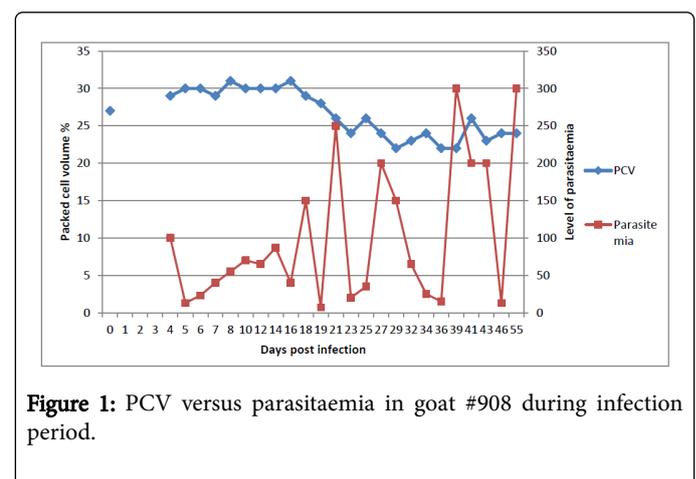

Figure 1: PCV versus parasitaemia in goat #908 during infection period.

In goat #713, PCV was gradually decreased from 28% to 19% within 64 days, post infection. The mean PCV value during the course of infection was 25.45% (Figure 2). High rectal temperature was observed on day 21 (42.2°C) The highest parasite burden, estimated by counting on woo test, was observed on day 5, 14, and 36 post infection. The rectal temperature on these days was, 40, 37.7 and 39.7°C respectively. Between day 39 and 48, the rectal temperature was stayed between 40.1 and 40.8. After day 50 the estimated parasite load was less than 100

and the rectal temperature was between 39.3 and 39.7. It seems that chronic infection was established after day 50 post infection.
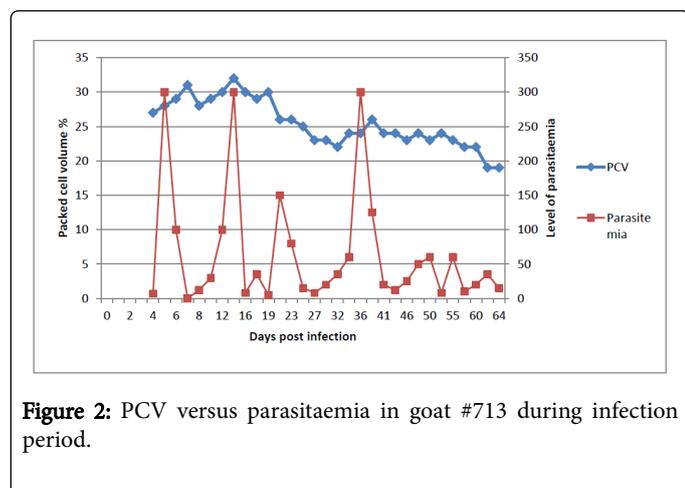

Figure 2: PCV versus parasitaemia in goat #713 during infection period.

The PCV value of goat #907 was gradually decreased from 28% to 14% within 64 days post infection. The mean PCV value during the course of infection was 21.26%. There was a rapid drop in PCV, from 27% to 16%, between day 16 and day 25 post infection (Figure 3). It is improved after that and starts to decline slowly to 14%. During the course of infection high rectal temperature was observed on day 32 (40.7°C). The highest parasite burden on woo test was observed on day 14, 21 and 36 post infection. The rectal temperature on these days was 39.6, 39.4 and 38.9°C respectively. The mean parasite count estimated by woo test during the course of infection was 80 parasites. After day 48 the parasite load was less than 100 and the rectal temperature was between 38.7 and 39.80C. It seems that chronic infection was established after day 50 post infection.
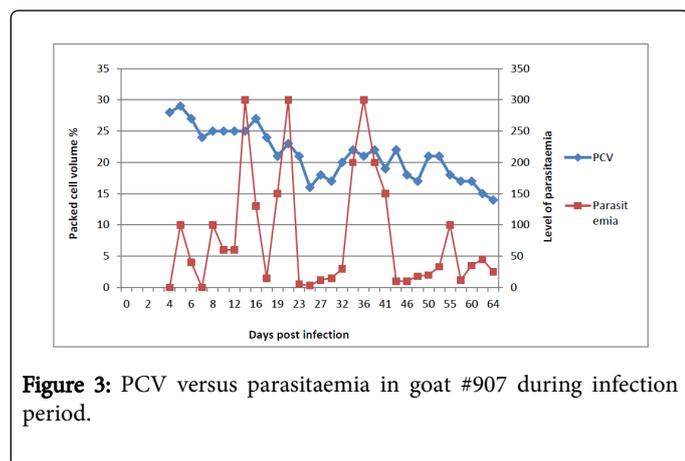

Figure 3: PCV versus parasitaemia in goat #907 during infection period.

Mice

For the first batch of mice, infected with 1×10^3 parasites in 0.3 ml dose volume, data was collected every other day and day of inoculation was recorded as day zero. Mouse 1 and 5 were positive on the second day with parasitaemia of 2.5×10^5 and 2×10^6 parasites per ml of blood respectively. Mouse 2 was positive with parasitaemia of less than 2.5×10^5 parasites per ml of blood. Mice 3 and 4 were negative. During data collection on the 4th day for mice 1, 2, 3, 4, 5, and 6 was 1.26×10^8, 6.3×10^7, 1.58×10^7, 3.16×10^7, 1.26×10^8 and 1.58×10^7 sequentially.

The survival period for the first batch of mice was less than one week. Mice number 1 and 5 were found died late on the 4th day, post infection. Their parasitaemia just before their death was 1.6×10^8 parasites per ml of blood for each mouse. Mice number 3 and 4 died early on the 5th day post infection and their parasitaemia was 1.58×10^7 and 3.16×10^7 respectively. Mice number 2 and 6 died late on the 5th day post infection and their parasitaemia was 6.3×10^7 and 1.58×10^7 respectively (Table 1).

The mean PCV volume of the infected group and the control group was 48% (95% CI: 47.05–48.92) and 47.61% (95% CI: 46.85–48.35) respectively. There was no significant change in PCV between infected and control group. There was no significant change in body weight during the course of infection (95% CI for infected group: 30.58–32.67, 95% CI for control group: 32.06-33.26). Results obtained from differential WBC count of both batch and control groups are summarized under Table 2.

In the case of the second batch of mice, infected with 1×10^3 parasites in 0.2 ml dose volume, data was collected in several hours interval. Mice number 2 died after 72 hours post infection and its parasitaemia was 5.01×10^8. Mice number 1 and 4 were euthanized after 72 hrs and their parasitaemia was 2.51×10^8, each. Mice number 3 and 5were euthanized after 93 hour post infection and their parasitaemia was 2.51×10^8, each (Figure 4). The mean PCV value for infected and control group was 43.7% (95% CI: 41.94–45.46) and 47.61 (95% CI: 46.85–48.38.) respectively. There was statistical significant change in PCV during the course of the infection. There was no significant change in body weight during the course of infection (95% CI for infected group: 31.123-32.547; 95% CI for control group: 32.06-33.26). The post mortem examination indicated that there were no visible inflammatory or pathological changes on examined tissues and organs. Organs examined include lung, heart, liver, spleen, kidney and intestinal organ. The survival period for the second batch of mice was also less than one week. Results obtained from differential WBC count are summarized under Table 2.

Day after infection	Mice 1	Mice 2	Mice 3	Mice 4	Mice 5	Mice 6
0	0	0	0	0	0	0
2	5.4	0	0	0	6.3	0
4	8.1	7.8	7.2	7.5	8.1	7.2

Table 1: Progress of parasitaemia on first batch mice post infection.

Mice group	Mean (95% CI)		
	Neutrophil Lymphocyte Monocytes		
First batch infected	36.94 (34.28-39.61)	55.78 (53.39-58.39)	6.89 (5.85-7.92)
Non infected control	32.17 (28.66-35.68)	60.72 (57.69-64.36)	6.78 (5.52-5.04)
Second batch infected	40.7 (37.49-43.91)	51.58 (49.31-54.39)	7 (5.83-8.17)

Table 2: Result obtained for differential leukocyte count on both batch of infected and non-infected mice.
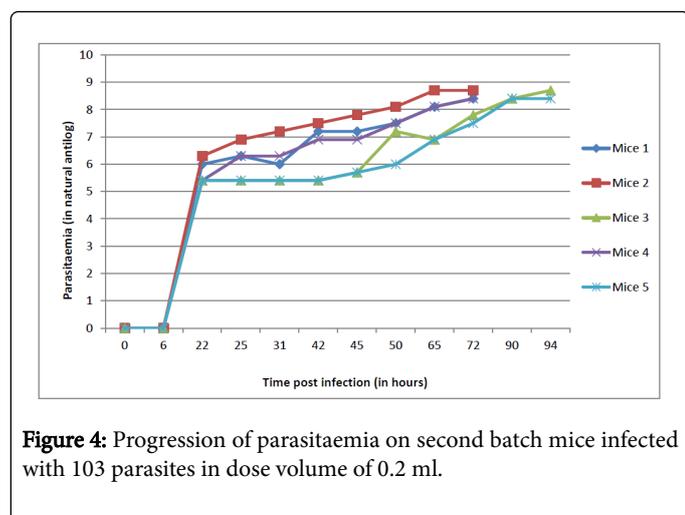

Figure 4: Progression of parasitaemia on second batch mice infected with 103 parasites in dose volume of 0.2 ml.

The mean neutrophils at confidence interval of 95% of second batch infected mice were much greater than the first batch infected and uninfected mice (Table 2). This change was may be due to increased sampling frequencies and stress related with sampling.

Discussion

Goat

The present study was performed with an attempt to observe the effect of monomorphic Trypanosoma brucei rhodesiense on goats and on Swiss White mice. The nature of the investigation was a pilot study and it was not standardized for several reasons, in the case of goat inoculation. To count, economic constriction is the major. The study was primarily concerned with determining the reasonable dose of parasite that should be inoculated on goats to establish chronic infection without overwhelming the host immunity, which could lead to early mortality, for intended future study. In the current study, our finding indicated that infection can be established with the study parasite at a dose of 1000 parasite per volume of inoculums in less than one week and infection could be run for about two months with a parasitaemia of less than 2.5×10^5. Similar trends of progression in parasitaemia were observed in all trypanosome infected three goats. This result is similar with what is observed by Abebayehu [15] on goats infected with *T. congolense*. In animals infected with blood stream forms of trypanosomes peak parasitaemia can occur within a few days post infection. The pre-patent period with *T. brucei* is less well defined but the parasites can sometimes be detected in the blood as early as 3 to 4 days after exposure to the infection [16] and acute *T. brucei* infection have been reported in laboratory experiments [17]. The

obtained experimental result also agrees with the work of Adeiza et al. [18], that indicates the pre-patent period for *T. brucei* infected goats had a mean of 4.6 days, while the *T. vivax* infected goats had a mean of 5.3 days.

Many of the clinical and pathological manifestation showed in the current study are common to all domestic animals infected with trypanosomes, irrespective of the species of trypanosome involved [19]. The disease shows different clinical signs depending on the virulence of the infection trypanosome, the infective dose and immune status of the animals, age and breed of the host [16,19]. Fever is guide to the presence of infectious disease including trypanosomosis [16,19,20], which is due to the reaction between pathogens and body defenses. The result revealed that, increase in parasites population corresponded with the rise in body temperature. The experimental result also agreed with [16] that following the invasion of the blood by actively dividing trypanosomes is associated with increased body temperature and the initial parasitaemia and fever usually persist for several days before a trypanolytic crisis occur after which parasitaemia reduced and the temperature returns to physiological value.

In the current study the change in PCV was also fall gradually as what has been observed by Abebayehu, [15] and ILRAD [21]. This progressive fall in PCV value during trypanosome infection has also been described in different breeds of cattle, sheep and goats [22]. Despite the importance of anemia, the exact mechanisms underlying its induction remained unsolved [23]. In the current study even though the mechanism of development of anemia was not investigated, some studies showed that in vitro VSG-sensitized RBC can be lysed by VSG-specific antibodies [24]. This author suggested that anemia may be linked to B-cell responses and that antibody mediated lysis may be a contributing factor in vivo [24]. Other studies have suggested that trypanosomes themselves release components which directly lyse RBC [25]. Other possible factors include the role of non-specific hemolytic factors, which might facilitate the distraction of normal red blood cells by macrophages was also indicated by Murray and Dexter [22].

Except only on few cases, the parasite burden on each goat was less than 2.5×10^5 parasites per ml of blood. This may suggest that the parasite proliferation is well regulated or more parasites were dwelling in the tissue during the course of the infection [26].

Mice

Infected mice develop similar but not always identical symptoms of disease as livestock. Infections of inbred mice are a useful disease model, as long as one keeps in mind that there are differences in the physiology of mice, humans and livestock. In the current study, the survival period of infected mice was less than one week. This result was similar with what have been observed by Magez et al. [26]. On their

study, a different strain of monomorphic parasite clones was used to study the role of B-cell in causing anemia [26]. The parasite had killed some of the mice within 5 days. Similar results were also obtained by this workers when they tried the parasite on different in breed mice genetically modified for this study [26]. On this study it has been suggested that through their accelerated exponential growth, these parasites reached a level of virulence that is lethal before an *in vivo* efficient B-cell response can be initiated. The current study supports the notion that antibodies can only exert their full antitrypanosome activity in an immunological environment that develops later than day 6. At this early time point, activated macrophages play a crucial role in parasite destruction via the secretion of inflammatory molecules such as nitric oxide (NO) and TNF, and through phagocytosis of damaged and opsonized parasites [26]. However, a monomorphic parasite, like the one used in the current study, can overwhelm distractions that is caused by macrophages and its secreted toxic substance. It is indicated that exponential parasite proliferation growth, resulting in parasitaemia levels up to 2×10^9 parasites per ml is associated with eventual death of experimentally infected mice. Mice which were able to control the parasite proliferation survive longer from those unable to do so [26].

It has been long suggested that breed of mice contribute to the outcome of trypanosome infection. It has been indicated that different strains of mice can show different degrees of susceptibility to trypanosome infection [27,28]. Experiment done on BALB/c and C57BL/6 mice, infected with *T. congolense* has shown that BALB/c mice are highly susceptible and C57BL/6 mice are relatively resistant to *T. congolense* infections. When these mice breed are infected intraperitoneally (i.p) with 10^3 organisms of *T. congolense* clone TC13, BALB/c mice had a mean survival time of 8.5-0.5 days, whereas C57Bl/6 mice survived for more than 100 days [29,30]. This is one of the reasons which lead us to test the effect of *T. b. rhodesiense* (EATRO 3 Et at 1.2) on Swiss white mice, which are commonly used in trypanosome research carried in Ethiopia. These mice survive experimental infection of *T. congolense*, isolated from different tsetse infested area of Ethiopia, for more than one month [31]. What was observed in survival period of this Swiss whit mice can be also attributed to their breed.

The short survival period of infected mice observed in this study can also be explained by the fact that trypanosomosis is a complex disease and inflammation could be one of the many reasons. It has been shown that susceptible BALB/c mice infected with *T. congolense* succumb to the infection due to the development of systemic inflammatory response syndrome (SIRS) within 10 days of infection. This inflammatory syndrome is associated with hyper activated macrophages, an outburst of cytokine release, enlarged capillary bed, decreased blood pressure, drop of body temperature, hypomotility and piloerection towards the terminal stage [32].

In the current study, despite high parasitaemia level, there was no significant change in the PCV level of first batch infected mice. This is similar with what has been explained by Magez et al. [26]. On their work they have showed that there is no correlation between level of parasitaemia and anemia. A comparison study done on anaemia and parasitaemia between A/J and more resistant C57BL/6 mice revealed that anaemia development was more severe in the C57BL/6 strain, despite the fact that this strain acquired lower parasitaemia and survived longer after infection than A/J mice [33]. A comparison of different host-parasite combinations also revealed that there is no correlation between pathology and survival [34]. Such data suggest

that anaemia is a consequence of host responses to the infection, and not directly induced by the parasite products. For example, studies with *T. brucei* infected C3H/He mice suggested an involvement of nitric oxide (NO) [35]. In some murine models, but not others, anaemia was mediated by TNF [36], which seems to achieve its function via binding with TNF [34]. The statistical difference observed in the second batch of mice, however, could be due to stress factors that are caused by frequent blood sampling and handling of mice.

Even though the post mortem examination in the current study do not show visible inflammatory or pathological changes, the pathogenesis of *Trypanosoma brucei* sub species is well described by Victor and Peter [37] and Taylor and Authié [38]. They have described the involvement of many tissues, including the nervous system. Most of the information on changes in the immune response and their possible contributions to the pathogenesis has been obtained in the animal models infected with *T. b. brucei*. For example, in rat experimentally infected with *Trypanosoma brucei*, trypanosomes were found localized extravascularly in the interstitial tissues of the myocardium and were associated with focal loss of myocardial fibers and a mononuclear myocarditis [39]. Other vital organs or systems, which are commonly affected, include the skeletal muscle, central nervous system [40], endocrine organs and reproductive systems [41-45].

Conclusion and Recommendations

Trypanosomosis is a disease of extracellular flagellated protozoan parasite, predominantly transmitted by the bite of the haematophagic tsetse fly and it cause a devastating disease to both human and livestock. It has been hypothesized that small ruminants play a carrier role for human infecting trypanosomes without being affected themselves by the disease. The current study indicated that goats are susceptible to laboratory strain of *Trypanosoma brucei rhodesiense* and showed recognized clinical sign of African Animal Trypanosomosis with, fluctuating parasitaemia, progressive reduction in PCV value, and poor body conditions with enlarged regional lymph nodes. The current study also indicated that few thousand of the study parasite are enough to establish chronic infection on goats without overwhelming the host. The current study also showed that Swiss white mice are highly susceptible to the study parasite and they survive only for few days after infection. Based on the current study the study parasite was more pathogenic on Swiss white mice than on goat.

Based on the above conclusion the following recommendations are forwarded.

Further in depth study should be done on different breeds of goat to investigate impact of the study parasite, using standardized method.

Further study should also be done using immunological and histopathological parameters to better understand the pathophysiology of Swiss white mice.

This experimental study should be conducted under organized project and the result should be published for the public.

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