

Impacts of *Trypanosoma vivax* on Experimentally Infected Calves and Goats in Bishoftu, Ethiopia

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Rec date: March 13, 2018; Acc date: April 19, 2018; Pub date: April 21, 2018

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Abstract

Four female calves aged of 1 year to 1 year and 4 months which weighing 86-100 kg and three male goats weighing 22-31 kg and ages of less than one year were included in the study. All experimental animals were infected intravenously with 1 ml (concentrated) blood containing the wild strain of *T. vivax* brought from Gibe valley. Fresh blood sample of each animal was collected directly from the jugular vein for 84 days in two days interval. The mean PCV was 34%, 33%, 35.5% and 33.25% respectively, for week one, week two, three and week four before inoculation for calves and 32.8%, 34.2%, and 35.8% for goats. The mean PCV started decreasing between 4-6 days post inoculation for all calves to 21.5±1.1% and then sharply decreased to 13±0.5% in day 13-17 post inoculation. Between days 19-24 post inoculation the mean PCV was increased to 21±2.2 and decreased to 14.6±1.14 between 26-35 days post inoculation. Thereafter the PCV decreased continually and attained the severely lowest value (10.5±0.7) between 66-78 days post inoculation. For goats the mean PCV decreased to 20±0.08% by day 6 post inoculation and then drop down in PCV attained its lowest (15±0.03%) by day 14 post inoculation. Thereafter throughout the course of the disease the mean PCV makes up and down waves till the second lowest mean PCV was recorded at day 37 post inoculation. The lowest PCV was beyond 37 days post inoculation for goats but it was beyond 66 days post inoculation for calves. The wave of up and downs was almost similar in two species even though the numbers vary. All infected calves and goats similarly developed progressive neutropenia, an initial lymphocytosis, monocytosis and eosinophilia. In general as the course of infection increased severe pan leukopenia was observed and this could cause immunosuppression which intern could be due to reduced myelopoiesis for both species (the exact number of leukocytes vary for calves and goats). In general neutropenia and monocytosis continues for longer period than other leukocytes and these leukocytes if studied and amalgamated with other factors might help in diagnosis of animal trypanosomiasis. The weight loss was progressive for calves and goats and as the course increased loss was worsened in both species.

Keywords: Calves; Goats; Leukocytes; PCV; *Trypanosoma vivax*; Weight

Introduction

Agriculture is the mainstay of the Ethiopian economy; it employs over 80% of the adult population and account for 45% of the GDP and 85% of the export earnings. Livestock production performs several functions primarily as source of household incomes, food and animal drought power for livestock producers. However, livestock production has faced many constraints one of which include animal diseases and in particular trypanosomosis has exerted adverse effect on the whole pattern agricultural activities [1].

Trypanosomosis in domestic livestock causes a significant negative impact in food production and economic growth in many parts of the world, particularly in sub-Saharan Africa and it has greatly hampered people and animals settlement [2]. Trypanosomiasis is one of the most important serious diseases of livestock and humans in worldwide which cause serious disease in domestic animals and human beings in sub-Saharan Africa. Tsetse transmitted trypanosomosis affects 37 sub-Saharan countries, an estimate of 160 million cattle and 260 million sheep and goats are kept in this area of risk extending over 10 million km² of land [3].

Bovine trypanosomosis is clinically none describable disease with many clinical manifestations, in which the course of the disease may run from a chronic long lasting to an acute and rapidly fatal one based on the rate of infestation and the immune status of the animal [4,5]. The disease has economic important due to loss of condition, reduction in milk yield, decrease capacity of work and it may affect the quality of the semen in bulls and also it may cause irregular estrus, abortion and still birth in cows [6,7].

Six species of trypanosomes were recorded in Ethiopia and the most important trypanosomes, in terms of economic loss in domestic livestock are the tsetse-transmitted species: *T. congolense*, *T. vivax* and *T. brucei*. The closely related *T. brucei* subspecies, *T. b. rhodesiense* causes human sleeping sickness. The other *Trypanosoma* species of economic importance are *T. evansi* of camels and *T. equiperdum* of horses [8]. Trypanosomosis caused by *Trypanosoma vivax* is a debilitating and usually fatal disease of domestic ruminants in Africa [9,10].

In Ethiopia, the prevalence of mechanically transmitted *T. vivax* and *T. evansi* reported by various workers has been indicated the wider distribution in the country and the diseases impact due to both parasite is substantial. The presence of mechanical vectors, existence of reservoir hosts, and the involvement of wider host range in parasites, the various agro-climatic zones and the poor veterinary infrastructure

would undoubtedly ensure the existence of *T. vivax* and *T. evansi* in Ethiopia [11]. Trypanosomosis complex was described by the world health organization (WHO) as serious diseases lacking effective control measure and all mammalian species are susceptible to the infection [12,13].

Accurate diagnosis of trypanosome infection in livestock is required for a proper understanding of the epidemiology of the disease in any geographical locality. However, high parasitemia are usually evident only in early infections, and in chronic phase of the disease, parasitemia apparently be absent from the blood for long intervals. This is due to the ability of the trypanosome to establish the prolonged infections attributed to the phenomenon of antigenic variation [14]. Hence, a diagnostic method with high degree of sensitivity and specificity is required like PCR, besides direct parasitological diagnostic methods with varying degree of sensitivity and specificity are available for trypanosomosis [15]. Therefore, this study was undertaken with the objectives of study on pattern of changes in PCV in relation to the parasites in the blood to evaluate the PCV ranges at which the chance of getting parasites from blood increased and study on pattern of changes in WBC and to evaluate whether any of the leukocytes help in diagnosis of chronic trypanosomosis.

Materials and Methods

Study animal

Four female calves aged 1-1.4 years and weighing 86-100 kg and three male goats were included in the study. Animals were bought from local market and they are local breed. These animals were kept in Addis Ababa University school of Veterinary Medicine fattening and dairy farm plant which is found around Lake Babo Gaya. Experimental animals were maintained on the same diet such as straw, wheat bran and water provided with water trough. All the animals were kept in fly proof stable to avoid parasite transmission and external transmission. They were screened for haemoparasitism, helminthiasis and other common infections before the study by physical, blood and faecal examination after which they were treated with Albendazole, long acting Oxytetracycline, Diaminazine aceturate and diazinon sprayed against ectoparasite infestation.

Study design

The study design was Experimental study. Animals were grouped in to two based on their species. Before starting the study, they were checked, if they were free from a disease. The animals were followed for 84 days and then change in disease status was recorded during the study time.

Inoculation of parasite (*T. vivax*) and blood sample collection

Wild Strain of *T. Vivax* used for this study was collected from cattle found around Gibe Valley and the media used for transportation and preservation was cryo medium and transported with liquid nitrogen. All calves were infected with 1 ml blood intravenously. A fresh blood sample was collected directly from the jugular vein of calves and goats into a vacutainer test tube having a sufficient amount of EDTA anti coagulants.

Haematological examination

For haematological examination 5 ml of blood sample was collected from jugular vein of each animal. Two haematocrit capillary tubes were filled with collected blood sample at 3/4 of the height and sealed with Cristaseal. The first capillary tube was used to measure PCV and conducted Woo's test. The PCV was measured after the capillary tubes containing blood were centrifuged for 5 min at 12,000 rpm in microhematocrit centrifuge for the determination of anemia and comparison of animal before and after infection. At the meantime, the prepared capillary tube was placed in the viewer chamber and a drop of water added on top of the capillary tube and covered with cover slip (Woo's test performed) and then searching for viable parasites [16]. The second capillary tube was then cut; the Buffy coat and upper most layers of the red blood cells will be poured on a slide and covered with a 22 × 22 mm cover slip and examined under microscope for the presence of Trypanosomes. For the laboratory tests blood sample was taken for 84 days in two-day intervals.

Differential leukocyte count

Thin smear was prepared after blood was collected and mixed gently. This was done by dropping a drop of blood on one side of glass slide and spreading it by another glass slide which is placed at 45 Co inclinations. The slide waved in the air to dry and identified by writing identification number of animal on thick end of the smear with a pencil. The slide was stained with dip quick stain. The smear was dipped in solution (I) of quick dip used for fixative five times quickly, then dipped into solution (II) again five times quickly. Yet again, it was dipped for the third times in solution (III) five times quickly and the smear was air-dried. Stained blood smear was examined under oil immersion objective for accurate cell identification and careful studies of abnormal blood cells were indicated. The examination was started at the thin end of the smear and a systematic meander of the slide was made, a recount of the same field is avoided [17].

Body weight and rectal temperature examination

Rectal temperature was determined by means of a short blunt-bulb clinical thermometer which records the highest temperature reached and body weight was measured by measuring heart girth and length of animal.

Data analysis

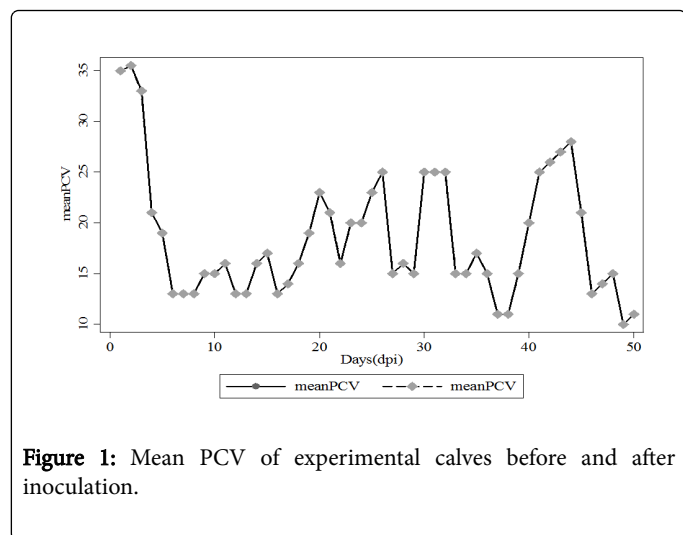
Data was recorded and entered to Microsoft Excel sheet and analyzed by using STATA version 11; Descriptive statistics were used.

Results

Packed cell volume (PCV) of calves

The mean PCV was 34%, 33%, 35.5%, and 33.25% respectively, at the first week, second week, third and fourth week before inoculation. The mean PCV started decreasing between 4-6 days post inoculation for all calves to 21.5 ± 1.1 and then sharply decreased to 13 ± 0.5 in day 13-17 post inoculation. Between days 19-24 post inoculation the mean PCV was ascended to 21 ± 2.2 and decreased to 14.6 ± 1.14 between 26-35 days post inoculation. Thereafter the PCV was decreased continually and attained the severely lowest value (10.5 ± 0.7) between 66-78 days post inoculation (Figure 1).

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Leukocyte changes in calves

The leukocyte changes observed were characterized by relative short initial lymphocytosis and longer monocytosis and decrease in the neutrophil counts. All infected calves similarly developed progressive neutropenia and the smallest mean neutrophil count (16 ± 9.55) was observed 21 days (average) post inoculation. Subsequently, the cyclic pattern of neutropenia continued throughout the course of study. However, its peak was much lower than the normal count. An initial lymphocytosis was observed in three of the four infected calves but later on in the course of infection lymphopenia with alternate lymphocytosis continues throughout the course in all calves. The first few days following infection constant monocytosis was observed and continues for long period through the course of infection than any other WBC counts, but as the animals' condition deteriorated the leukocytes including the monocytes revealed diminishing. Eosinophils was also started increasing few days after inoculation but comparing to the monocytes the eosinophils started declining in a shorter days post inoculation (Figure 2). In general as the course of infection increased severe panleukopenia was observed and this could cause immunosuppression which intern could be due to reduced myelopoiesis.

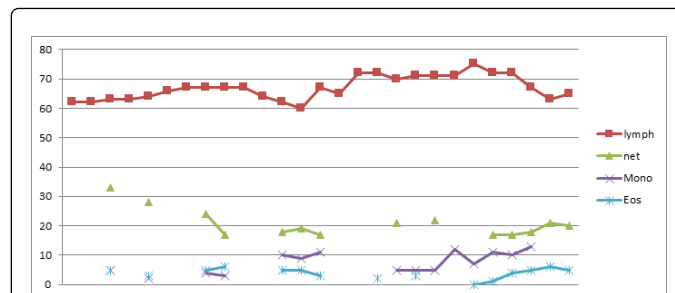


Figure 2: Pattern of leukocyte change before and after inoculation.

Post infection weight loss in calves

In all infected calves the weight loss was started at an average of day 9 post infections and thereafter the weight loss continued progressively. Severe weight loss was recorded after 30 days post inoculation and an average weight loss of 5 Kg was observed between day 30 to 39 post inoculations. As the days post inoculation continued the weight loss was even severely aggravated (Figure 3).

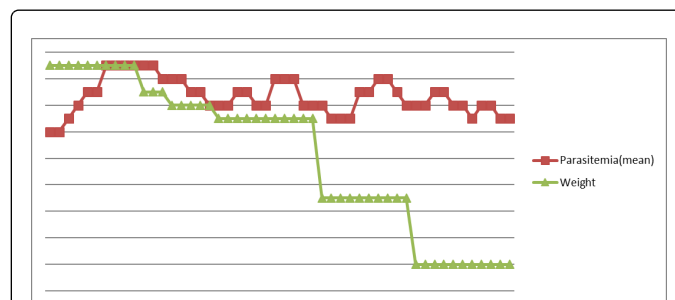


Figure 3: Pattern change before and after inoculation.

Goats packed cell volume (PCV)

The mean PCV of goats in the first few days before inoculation were 32.8%, 34.2%, 35.8%. However, the mean PCV were decreased to 20% at the day 6 post inoculation and then drop down in PCV reached its lowest (15%) at the day 14 post inoculation. After this day throughout the course of the disease the mean PCV was up and down till the second lowest mean PCV was recorded at day 37 post inoculation (Figure 4).

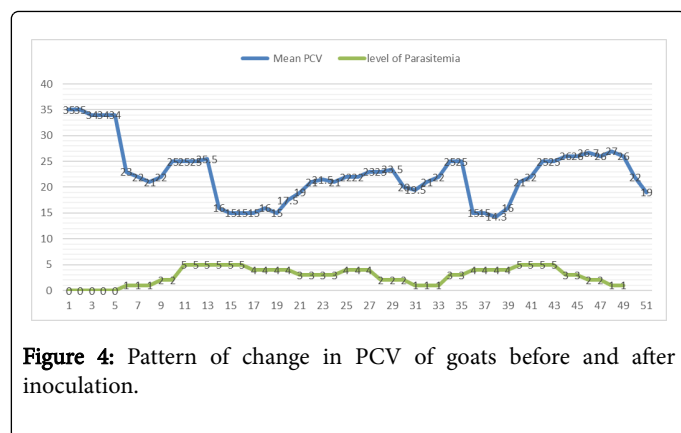


Figure 4: Pattern of change in PCV of goats before and after inoculation.

Goats weight loss before and after inoculation

All the goats included in the experiment showed increasing of weight gain before inoculation of the parasite. However, the weight started moving downward following inoculation and at day 13 following inoculation of the parasite weight decreased by 1 kg. Likewise weight loss continued progressively irrespective of the parasitemia level (Figure 5).

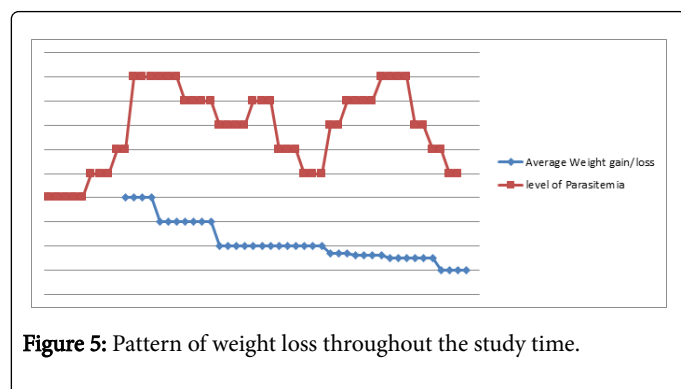


Figure 5: Pattern of weight loss throughout the study time.

Goats leukocyte changes

In all experimental goats with a similar manner an initial lymphocytosis followed by lymphopenia was observed. The infected animals were developed neutropenia, eosinopenia and monocytosis throughout the course of the disease. As the days post inoculation increased variation in individual leukocytes count was observed. Higher levels of leukopenia were observed throughout the study and this might be attributed to a reduced myelopoiesis (Table 1).

Lymphocytes%	After infection	Normal value
Neutrophils (%)	26.5 ± 0.08	33.99 ± 0.59
Lymphocytes (%)	31 ± 0.21	61.69 ± 0.59
Monocytes (%)	6.33 ± 0.39	1.98 ± 0.11
Eosinophils (%)	1.7 ± 0.2	2.69 ± 0.18
Basophils (%)	NO	0.22 ± 0.03

WBC (103)	4.54 ± 0.54	11.32 ± 0.27
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Table 1: White blood cells differential count (Mean ± SD) before and after inoculation. No=Not observed.

Discussion

It is known that the development of anemia is the most reliable indicator of the progress of trypanosome infection in cattle but it can also be assumed that numerous concurrent diseases and nutritional factors interfere with anemia development [18]. During PCV determination a value of 24-36% was considered to be normal [19] but anemic if below 24%. In both calves and goats in this study anemia is the principal clinical finding seen and this is attributable to the extra vascular destruction of RBCs [20]. And is possibly the result of increased red cell destruction by phagocytosis in the spleen, lungs, haemal nodes and bone marrow [8].

In present study the mean PCV value was 35.5% before infection and immediately following infection with *T. vivax* the PCV starts declining and decreased continually and attained the severely lowest value (10.5+0.7) between 66-78 days post inoculation. Camus and Martrenchar reported a Guyanese strain of *T. vivax* that was pathogenic for Brahman zebu [21]. In their study, an experimental infection of one-year-old cattle was followed by moderate and transitory fever, drop in PCV, and quick and marked weight loss of 10 to 17 kg within one month, compared with non-infected animals. The present finding also agrees with the result of Daniel et al. that stated severe anemia mostly associated with *T. vivax* infections as compared to other species of trypanosomes [22]. The decline in PCV also agree with result of Abebe the initial fall in PCV value is associated with the first wave of parasitemia [8]. In the early stage of the disease, which can last up to 12 weeks in cattle the main features are a fluctuating parasitemia together with anemia. It was observed in this study that the pattern of decline in PCV was not constant and sometimes the PCV starts increasing and then shortly decreasing even if the animals were positive for parasites and throughout the study periods all animals remained parasitemic. The severity of the anemia is directly related to the level of parasitemia [23]. High parasitemia are usually evident only in early infections and in chronic phase of the disease [14].

The leukocyte changes observed were characterized by relative short initial lymphocytosis and longer monocytosis and decrease in the neutrophil counts. The pattern of change of Neutrophils and Monocytes were more constant than any other leukocytes in this study. In all infected calves the neutropenia continued to 21 days (average) post inoculation and thereafter the mean neutropenia made wave of up and downs a peak of which is still lower than the normal count. The monocytosis was observed and continued for long period through the course of infection than any other leukocyte but totally leucopenia was observed. This finding is inline with the other works Anosa, Esievo and Jenkins [24-26]. In the present study progressive weight loss was observed and as the days post inoculation continues the weight loss was severely decreased with average weight loss of 5 kg in midway and 10 kg as the course continued. This finding is in agreement with others work; Camus and Martrenchar reported even quick and marked weight loss of 10 to 17 kg within one month [21]. Okech et al. reported weight loss of 24 kg 6 weeks post inoculation [27]. Study conducted by Silva et al. reported lethargy, weakness, substantial weight loss in relatively short time following *T. vivax* infection [28].

Even though current study was conducted on two different species, almost all the measured parameters were common to calves and goats infected with trypanosomes. In present study infected goats shows fever, anemia (low PCV) values and progressive weight loss. Fever is a sign of the presence of infectious disease including trypanosomosis which is due to the reaction between pathogens and body defenses [29]. The experimental result also agreed with that following the invasion of the blood by actively dividing trypanosomes is associated with increased body temperature and initial parasitemia and fever usually persist for several days before a trypanolytic crisis occur after which parasitemia reduced and the temperature returns to physiological value [23].

The gradual fall of PCV in goats in the current study was also agreed with that of Tadesse [30]. This progressive fall in PCV value during trypanosome infection has also been described in different breeds of sheep and goats [31]. Despite the importance of anemia, the exact mechanisms underlying its induction remained unsolved [32]. In the current finding 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 [33]. This author suggested that *in vivo* anemia may be linked to B-cell responses and that antibody mediated lysis may be contributing factor. Other studies have suggested that trypanosomes themselves release components which directly induce lysis of RBC. Likewise 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 [31].

In a similar manner to that of calves in goats the change in differential leukocyte was initial lymphocytosis followed by lymphopenia. The infected animals developed neutropenia that continued for longer time before fluctuation and eosinopenia and as well as monocytosis which was constant for longer period. In general in the first two weeks there were total leukocytosis particularly because of lymphocytosis and monocytosis but on wards total leucopenia onsets as the animal's body getting deteriorated. This finding is inline with other works Dagnachew who stated that the infected goats showed a sharp increase in leukocytes in the first few weeks after inoculation in goats but short in duration in the acute phase [34].

This work revealed that progressive weight also observed in goats and this result agreed with Batista et al. who showed the low body scores were attributed to the weight loss that is always associated with high parasitemia and anemia [35]. Small ruminants can be asymptomatic carriers and consequently are an important source of *T. vivax* for all others ruminants [35].

African animal trypanosomiasis (AAT) or nagana causes gradual health decline in infected livestock, reduces milk and meat production, increases abortion rates, and animals eventually succumb to the disease. This has an enormous impact on the livelihood of farmers who live in this area, as infected animals cannot be used to plough the land, and keeping cattle is only feasible when the animals are kept under constant prophylactic treatment with trypanocidal drugs. The economical impact imposing by the disease directly affects the milk and meat productivity of animals, reduce the birth date and increase the abortion rates as well as mortality rate; all of these affect the herd size and hared composition.

Conclusion

The PCV started decreasing with in the first week of inoculation and continued decreasing to the third week post inoculation and latter on the PCV started ascending for short time period and then started decreasing followed by short increase in PCV and making waves of ups and downs. For all calves the severely lowest value of PCV were beyond day 66 post inoculation and was beyond 37 days for goats. In this study the animals remained positive for parasites whenever the lowest the PCV was recorded. We concluded that measuring the PCV helps diagnosis but cannot be sole diagnostic by itself as the PCV shows great variation. Following infection leukocytosis because of lymphocytosis and monocytosis occurred but as the course of the disease continue pan leukopenia was characteristic for all calves. Neutropenia and monocytosis persists for a considerably long time through the course than other leukocytes. The patterns of changes of hematological values were almost similar for goats and calves although there was no species difference.

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