ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

EFFECTS OF TRYPANOSOMOSIS ON HEMATOLOGICAL AND PLASMA BIOCHEMICAL REFERENCE PARAMETERS OF SMALL RUMINANTS IN SOUTH WEST ETHIOPIA

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TESFAYE MULATU

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ABBREVIATIONS

μl	micro liter
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotranferase
CSA	Central Statistics Authority
g /dl	gram per deciliter
GGT	Gama-glutamyl transferase
GOT	Glutamic oxaloacetic transaminase
GPT	Glutami pyruvic transaminase
ILCA	International Livestock Centre for Africa
ILRAD	International Laboratory for Research on Animal Diseases
ILRI	International Livestock Research Institute
ISCTRC	International Scientific Council for Trypanosomiasis Research and Control
LDH	Lactate dehydrogenase
m.a.s.l	meters above sea level
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
mg /dl	milligram per deciliter
ml	milliliter
mmol /L	millimole per liter
MOARD	Ministry of Agriculture and Rural Development
nm	nanometer
NTTICC	National Tsetse and Trypanosomiasis Investigation and Control Center
OIE	Office International Des Epizooties
PCV	Packed Cell Volume
RBC	Red Blood Cell
SD	Standard Deviation
IU/L	International Unit per liter

ABSTRACT

A study was carried out to investigate the effect of trypanosomosis on the hematological and plasma biochemical parameters of small ruminants (sheep and goat) at Gera and Seka-Chekorsa districts in the Gojeb valley of Ghibe Omo river system, Jimma Zone, Oromiya region in the southwest of Ethiopia. The mean hematological values of RBC (11.8±2.8 $X10^6$/µl), PCV (24.2±4.1%), MCV (21.3±4.7fl), neutrophil (30.6±12.8%), monocyte (3.2±2%) and basophil (0.8±0.9%) for small ruminants (sheep and goats) raised in high tsetse challenge area were found to be smaller than the values of RBC (12.7±3.6 $X10^6$/µl), PCV (26.8±9.8%), MCV (22.3±8.4fl), neutrophil (32.7±10.5%), monocyte (4.6±3.2%) and basophil (0.6±0.8%) in low tsetse challenge area and the variation was statistically significant ($P < 0.05$). Smaller values of PCV and MCV in the hematological parameters of small ruminants in the high challenge area could be the cause of microcytic type of anemia. Significant differences ($P < 0.05$) were also observed between sheep and goat species within the high challenge area in the values of MCV (21.9±4.7fl for sheep, 20.7±4.6fl for goats), neutrophil (31.8±12.8% for sheep, 29.2±12.6% for goats) and monocyte (2.9±1.7% for sheep, 3.5±2.3 %for goats) where the values were found to be higher in sheep than goats. In the biochemical analysis values for AST (84.8±25.8IU/L), ALT (13.5±6.5IU/L), creatinine (1.1±0.2mg/dl), and total protein (4.9±1.6g/dl) were smaller in small ruminants (sheep and goats) raised in high tsetse challenge area than the values of AST (116.5±44.9IU/L), ALT (19.1±9.1IU/L), creatinine (1.2±0.3mg/dl), and total protein (7.6±2.9g/dl) in low challenge area and the difference in values between the areas were significant ($P < 0.05$). Within the high challenge area significant difference in some biochemical parameters ($P < 0.05$) were also observed between sheep and goat species whereby the values of AST (91.3±25IU/L), ALT (14.3±7.3IU/L), and creatinine (14.3±7.3mg/dl) were found to be higher in sheep than the values of AST (80.3±46IU/L), ALT (12.6±5.4IU/L), and creatinine (1.1±0.2mg/dl) in goats. The study also showed higher values of cholesterol (94.5±32.5mg/dL) in small ruminants (sheep and goats) raised in high tsetse challenge area than the values of cholesterol (61.7±42.1mg/dL) in low challenge area whereas the values for triglycerides (93.4±43.7mmol/L) were found to be smaller than the values for triglycerides (107.5±37.4mmol/L) in the low challenge area and the difference in both cases were statistically significant ($P < 0.05$). Thus it can be concluded from the study that trypanosomosis has a prominent effect on the hematological and plasma biochemical

parameters of small ruminants in the high tsetse challenge areas of South Western parts of Ethiopia and attention should be given in utilizing the values of these parameters for assessing the physiological status of animals for diagnostic purpose.

Key words: Trypanosomosis, small ruminant, hematological parameters, biochemical parameters, southwest of Ethiopia

1. INTRODUCTION

Trypanosomosis is a disease caused by several species of protozoan parasites of the genus *Trypanosoma*, which differ in host range, severity of disease and other biological criteria (Masiga *et al*., 2002). In Africa, the disease is mainly transmitted by tsetse flies (*Glossina* species) in an area of about 7-10 million km^2 and still it is one of the most serious threats to health of man and a serious obstacle to the development of agricultural industry (Molyneux and Ashford, 1983; FAO, 1979). In Ethiopia tsetse born trypanosomosis is excluding some 180,000-200,000 km^2 of agriculturally suitable land in the west and southwest of the country, and about 14 million head of cattle, an equivalent number of small ruminants, nearly 7 million equines and 1.8 million camels are at the risk of contracting trypanosomosis at any one time (Langridge, 1976; MOA, 1996; MOARD, 2004). Moreover, new settlements of people in tsetse occupied areas have brought livestock in closer contact with tsetse and trypanosomosis and triggered serious disease outbreaks with heavy morbidity and mortality losses in the newly introduced stock in particular. Thus trypanosomosis is one of the major impediments to livestock development and agricultural production in contributing negatively to the overall development in general and to food self-reliance efforts of the country in particular (Lemecha *et al.,* 2006).

Small ruminants (sheep and goats) in Ethiopia form an important component of the livestock system; they provide meat, milk, hides and skin. The adaptive features such as feeding behavior, feed efficiency and disease tolerance, make goats to thrive on natural resources left untouched by other ruminants. The small size and efficient reproductive performance also cause goats to be considered as a "current account" or working capital for pastoralist and small subsistence farmers (MacFarlane *et al.*, 1982). For many years, it was widely believed that goats and sheep were little affected by trypanosomosis (Stephen, 1970). Nevertheless, there is evidence that goats and sheep naturally acquire trypanosomal infections. In a review of trypanosomosis in sheep and goats Griffin (1978) indicated that many of the accounts of naturally occurring trypanosomosis in sheep and goats came from East Africa caused by *Trypanosome congolense*. Hecker (1994) found that nutritional supplementation delayed but not prevent the establishment of trypanosomal infections in sheep exposed to high trypanosomosis risk in Northern Côte d’Ivoire. Studies conducted in neighboring countries of Ethiopia with similar ecological situations indicate that levels of

infection in small ruminants can be significant and an increase in tsetse numbers which occurred one month after substantial rainfall was followed by an increase in the prevalence of trypanosomosis in small ruminants (Griffin and Allonby, 1979). The changes in the blood lipids observed in infected sheep appeared to be related with trypanosome infection (Katunguka *et al*., 1992).

In Ethiopia although the epidemiology of trypanosomosis in sheep and goats in the tsetse-infested areas is not well documented. There is evidence that in some parts of the country trypanosome infections have been detected in small ruminants. In Wolyta of Southern region it was demonstrated that trypanosomosis infections in goats was 4.8% whereas an investigation carried out in three zones of Oromia regions; Jimma, Wellega and Illubabor showed an infection rate of 2.4% in sheep and 2.7% in goats (TCS, 1983). Recent study conducted by Abebe and Dinka (2005) in South West Ethiopia also revealed an infection rate of 7.7% in sheep and 3.6% in goats. Thus trypanosomosis in sheep and goats is an important disease and small ruminants serve as potential reservoir of infection for other animals.

Blood is an important and reliable medium for assessing the health status of individual animals. The high metabolic demands of the rapidly dividing trypanosome arise during the infections are fulfilled in the blood. During these periods there are major changes in glucose and a range of other substrates and catabolites. There is an overall reduction in both erythrocytes and white blood cells. In general the blood composition and it's hematological and biochemical parameters become profoundly disturbed (Maudlin *et al*., 2004).

Even though understanding of these hematological and biochemical parameters in different breeds of animals during infection with trypanosome parasite has a paramount importance in establishing baseline information for assessing the physiological status of animals for diagnostic purpose. In Ethiopia works attempted on small ruminants to determine the effect of trypanosomosis on the hematological and biochemical parameters is still scarce.

Therefore the objective of this study is:

- To investigate the effect of trypanosomosis on the hematological and biochemical parameters of small ruminants (sheep and goats) raised under local husbandry practice in South Western tsetse challenge areas of Ethiopia.

2. LITERATURE REVIEW

2.1 Parasite

Trypanosomes are spindle-shaped organisms very variable in size from 15µm to 100µm depending on species and sub genus. The characteristic morphological forms are trypanomastigotes, typically found in the blood and tissues of vertebrate hosts, and the epimastigote forms which are found in the invertebrate vector. The infective forms which are produced in the vector are known as metacyclic and they are trypanomastigotes in configuration (Molyneux and Ashoford, 1983).

In Ethiopia four tsetse borne trypanosome species, *T. congolense*, *T. vivax* and *T. brucei brucei* of livestock and *T. brucei rhodesiense* of humans have been identified and their distribution and frequency in hosts recorded. According to Langridge (1976) the two commonest trypanosomes of livestock in Ethiopia are *Trypanosoma vivax* and *T. congolense*. When livestock is in contact with tsetse the transmissions were cyclical and *T. congolense* was predominant but when livestock were not in contact with tsetse *T. vivax;* which can be transmitted mechanically became dominant (Langridge, 1976). From the genus *Trypanosoma* the disease is mainly caused by *Trypanosoma congolense, T. vivax,* and to a lesser extent *T. brucei brucei*, *T. uniforme, T. simiae* and *T. suis* are other less common tsetse-transmitted species (OIE, 2002).

2.2. Vector

The vectors are haematophagous arthropods (insects and arachnids) but leeches (*Hirudinae*: *Annelida*) act as vectors of some fish and amphibian trypanosomes. In Africa, the disease is mainly transmitted cyclically by tsetse flies (*Glossina* which belong to the family *Glossinidae*, order *Diptera*). Living Glossina is restricted to Sub-Saharan Africa north of Kalahari and there are 22 described species (Molyneux and Ashoford, 1983). Five species of tsetse are found in Ethiopia: *Glossina longipennis*, *G. morsitans submorsitans*, *G. pllidipes*, *G. fuscipes fuscipes* and *G. tachinoides*, and are confined to the Southern and South-western regions of the country (Langridge, 1976; MOA, 1996; MOARD, 2004).

Biting flies such as *Tabanidae*, *Stomoxyinae* and *Hippoboscidae* are capable of mechanically transmitting trypanosomes in their mouth parts if they feed on more than one host within a short interval. This is how *T. vivax* spread in areas out side the tsetse belt in Africa, Central and South America. Mechanical transmission can also occur through contaminated needles, syringes and surgical instruments and in carnivores feeding on infected animals. Transmission of *T. equiperdum* during coitus between mares and stallions has been also indicated. In addition, intrauterine infections occasionally occur with different species of trypanosomes (Molyneux and Ashoford, 1983; Radostits *et al*., 1994).

2.3. Blood and importance of determining hematological and biochemical parameters

Blood is one of the most important and frequently examined indexes of physiological and pathological alterations in animals and human (Andrews *et al*., 1990). Whole blood comprises fluid and cells. The fluid component is plasma and comprises about 90 % water and 10 % dissolved constituents, such as proteins, carbohydrates, vitamins, hormones, enzymes, lipids, salts, waste materials, antibodies and other ions and molecules. The cellular component is made up of red blood cells (RBC), or erythrocytes; white blood cells (WBC), or leukocytes; and platelets, or thrombocytes (Colville, 2003).

The trypanosome infections are generally characterized by anemia, leucopenia, thrombocytopenia as well as biochemical aberrations such as hypoglycemia, elevated blood urea nitrogen and hyperglobulinaemia (Anosa, 1988).

2.3.1. Blood cells (erythrocyte and leukocyte)

The red blood cells are derived from the erythroblasts, which are continuously being formed from the primordial stem cells in the bone marrow. In developing into a mature red cell, the primordial stem cell moves through a series of stages: erythroblast to normoblast to reticulocyte and finally to erythrocyte. During its transformation from normoblast to reticulocyte, the red blood cell loses its nucleus and hemoglobin synthesis begins at the erythroblast stage and continues until the cell becomes an erythrocyte. The four polypeptide chains of hemoglobin attached to a heme unit, which in turn, surrounds an atom of iron that binds oxygen. Therefore, the function of red blood

cell, facilitated by the hemoglobin molecule, is to transport oxygen to the tissues .In addition, hemoglobin binds some carbondioxide and carries it from the tissues to the lungs (Porth, 1990).

The leucocytes, or white blood cells, which are larger and less numerous than the red blood cells, develop from the primordial stem cell located in the bone marrow and lymphoid tissue serve to protect invasion by foreign agents. There are two types of white blood cells, granular (neutrophils, eosinophils, and basophils) and non granular leukocytes (monocytes and lymphocytes) (Porth, 1990).

2.3.2. Importance of determining hematological parameters

The red blood cell count (RBC) measures the total number of RBCs in cubic millimeter of blood. The hemoglobin (grams per 100 ml of blood) measures the hemoglobin content of the blood. The packed cell volume (PCV) which can be determined by the micro-haematocrit centrifugation technique used as a quantitative expression of anemia. Packed cell volume is a most useful index in assessing the progress of trypanosomiasis and the degree of anemia. Additionally, PCV is a reliable indicator of the extent and severity of infection as well as the performance of the host (ILCA, 1986). The mean corpuscular volume (MCV) reflects the volume or size of red cells. The MCV falls in microcytic anemia and rises in macrocytic anemia. The mean corpuscular hemoglobin concentration (MCHC) is the concentration of hemoglobin in each cell and it decreases in hypochromic anemia. Mean cell hemoglobin (MCH) refers to the mass of red cell and is less useful in classifying anemias. Determination of PCV, MCV, MCHC, and MCH, signify not only anemic disorders, but also show imbalances induced by a deficiency of ions and plasma proteins in the blood (Coles, 1986; Josh *et al*., 1991).

2.3.3. Plasma enzymes and other biochemical components

Enzymes are protein catalysts synthesized by all living things. As catalysts their only biologic activity is to alter the rate at which equilibrium is established between reactants and their products. Plasma (serum) enzymes can be placed into two distinct classes. The first consists of the plasma-specific enzymes which are those that have a definite and specific function in plasma. Their normal site of action is in the plasma, and they are present in higher level in plasma than in most tissue cells. The second class consists of the non plasma specific enzymes which are present in concentrations much lower than their concentration in certain tissues. This group is divided

into two sub classes: (1) enzymes associated with cellular metabolism and (2) enzymes of secretion. Enzymes of cellular metabolism are located within tissue cells and are present there in high concentrations as long as the cell remains healthy and the membrane is intact. The level of these enzymes in extracellular fluid and plasma is low. Secretory enzymes are rapidly disposed of through excretory channels such as the intestinal tract, urine, and bile and by inactivation and degradation their normal plasma levels are relatively low and are constant (Coles, 1986).

Alterations in serum enzyme activity due to malfunctioning of the liver occur as a result of three processes: (1) an elevation of enzymes due to disruption of hepatic cells as a result of necrosis or as a consequence of altered membrane permeability. Included in this group are the enzymes alanine amino transferase (ALT) (formerly known as glutamic pyruvic transaminase [GPT]), aspartate amino transferase (AST) (formerly called glutamic oxaloacetic transaminase [GOT]), and lactic dehydrogenase (LDH). (2) A decrease in concentration in the serum resulting from impaired synthesis by the liver (choline esterase). (3) An elevation in enzyme levels due to cholestasis. The enzymes affected include alkaline phosphatase (ALP), Gama-glutamyl transferase (GGT), and leucine amino peptidase (LAP) (Coles, 1986).

The only sugar normally found in blood is glucose, which is stored in the form of its polymer, glycogen. Normally, glycogen is found only in the intracellular form, whereas glucose is found almost exclusively in extracellular fluids. The level of glucose is maintained within a relatively narrow range and is controlled by several factors including: (1) hepatic and renal uptake and release of glucose, (2) glucose removal by the peripheral tissue, (3) effects of hormonal influences on these processes, and (4) intestinal absorption of glucose, which has only a temporary effect on blood levels (Coles, 1986).

The total plasma protein measurement which includes fibrinogen values may be affected by altered hepatic synthesis, rapid albumin breakdown or excretion during disease.

Creatinine is irreversibly formed from creatine that is found in skeletal muscle, as part of muscle metabolism. It rapidly diffuses out of the muscle cell into the blood. The creatinine in blood is filtered through the glomeruli and eliminated in urine with out renal tubular reabsorption (Colville, 2003; Meyer and Harvey, 1998).

Lipids, including cholesterol and triglycerides serve as hormones or hormone precursors, an aid to digestion, they provide energy storage and metabolic fuels, act as functional and structural components in biomembranes in man, animals and their parasites, and form insulation to allow nerve conduction or to prevent heat loss (Stein, 1987). Triglycerides are simple lipids and combine with cholesterol, phospholipids and plasma proteins to form protein-lipid complexes, known as lipoproteins, in plasma (Kaneko, 1980; Milne, 1990).

Importance of determining the level of alanine aminotransferase (ALT)

ALT is a cytoplasmic enzyme that catalyzes the transamination of alpha-ketoglutarate and L-alanine, forming glutamate and pyruvate (Bain, 2003; Kaufman and Greene, 1993). The highest levels of ALT are found in hepatocytes and striated (skeletal and cardiac) muscle cells. Therefore, increased plasma ALT level can accompany hepatocellular injury or necrosis of striated muscle as a result of escape from cytosol (Valentine *et al.*, 1990). Mechanisms of increased level of ALT in plasma include enzyme release from damaged cells or increased enzyme synthesis (Stockham and Scott, 2002).

Importance of determining the level of aspartate aminotransferase (AST)

AST occurs in a wide variety of tissues including liver hepatocytes, cardiac muscle, skeletal muscle, brain, kidneys, lungs, pancreas, erythrocytes and leukocytes, with highest concentration found in liver and skeletal muscle. When disease or injury affects these tissues, the cells are destroyed and AST is released in to the blood stream (Coles, 1986).

Importance of determining the level of serum alkaline phosphatase (ALP)

Phosphatases are agents that hydrolyze phosphoric easters with the liberation of inorganic phosphate. Alkaline phosphatase (ALP) is widely distributed in the body, and is found in high concentrations in bone, intestinal mucosa, renal tubule cells, liver, and placenta (Coles, 1986). In the serum ALP is found in small amount, but when largely elevated it particularly indicates bone or liver disease primarily of an obstructive nature (cholestatic), i.e. involving the biliary drainage system, the alkaline phosphatase will be the first and foremost enzyme to be elevated, unlike

aminotransferase elevation that indicates hepatocyte damage. In bone, elevated level signifies bone cancer or rickets (Coles, 1986; Colville, 2003).

Importance of determining the level of blood glucose

Determination of blood glucose is important in sheep or cattle in which ketosis is suspected, and in young pigs suspected of having a hypoglycemia associated with starvation. In aged animals, as it will often reveal the existence of a disease entity not previously suspected glucose estimation is included in biochemical profiles (Coles, 1986).

Importance of determining the level of total protein

Plasma protein alterations are not usually specific for a particular disease condition. However, certain alterations in the total concentration or a variation in the components comprising the total plasma protein may be of significant both diagnostically and prognostically. Any abnormality in plasma proteins indicates that some pathologic, physiologic, or other induced factor is responsible. Total protein concentrations are especially valuable in determining an animal's state of hydration. Total protein concentrations also are useful as initial screening tests for patients with edema, ascites, diarrhea, weight loss, hepatic and renal disease, and blood clotting problems (Colville, 2003; Coles, 1986).

Importance of determining the level of creatinine

Blood creatinine levels are used to evaluate renal function, based on the ability of the glomeruli to filter creatinine from blood and eliminate it in urine. However it is not accurate indicator of kidney function, because nearly 75 per cent of the kidney tissue must be non functional before blood creatinine levels rise (Colville, 2003).

Importance of determining the level of Lipids (cholesterol and triglycerides)

Assay of triglycerides is one of the best method for diagnosing hyperlipemia, which is a syndrome characterized by negative energy balance and rapid mobilization of fatty acids derived from adipose tissue. The mobilized fatty acids ultimately result in fatty liver and subsequent hypertriglyceridemia (Forhead, 1994; Kaneko, 1980). Decreases in plasma levels of

cholesterol and serum phospholipids have been reported in sheep infected with *Trypanosome congolense* (Katunga-Rawakishaya *et al*., 1991) and in man infected with *T. brucei* (Huet *et al*., 1990). These have been attributed to the fact that trypanosomes take up lipids for growth in the infected host (Katunga-Rawakishaya *et al*., 1991).

2.4. Factors affecting hematological and biochemical parameters

Various factors influence the values of hematological and biochemical parameters in both healthy and diseased animals. These include sex, species or breed of the animal, age of the animal and altitude, and physiological factors. Physiologic factors to be considered includes: age of the animal, breed or species of the animal (Coles, 1986).

2.4.1. Sex

Males tend to have slightly higher RBC count, Hg concentration and PCV than females in different species of animals. The differences however, were not statistically significant and of no practical importance (Bekele 1987; Sekar *et al.,* 1990; Jain, 1993; Sherman and Mary 1994). The slight sex difference existed in leukogram was also reported by (Coles, 1986; Fufa, 1999) and showed that females have higher WBC count than males.

2.4.2. Species/breed

Species variation is marked, ranging from a predominance of lymphocytes in bovine to a prepond erance of segmented neutrophils in canine blood. The influence of breed on hematological parameters has been reported by Coles (1986), Jain (1993) and Fufa (1999). For example, Zebu cattle have more erythrocytes; smaller mean corpuscular and packed cell volumes and a lower hemoglobin level than the Scotch Highland breed (Coles, 1986).

2.4.3. Age

The occurrence of significant age difference in both total WBC and differential leukocyte counts were reported by Jain (1993) where the total WBC count and absolute lymphocyte number were highest in young growing animals with gradual reduction in advancing age. New borne have large erythrocytes of fetal origin, which is later, replaced by smaller cells with reduced MCV

value. The erythrocyte size will become representative of the normal adult of that species by the age of 2-12 months and the extent of decrease in lymphocyte with age is useful when interpreting the degree of stress imposed by diseases. In sheep the number of erythrocytes increases from approximately 7.5 million/µl at one week of age to over 14 million/µl at eight weeks of age. In the goat, the total erythrocyte count is relatively low at birth (7 to 8 million/µl) , reaches its highest level at about eight months of age and gradually falls to stabilize at about 11 million/µl by three years of age. The MCV is higher at birth (45 fl) and declines as the animal ages (Coles, 1986).

2.4.4. Altitude

Animals at high altitude have a greater number of erythrocytes, larger PCV values and higher concentrations of hemoglobin than comparable animals raised at lower altitude (Coles, 1986).

3. MATERIALS AND METHODS

3.1. Study area

The study was carried out at Gera and Seka-Chekorsa districts in the Gojeb valley of Ghibe Omo river system, Jimma Zone, Oromiya region in the southwest of Ethiopia between 07°24'-08°04' N and 036°17'-036°57'E with altitude range of 1290-1800 meter above sea level(Fig.1). In the study area the high tsetse challenge area composes of 12 peasant associations which lie mainly in the valley floor between 1290-1600 meter above sea level and the low challenge area situated between 1600-1800 meter above sea level altitudinal range and composes 12 peasant associations from the escarpment of the valley. Tsetse and trypanosomosis survey results from the 2004 annual report of NTTICC referred for identifying relatively the high and low tsetse challenge areas. According to a population census of CSA the human population in the study area (in both districts of Gera and Seka-Chekorsa) amounts to 1.3 million (or 130467 house holds). The livestock population is estimated at close to 88,257 cattle and 149,746 small ruminants (sheep and goats) (CSA, 2004).

The valley has a pronounced dry season from November to February with rainfall occurring between March and April and from late May to October. The mean maximum temperature ranges from 29.8°C to 44°C. Temperatures are generally lower from June to August and highest from October to May (Leak *et al.,* 1993). The predominant type of vegetation is Combretaceous woodland and the most common trees are *Combretum spp, Terminalia spp, Philostigma spp, Albiza spp, Gardenia spp, Garewia spp,* and *Stereospermum spp* with *Hyperrhenia spp* grass in between. Trypanosomosis is endemic and the area is infested with two tsetse species: *Glossina pallidipes* and *G. fuscipes fuscipes*.

Hosts of tsetse flies in the study area include; warthog (*Phacochoerus aethiopicus*), bush-buck (*Tragelaphus scriptus*), buffalo (*Syncerus caffer*) and hippopotamus (*Hippopotamus amphibious*).

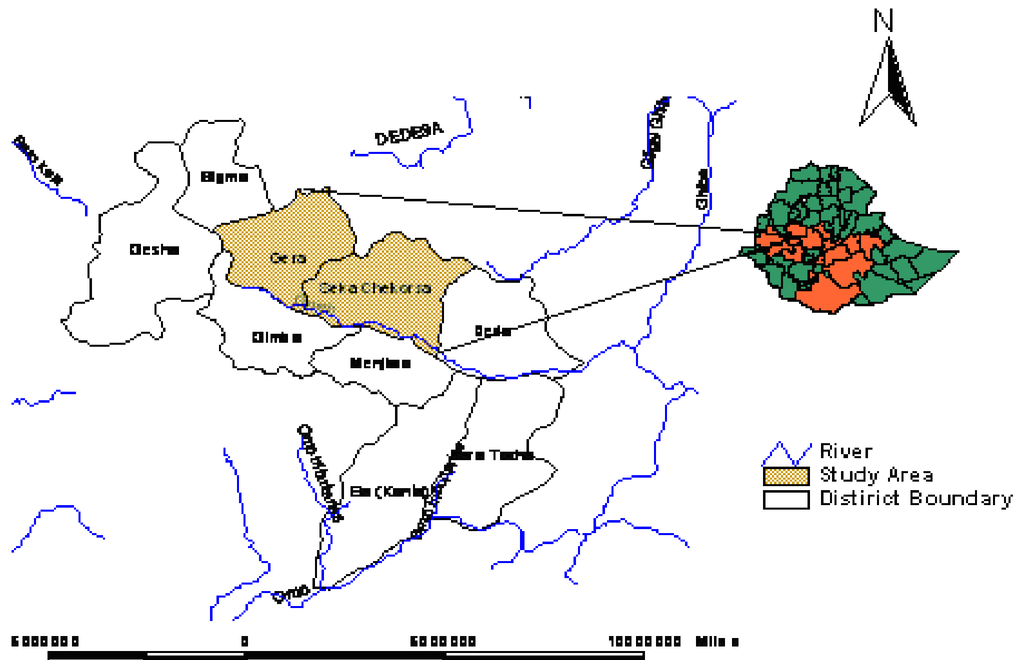

Figure 1. Map showing the study area

Source: Derived from 2004 annual report of National Tsetse and Trypanosomiasis Investigation and Control Center, (NTTICC)

3.2. Study population

Local breeds of sheep and goats raised under native husbandry practice in the study areas were used as a sampling population.

The study was conducted on the randomly selected 237 sheep (176 male and 61 female) and 213 goats (89 male and 124 female) from high tsetse challenge areas; 218 sheep (87 male and 131 female) and 232 goats (60 male and 172 female) from low tsetse challenge areas. All sampling units were tagged and sampling carried on after two weeks of deworming with broad spectrum Albendazole 300mg at a dose of 7.5mg/kg of body weight.

3.2.1. Type of study and sample size determination

A cross-sectional study was conducted to investigate the effect of trypanosomosis on the hematological and biochemical parameters of small ruminants (sheep and goats) raised under

native husbandry practice. The desired sample size for the study was calculated using the formula:

$$n = \frac{1.96^2 \text{ X P expected (1-P expected)}}{d^2}$$

(Thrusfield, 2005), with 95% confidence interval and at 5% absolute precision. Assuming 2.4% average prevalence of trypanosomosis for sheep and 2.7% for goats in high challenge South western high tsetse challenge areas (TCS, 1983) and 237 sheep and 213 goats totally 450 small ruminants (sheep and goats) were sampled randomly from households of Gera and Seka-Chekorsa districts. From low tsetse challenge areas 218 sheep and 232 goats totally 450 small ruminants (sheep and goats) were also sampled randomly from households of Gera and Seka-Chekorsa districts. Therefore, a total of 900 small ruminants were sampled for the study.

3.3. Study methodology

Blood samples were collected from jugular veins of study animals under least excitement into test tubes containing ethylene diamine tetra acetic acid (EDTA) to obtain un-coagulated blood for parasitological and hematological analysis. The blood samples were later centrifuged at 1,200 G for 10 minutes at 37 ^{o}C and the plasma obtained kept at –20 ^{o}C until biochemical analysis.

3.3.1. Parasitological analysis

Blood from EDTA coated vacationer was drown into plain microhaematocrit capillary tube; the tube was sealed and centrifuged for five minutes. Packed red cell volume (PCV) was measured with microhaematocrit reader before the tube was cut about 1 mm below the buffy coat. Fresh preparations of the buffy coat were examined microscopically under phase contrast illumination for the presence of live trypanosomes (Murray *et al.*, 1977). Giemsa stained thick and thin blood smears were also prepared and examined microscopically.

3.3.2. Haematological analysis

Total erythrocytic counts and total leukocytic counts were determined with the aid of Neubaur counting chamber (Haemocytometer) after the blood was diluted 1:200 Hayem‘s solution and

1:20 WBC diluent (1 ml Glacial acetic acid, and 1ml methylene blue, both diluted to 100 ml with distilled water) respectively. Thin blood smears were stained with Wright's stain for differential leukocyte counts which were based on 100 cells per slide and the total number of leukocytes; eosinophils, lymphocytes, neutrophils, basophils and monocytes were obtained. Hemoglobin concentration was determined by Sahl's (acid haematin) method (Benjamin, 1978). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were obtained by calculation from PCV, Hb concentration and RBC counts (Annex 1) (Jain, 1986).

3.3.3. Biochemical analysis

The levels of plasma alanine aminotransferase (ALT), aspartate aminotansferase (AST), alkaline phosphatase (ALP), creatinine, total protein, cholesterol, tryglicerides and glucose were analyzed using photometer 5010 (Robert Riele GmbH & Co KG, Germany, 2002) and commercially available kits (Annex 1).

The level of plasma enzymes was expressed in U/L. The level of plasma AST (GOT) and ALP was determined using kits (Biocon Diagnostik, Germany), whereas the level of plasma ALT (GPT) was obtained using commercial (Human, Germany) kits. Kinetic method was employed for the determination of the level of AST, ALP and ALT. The absorbance of ALP was read at 400 nm wavelength, while that of AST and ALT were read at 340nm wavelengths.
Plasma creatinine concentration (mg/dl) was determined by kinetic colorimetric assay using a kit (Biocon Diagnostik, Germany). The absorbance was read at 492nm wavelength. The concentration plasma triglycerides (mmol/L), whose absorbance was read at wavelength of 546nm, was determined by enzymatic colorimetric test using a commercially available kit (Human, Germany). The level of total protein (g/dl) in the plasma read at wavelength of 546nm, was determined by colorimetric assay using commercially available kit (Biocon Diagnostik, Germany).

3.3.4. Sampling tsetse flies

Tsetse flies were caught using 20 monopyramidal traps sited in tsetse suitable habitats for three days where sheep and goats grazed and browsed. The traps were baited with 1-octen-3-ol, aceton,

and cow urine. The number of tsetse of each sex and species caught in each trap was recorded and the apparent densities of tsetse calculated as the number of tsetse caught per trap per day.

3.4. Data analysis

Data obtained were entered into Microsoft Excel spreadsheet and then by the use of SPSS (Version 13.0, 2004) statistical package the data were subjected to one-way ANOVA, to compare the factors affecting the measured hematological and biochemical parameters after setting the level of significance at 0.05. The factors included the effects of area (high tsetse challenge and low challenge areas), species (sheep and goat), sex (female and male), and trypanosome infection (trypanosome positive and negative). The variables were RBC, PCV, Hb, MCV, MCH, MCHC, WBC, neutrophil, lymphocyte, monocyte, eosinophil, basophil, AST/GOT, ALT/GPT, ALP, creatinine, total protein, cholesterol, triglycerides and glucose.

4. RESULTS

4.1. Parasitological and Entomological results

Twenty four cases were found to be positive for trypanosome parasite from the total 900 sampled study population. High prevalence rate of trypanosome (4.9%) from the total 450 sampled animals was detected in the high challenge study area than from low challenge area (0.4%) with equal size sampled animal (450). In the high challenge area the prevalence rate was higher in sheep (4%) than goats (0.9%) and from the identified *Trypanosome* species *Trypanosome congolense* was the dominant one (54.6%), followed by *T. vivax* (18.2%) and the rest was *T. brucei* (13.6%) and mixed infection of *T. congolense* and *T. vivax* (13.6%). In the low tsetse challenge study area the prevalence rate of trypanosome was very low (0.4 %) and the identified parasite was *T. vivax*.

In the entomological survey carried out to check the current situations of tsetse flies in the study areas a total of 607 flies with an apparent density of 10.1 flies per trap per day were trapped in the high challenge area. From the flies caught 60.8% was *Glossina pallidipes* and 39.2% was *G. fuscipes fuscipes*. Earlier work in Ghibe valley (Lemecha *et al.,* 2006) conducted up on these species reported that the fly infection rate is higher in *G. pallidipes* than *G. fuscips fuscips*. In the low tsetse challenge areas there was no trap found capturing tsetse flies except other biting flies such as stomoxys, tabanus and haematopota.

4.2. Hematological and biochemical result

Results of hematological and biochemical values (Mean ± S.D) of small ruminants by area (high and low tsetse challenge); by species and by sex are shown as follows.

4.2.1. Hematological result

The hematological (Mean ± S.D) values of small ruminants (sheep and goats) raised in high and low tsetse challenge areas are presented in tables 1-6.

Table 1: Values of hematological parameters of small ruminants raised in high and low tsetse challenge area of South West Ethiopia as affected by area

Hematological Parameters	Area	n	Mean ± SD	95% CI for mean	P value
PCV	High challenge	450	24.2±4.1 (%)	23.8-24.6	0.000
	Low challenge	450	26.8±9.8 (%)	25.9-27.7	
RBC	High challenge	450	11.8±2.8countsX10^6/ µl	11.5-12.1	0.000
	Low challenge	450	12.7±3.6counts X10^6/ µl	12.4-13.1	
Hb	High challenge	450	9.9±1.6 gm/dl	9.8-10.1	0.076
	Low challenge	450	10.2±3.4 gm/dl	9.9-10.6	
MCV	High challenge	450	21.3±4.7 fl	20.9-21.8	0.034
	Low challenge	450	2.3±8.4 fl	21.5-23.1	
MCH	High challenge	450	8.8±2.4 pg	8.6-9.1	0.692
	Low challenge	450	8.8±4.7 pg	8.3-9.2	
MCHC	High challenge	450	41.5±5.9 gm/d	41.0-42.1	0.069
	Low challenge	450	39.7±20.5gm/d	37.8-41.6	
WBC	High challenge	450	13.9±4.4 counts X 10^3 /µl	13.5-14.3	0.198
	Low challenge	450	14.3±3.7 counts X10^3/µl	13.9-14.6	
Neutrophil	High challenge	450	30.6±12.8 (%)	29.4-31.8	0.007
	Low challenge	450	32.7±10.5 (%)	31.7-33.6	
Lymphocyte	High challenge	450	57.9±13.7 (%)	56.6-59.1	0.635
	Low challenge	450	58.3±11.3 (%)	57.2-59.3	
Monocyte	High challenge	450	3.2±2 (%)	3.0-3.4	0.000
	Low challenge	450	4.6±3.2 (%)	4.3-4.9	
Eosinophil	High challenge	450	7.6±4.1 (%)	7.2-8.0	0.000
	Low challenge	450	4±4 (%)	3.6-4.3	
Basophil	High challenge	450	0.8±0.9 (%)	0.7-0.8	0.005
	Low challenge	450	0.6±0.8 (%)	0.5-0.7	

The smaller values of RBC, PCV, MCV, neutrophils, monocyte, and higher values of eosinophil and basophil in high challenge area than in low challenge area show statistically significant difference ($P < 0.05$). The values for Hb, MCH, MCHC, WBC, and lymphocyte, were not significantly different ($P > 0.05$) (Table 1).

Table 2: Values of hematological parameters of small ruminants raised in high tsetse challenge area of South West Ethiopia as affected by species

Hematological Parameters	Species	n	Mean ± SD	95% CI for mean	P value
RBC	Sheep	237	11.7±2.8countsX10^6/ µl	11.3-12.0	
	Goat	213	11.9±2.9countsX10^6/ µl	11.5-12.3	0.316
PCV	Sheep	237	24.6±4 (%)	24.0-25.1	
	Goat	213	23.8±4 (%)	23.2-24.4	0.054
Hb	Sheep	237	10±1.6 gm/dl	9.8-10.2	
	Goat	213	9.8±1.6 gm/dl	9.6-10.0	0.153
MCV	Sheep	237	21.9±4.7 fl	21.2-22.5	
	Goat	213	20.7±4.6 fl	20.1-21.3	0.008
MCH	Sheep	237	9±2.5 pg	8.7-9.4	
	Goat	213	8.6±2.2 pg	8.3-8.9	0.061
MCHC	Sheep	237	41.3±5.8 gm/d	40.5-42.0	
	Goat	213	41.8±6.1 gm/d	41.0-42.6	0.342
WBC	Sheep	237	13.6±4.4 countX10^3/ µl	13.1-14.2	
	Goat	213	14.3±4.4 countX10^3/ µl	13.7-14.9	0.121
Neutrophil	Sheep	237	31.8±12.8 (%)	30.2-33.4	
	Goat	213	29.2±12.6 (%)	27.5-30.9	0.031
Lymphocyte	Sheep	237	56.9±13.8 (%)	55.1-58.7	
	Goat	213	58.9±13.6 (%)	57.1-60.8	0.117
Monocyte	Sheep	237	2.9±1.7 (%)	2.7-3.1	
	Goat	213	3.5±2.3 (%)	3.2-3.8	0.005
Eosinophil	Sheep	237	7.6±4.5 (%)	7.0-8.2	
	Goat	213	7.6±3.6 (%)	7.1-8.0	0.892
Basophil	Sheep	237	0.7±0.8 (%)	0.6-0.8	
	Goat	213	0.8±1 (%)	0.7-1.0	0.099

Within the high challenge area values for MCV, neutrophil and monocyte were higher in sheep than in goats and the difference were significant ($P < 0.05$) (Table 2). There was significant difference ($P < 0.05$) in values of RBC, MCV, and MCH between sexes in sheep in the high challenge area where by MCV and MCH are higher in males than females and the value for RBC was higher in females than males (Table 4). There was also significant variation ($P < 0.05$) in the values of RBC, Hb, MCV, MCH and monocyte between sexes in goats with in the high challenge area and Hb and MCV were higher in males than females whereas RBC and MCH were higher in females than males (Table 5).

Table3: Values of hematological parameters of small ruminants raised in low tsetse challenge area of South West Ethiopia as affected by species

Hematological Parameters	Species	n	Mean ± SD	95% CI for mean	P value
RBC	Sheep	218	10.3±2.5counts$X10^6$/µl	10.0-10.7	
	Goat	232	15±3counts$X10^6$ /µl	14.6-15.4	0.000
PCV	Sheep	218	25.6±4.2 (%)	25.0-26.1	
	Goat	232	27.9±12.9 (%)	26.2-29.5	0.013
Hb	Sheep	218	10.7±4.8 gm/dl	10.1-11.3	
	Goat	232	9.8±1 gm/dl	9.7-9.9	0.007
MCV	Sheep	218	25.7±5 fl	25.0-26.3	
	Goat	232	19.1±9.7 fl	17.7-20.4	0.000
MCH	Sheep	218	10.9±5.8 pg	10.1-11.6	
	Goat	232	6.8±1.4 pg	6.6-6.9	0.000
MCHC	Sheep	218	42.9±28.5 gm/d	39.1-46.7	
	Goat	232	36.7±5.7 gm/d	35.9-37.4	0.001
WBC	Sheep	218	13.3±3.1count$X10^3$/ µl	12.9-13.7	
	Goat	232	15.2±4 count$X10^3$ / µl	14.7-15.7	0.000
Neutrophil	Sheep	218	34.3±10.8 (%)	32.9-35.8	
	Goat	232	31.1±9.9 (%)	29.8-32.4	0.001
Lymphocyte	Sheep	218	56.7±11.2 (%)	55.2-58.2	
	Goat	232	59.7±11.1 (%)	58.3-61.1	0.005
Monocyte	Sheep	218	4.6±4 (%)	4.1-5.2	
	Goat	232	4.6±2.2 (%)	4.4-4.9	0.988
Eosinophil	Sheep	218	3.8±3.7 (%)	3.3-4.3	
	Goat	232	4.1±4.3 (%)	3.5-4.6	0.500
Basophil	Sheep	218	0.7±0.9 (%)	0.5-0.8	
	Goat	232	0.6±0.8 (%)	0.4-0.7	0.172

Table 4: Values of hematological parameters of sheep species raised in high tsetse challenge area of South West Ethiopia as affected by sex

Hematological parameters	Sex	n	Mean ± SD	95% Confidence Interval for Mean		P value
				Lower Bound	Upper Bound	
RBC	Female	61	12.6±2.6countsX10^6/µl	12.0	13.3	0.0016
	Male	176	11.3±2.8countsX10^6/µl	10.9	11.7	
PCV	Female	61	25.1±3.5 (%)	24.3	26.0	0.1807
	Male	176	24.3±4.2 (%)	23.7	25.0	
Hb	Female	61	10.1±1.4 gm/dl	9.8	10.5	0.6045
	Male	176	10±1.6 gm/dl	9.8	10.2	
MCV	Female	61	20.5±3.7 fl	19.5	21.4	0.007
	Male	176	22.4±4.9 fl	21.6	23.1	
MCH	Female	61	8.3±1.9 pg	7.8	8.8	0.0068
	Male	176	9.3±2.7 pg	8.9	9.7	
MCHC	Female	61	40.6±5.7 gm/d	39.1	42.0	0.2748
	Male	176	41.5±5.8 gm/d	40.7	42.4	
WBC	Female	61	13.2±4.8 countX10^3/ µl	12.0	14.5	0.4448
	Male	176	13.8±4.3 countX10^3/ µl	13.1	14.4	
Neutrophil	Female	61	32.7±13 (%)	29.5	36.0	0.5145
	Male	176	31.5±13 (%)	29.6	33.4	
Lymphocyte	Female	61	54.9±14 (%)	51.3	58.5	0.1863
	Male	176	57.6±14 (%)	55.6	59.6	
Monocyte	Female	61	3.2±1.8 (%)	2.7	3.6	0.2086
	Male	176	2.9±1.6 (%)	2.6	3.1	
Eosinophil	Female	61	8.4±4.5 (%)	7.3	9.6	0.0963
	Male	176	7.3±4.5 (%)	6.7	8.0	
Basophil	Female	61	0.6±0.8 (%)	0.4	0.8	0.3916
	Male	176	0.7±0.8 (%)	0.6	0.9	

With in the low challenge area values for Hb, MCV, MCH, MCHC and neutrophil were higher in sheep than in goats). Whereas the values for RBC, PCV, WBC and lymphocyte were higher in goats than sheep and the difference were significant (P < 0.05) in both cases. The difference in the values of monocyte, eosinophil and basophil were not significant between species in low challenge area (P > 0.05) (Table 3). Significant difference (P < 0.05) where males have a higher value than females was seen only in the value of RBC with in sheep species in the low challenge area. The differences in the other hematological values between sexes with in sheep species were not significant in the low challenge area (P > 0.05). There was also significant variation (P < 0.05) between sexes in goats with in the low challenge area in the values of RBC and lymphocyte

where males have higher values than females and in MCHC and eosinophil where females have higher values than in males. The differences in the other hematological values between sexes with in goat species were not significant in the low challenge area (P > 0.05) (Table 6).

Table 5: Values of hematological parameters of goat species raised in high tsetse challenge area of South West Ethiopia as affected by sex

Hematological parameters	Sex	n	Mean ± SD	95% Confidence Interval for Mean		P value
				Lower Bound	Upper Bound	
RBC	Female	124	12.4±2.9counts$X10^6$/µl	12	12.9	0.047
	Male	72	11.5±2.92counts$X10^6$/µl	11	12.2	
PCV	Female	124	23.4±4.25 (%)	23	24.2	0.034
	Male	72	24.7±3.89 (%)	24	25.6	
Hb	Female	124	9.5±1.37 gm/dl	9.3	9.75	0.001
	Male	72	10.3±1.79 gm/dl	9.9	10.7	
MCV	Female	124	19.6±4.52 fl	19	20.4	0
	Male	72	22.3±4.64 fl	21	23.4	
MCH	Female	124	8±2.03 pg	7.7	8.41	0
	Male	72	9.4±2.29 pg	8.8	9.89	
MCHC	Female	124	41.3±6.2 gm/d	40	42.5	0.479
	Male	72	42±6.09gm/d	41	43.4	
WBC	Female	124	14.5±4.31 count$X10^3$/ µl	14	15.3	0.793
	Male	72	14.4±4.46 count$X10^3$/ µl	13	15.4	
Neutrophil	Female	124	29.8±13.4 (%)	27	32.2	0.895
	Male	72	29.5±11.8 (%)	27	32.3	
Lymphocyte	Female	124	57.9±14.5 (%)	55	60.5	0.431
	Male	72	59.5±12.8 (%)	56	62.5	
Monocyte	Female	124	3.7±2.52 (%)	3.3	4.19	0.021
	Male	72	2.9±1.91 (%)	2.5	3.39	
Eosinophil	Female	124	7.8±3.76 (%)	7.2	8.51	0.193
	Male	72	7.1±3.56 (%)	6.3	7.96	
Basophil	Female	124	0.8±0.98 (%)	0.6	1	0.907
	Male	72	0.8±0.99 (%)	0.6	1.04	

Table 6: Values of hematological parameters of goat species raised in low tsetse challenge area of South West Ethiopia as affected by sex

Hematological parameters	Sex	n	Mean ± SD	95% Confidence Interval for Mean Lower Bound	Upper Bound	P value
	Female	172	14.6±2.9counts$X10^6$ /µl	14.1	15	
RBC	Male	60	16.3±2.8counts$X10^6$ /µl	15.5	17	0.000
	Female	172	27.8±15 (%)	25.6	30	
PCV	Male	60	28±5 (%)	26.7	29.3	0.924
	Female	172	9.761±1 gm/dl	9.61	9.91	
Hb	Male	60	9.97±1.1 gm/dl	9.68	10.3	0.185
	Female	172	19.7±11 gm/dl	18	21.3	
MCV	Male	60	17.5±3.4 gm/dl	16.7	18.4	0.144
	Female	172	6.93±1.5 pg	6.71	7.15	
MCH	Male	60	6.28±1.1 pg	6	6.56	0.002
	Female	172	36.8±6 gm/d	35.9	37.7	
MCHC	Male	60	36.2±4.7 gm/d	35	37.4	0.487
	Female	172	15.3±4 count$X10^3$/ µl	14.7	15.9	
WBC	Male	60	15.1±3.9count$X10^3$/µl	14.1	16.1	0.793
	Female	172	31.5±10 (%)	30	33	
Neutrophil	Male	60	30.1±9.7 (%)	27.5	32.6	0.338
	Female	172	58.8±11 (%)	57.1	60.5	
Lymphocyte	Male	60	62.3±10 (%)	59.6	65	0.034
	Female	172	4.65±2.1 (%)	4.33	4.96	
Monocyte	Male	60	4.63±2.4 (%)	4.02	5.24	0.970
	Female	172	4.66±4.6 (%)	3.97	5.36	
Eosinophil	Male	60	2.43±2.4 (%)	1.82	3.05	0.000
	Female	172	0.56±0.8 (%)	0.44	0.69	
Basophil	Male	60	0.52±0.8 (%)	0.31	0.72	0.698

4.2.2. Biochemical result

The values for AST, ALT, creatinine, and total protein were higher in small ruminants (sheep and goats) raised in low tsetse challenge area than in high tsetse challenge area but the value for ALP was smaller in low tsetse challenge area than in high tsetse challenge area and the difference in values between the areas was significant ($P < 0.05$). The difference in the value of glucose in small ruminants (sheep and goats) was not significant between high and low tsetse challenge areas ($P > 0.05$) (Table 7).

Table 7: The biochemical values of sheep and goats raised in high and low tsetse challenge areas

Biochemical parameters	Area	n	Mean ± SD	95% CI for mean	P value
AST/GOT	High challenge	450	84.8±25.78 IU/L	82.4-87.1	0.000
	Low challenge	450	116.45±44.86 IU/L	112.3-120.6	
ALT/GPT	High challenge	450	13.52±6.5 IU/L	12.9-14.1	0.000
	Low challenge	450	19.07±9.1 IU/L	18.2-20.0	
ALP	High challenge	450	45.45±23.2 IU/L	43.3-47.6	0.000
	Low challenge	450	6.69±8.1 IU/L	5.9-7.4	
Creatinine	High challenge	450	1.113±0.2 mg/dl	1.09-1.13	0.000
	Low challenge	450	1.2±0.3 mg/dl	1.17-1.22	
Total protein	High challenge	450	4.94±1.6 g/dl	4.8-5.1	0.000
	Low challenge	450	7.6±2.9 g/dl	7.3-7.9	
Glucose	High challenge	450	42.75±13.1 mg/dL	41.5-44.0	0.183
	Low challenge	450	41.33±18.5 mg/dL	39.6-43.0	

Within the high challenge area values for AST, ALT, and creatinine were higher in sheep than in goats and the variation was significant ($P < 0.05$). The difference in the values of ALP, total protein, and glucose between species in the high challenge area was not significant ($P > 0.05$) (Table 8).

Table 8: The biochemical values of sheep and goats raised in high tsetse challenge areas

Biochemical Parameters	species	n	Mean ± SD	95% CI for mean	P value
AST/GOT	Sheep	237	91.3±25IU/L	88.1-94.4	0.002
	Goat	213	80.3±46 IU/L	74.1-86.5	
ALT/GPT	Sheep	237	14.3±7.3I U/L	13.4-15.3	0.006
	Goat	213	12.6±5.4 IU/L	11.9-13.4	
ALP	Sheep	237	47.4±23.7 IU/L	44.4-50.4	0.062
	Goat	213	43.3±22.4 IU/L	40.3-46.3	
Creatinine	Sheep	237	1.2±0.2 mg/dl	1.1-1.2	0.000
	Goat	213	1.1±0.2 mg/dl	1.0-1.1	
Total protein	Sheep	237	5.1±1.4 g/dl	4.9-5.2	0.099
	Goat	213	4.8±1.8 g/dl	4.6-5.1	
Glucose	Sheep	237	42±12.3 mg/dL	40.4-43.6	0.210
	Goat	213	43.6±13.9 mg/dL	41.7-45.4	

In the low challenge area the values for AST, creatinine and total protein were higher in sheep than in goats and the values for ALT and glucose were higher in goats than in sheep and the variations were significant (P < 0.05). The difference in the value of ALP between species in the low challenge area was not significant (P > 0.05%) (Table 9).

Table 9: The biochemical values of sheep and goats raised in low tsetse challenge area

Biochemical Parameters	Species	n	Mean ± SD	95% Confidence Interval for Mean		P value
				Lower Bound	Upper Bound	
AST/GOT	Sheep	218	131.7±47.3 (IU/L)	125.4	138	0.000
	Goat	232	102.1±37.2 (IU/L)	97.3	106.9	
ALT/GPT	Sheep	218	17.1±8 (IU/L)	16	18.2	0.000
	Goat	232	20.9±9.6 (IU/L)	19.6	22.2	
ALP	Sheep	218	6.5±8.9 (IU/L)	5.3	7.7	0.677
	Goat	231	6.8±7.4 (IU/L)	5.9	7.8	
Creatinine	Sheep	218	1.3±0.3 (mg/dl)	1.2	1.3	0.000
	Goat	232	1.1±0.3 (mg/dl)	1.1	1.2	
Total protein	Sheep	218	8.1±2.9 (g/dl)	7.7	8.5	0.001
	Goat	232	7.1±2.9 (g/dl)	6.8	7.5	
Glucose	Sheep	218	39.5±19.2(mg/dL)	36.9	42	0.037
	Goat	232	43.1±17.72(mg/dL)	40.8	45.4	

There was significant variation (P < 0.05) between sexes in sheep within the high challenge area in the values of ALT, ALP, total protein and glucose whereby males have higher values than females. The variations in the values of AST and creatinine were not significant between sexes in sheep within high challenge area (Table10). The difference in the values of ALT, ALP, creatinine, and total protein between sexes in sheep within the low challenge area were not significant (P > 0.05%).

Table 10: The biochemical values of sheep raised in high tsetse challenge area as affected by sex

Biochemical Parameters	Sex	n	Mean ± SD	95% Confidence Interval for Mean		P value
				Lower Bound	Upper Bound	
AST/GOT	Female	61	94.1±27.(IU/L)	87.2	101.0	0.308
	Male	176	90.3±24.2.(IU/L)	86.7	93.9	
ALT/GPT	Female	61	10.6±6.1.(IU/L)	9.0	12.1	0.000
	Male	176	15.6±7.2 .(IU/L)	14.6	16.7	
ALP	Female	61	34.5±20.8.(IU/L)	29.2	39.8	0.000
	Male	176	51.9±23.1.(IU/L)	48.4	55.3	
Creatinine	Female	61	1.2±0.2 (mg/dl)	1.1	1.3	0.099
	Male	176	1.2±0.2 (mg/dl)	1.1	1.2	
Total protein	Female	61	4.7±1.6 (g/dl)	4.3	5.1	0.025
	Male	176	5.2±1.3 (g/dl)	5.0	5.4	
Glucose	Female	61	38.6±10.62(mg/dL)	35.8	41.3	0.011
	Male	176	43.2±12.62(mg/dL)	41.3	45.1	

There was also significant variation (P < 0.05) between sexes in goats with in the high challenge area in the values of ALP, creatinine and glucose where the values were higher in males than females. In the values of AST, ALT and total protein between sexes in goats within high challenge areas were not significant (P > 0.05) (Table 11).

Table 11: The biochemical values of goats raised in high tsetse challenge area as affected by sex

Biochemical Parameters	Sex	n	Mean ± SD	95% Confidence Interval for Mean		P value
				Lower Bound	Upper Bound	
AST/GOT	Female	124	82.1±57.2(IU/L)	72.0	92.3	0.496
	Male	89	77.8±22.2(IU/L)	73.1	82.5	
ALT/GPT	Female	124	12.1±5.9(IU/L)	11.1	13.2	0.119
	Male	89	13.3±4.6 (IU/L)	12.3	14.3	
ALP	Female	124	39.2±24.4(IU/L)	34.9	43.5	0.002
	Male	89	49.0±18.1(IU/L)	45.2	52.8	
Creatinine	Female	124	1.0±0.2 (mg/dl)	0.9	1.0	0.000
	Male	89	1.2±0.2(mg/dl)	1.1	1.2	
Total protein	Female	124	4.7±1.9(g/dl)	4.4	5.0	0.323
	Male	89	5.0±1.7 (g/dl)	4.6	5.3	
Glucose	Female	124	39.2±11.6(mg/dL)	37.1	41.2	0.000
	Male	89	49.7±14.6(mg/dL)	46.6	52.8	

In goats in the low challenge area there was a significant ($P < 0.05$) variation only in the value of glucose between sexes whereby males (51.6±18.3mg/dL) have a higher value than females (40.1±16.5mg/dL). The value for glucose was higher in male (46±18.3mg/dL) sheep than females (35.1±18.5mg/dL) whereas the value for AST was smaller in male sheep (122.2±41.1IU/L) than females (138±50.2IU/L) and the difference was significant ($P < 0.05$).

Result of cholesterol and triglycerides

The biochemical; cholesterol and triglycerides (Mean ± S.D) values of small ruminants (sheep and goats) raised in high and low tsetse challenge areas are presented in Tables 12 and 13.

The values for cholesterol were higher in small ruminants (sheep and goats) raised in high tsetse challenge area than in low challenge area whereas the values for triglycerides were smaller than the low challenge area and the difference in both cases were significant ($P < 0.05$) (Table 12).

Table 12: Cholesterol and triglyceride values of small ruminants raised in high and low tsetse challenge area of South West Ethiopia

Biochemical Parameters	Area	n	Mean ± SD	95% confidence interval	P value
Cholesterol	High challenge	100	94.5±32.5 (mg/dL)	88.1-101	0.000
	Low challenge	100	61.7±42.1 (mg/dL)	53.3-70.0	
Triglyceride	High challenge	100	93.4±43.7 (mmol/L)	84.7-102.1	0.015
	Low challenge	100	107.5±37.4(mmol/L)	100.1-114.9	

With in the high challenge area the values for cholesterol were higher in goats than in sheep and the variation was significant between species ($P < 0.05$) whereas the variation in the values for triglycerides were not significant ($P > 0.05$) between species in the high challenge area (Table 13).

Table 13: Cholesterol and triglyceride values of small ruminants raised in high tsetse challenge area of South West Ethiopia as affected by species

Biochemical Parameters	Species	n	Mean ± SD	95% confidence interval	P value
Cholesterol	Sheep	50	85.9±25.1(mg/dL)	78.8-93.0	0.007
	Goat	50	103.1±36.8(mg/dL)	92.7-113.6	
Triglyceride	Sheep	50	92±42.3(mmol/L)	80.0-104.1	0.754
	Goat	50	94.8±45.5(mmol/L)	81.9-107.7	

The values for cholesterol were higher in males than females in sheep with in the high challenge area and the difference between sexes was significant ($P < 0.05$). The difference was not significant in the values for triglycerides between sexes in sheep species within the high challenge area ($P > 0.05$). In goats between sexes the difference was not significant ($P > 0.05$) in both the values of triglycerides and cholesterol.

Within the low challenge area there was only significant difference in the value of triglycerides between sexes in goat species ($P < 0.05$) whereby the value was higher in males

(122.3±36.8IU/L) than in females (92.2±33.6IU/L). The difference in the values of cholesterol between sexes within goat, and in the value of both cholesterol and triglycerides between sexes in sheep species were not significant in the low challenge area ($P > 0.05$).

5. DISCUSSION

In the current study the smaller mean hematological values of RBC (11.8±2.8X10^6/μl), PCV (24.2±4.1%), MCV (21.3±4.7fl), neutrophil (30.6±12.8%), monocyte (3.2±2%) and basophil (0.8±0.9%) for small ruminants (sheep and goats) raised in high tsetse challenge area than the values of RBC (12.7±3.6X10^6/μl), PCV (26.8±9.8%), MCV (22.3±8.4fl), neutrophil (32.7±10.5%), monocyte (4.6±3.2%) and basophil (0.6±0.8%) in low tsetse challenge area showed significant variation ($P < 0.05$). This suggests that differences exist in the values of hematological parameters between small ruminants raised in high and low tsetse challenge areas due to the effect of exposure to trypanosomosis which is higher in high tsetse challenge area than in the low tsetse challenge area. Smaller values of PCV and MCV in the hematological parameters of small ruminants in the high challenge area could be the cause of microcytic type of anemia. Significant differences ($P < 0.05$) were also observed between sheep and goat species within the high challenge area in the values of MCV (21.9±4.7fl for sheep, 20.7±4.6fl for goats), neutrophil (31.8±12.8% for sheep, 29.2±12.6% for goats) and monocyte (2.9±1.7% for sheep, 3.5±2.3% for goats) where the values were found to be higher in sheep than goat.

In the biochemical analysis values for AST (84.8±25.8IU/L), ALT (13.5±6.5IU/L), creatinine (1.1±0.2mg/dl), and total protein (4.9±1.6g/dl) were smaller in small ruminants (sheep and goats) raised in high tsetse challenge area than the values of AST (116.5±44.9IU/L), ALT (19.1±9.1IU/L), creatinine (1.2±0.3mg/dl), and total protein (7.6±2.9g/dl) in low challenge area and the difference in values between the areas were significant ($P < 0.05$). The smaller values of total protein and creatinine in this result for small ruminants in the high challenge area which are more exposed to trypanosomosis than those of low challenge area contradict with the increased protein and creatinine levels in monkeys experimentally infected with *T. b. gambiense* reported by Abenga and Anosa (2005).

Within the high challenge area significant difference in some biochemical parameters ($P < 0.05$) were also observed between sheep and goat species whereby the values of AST (91.3±25IU/L), ALT (14.3±7.3IU/L), and creatinine (14.3±7.3mg/dl) were found to be higher in sheep than the values of AST (80.3±46IU/L), ALT (12.6±5.4IU/L), and creatinine (1.1±0.2mg/dl) in goats.

In the present study values for cholesterol (94.5±32.5mg/dl) were found to be higher in small ruminants (sheep and goats) raised in high tsetse challenge area than the values of cholesterol (61.7±42.1mg/dl) in low challenge area whereas the values for triglycerides (93.4±43.7mmol/L) were found to be smaller than the values for triglycerides (107.5±37.47mmol/L) in the low challenge area and the difference in both cases were significant ($P < 0.05$). The findings of this study is in agreement with previous study where elevated levels of cholesterol in sheep experimentally infected with *T. b. brucei* and *T. congolense* (Taiwo *et al*; 2003). The difference was not significant in the values for triglycerides between sexes in sheep species within the high challenge area ($P > 0.05$).

With in the high challenge area the values for cholesterol were higher in goats (103.1±36.8mg/dl) than in sheep (85.9±25.1mg/dl) and the variation was significant between species ($P < 0.05$) whereas the variation in the values for triglycerides was not significant ($P > 0.05$).

The variations observed between the results of the current study and those from previous studies may be attributed to variation in breed, nutrition, environmental conditions or analytical techniques.

6. CONCLUSION AND RECOMMENDATIONS

In the present study the component of the hematological and plasma biochemical parameters in small ruminants (sheep and goats) showed remarkable difference between high and low tsetse challenge areas, between sheep and goat species and again with in the species between sexes. Thus trypanosomosis has a prominent effect on the hematological and plasma biochemical parameters of small ruminants in the high tsetse challenge areas of South Western parts Ethiopia and attention should be given in utilizing the values of these parameters for assessing the physiological status of animals for diagnostic purpose.

The findings of this study may serve as references in which alterations due to trypanosomosis in the hematological and biochemical parameters of small ruminants (sheep and goat) can be compared for assessing the physiological status of animals and for diagnostic purpose. Additionally, it also aids in establishing baseline information for diagnosis of the disease and selection of trypanotolerant breeds of small ruminants.

Taking in to account the above findings the following recommendations are forwarded:

- The findings of the study may serve as references for clinical use in which alterations in the physiological and health status of animals due to trypanosomosis can be compared between tsetse infested and tsetse free areas.
- Further investigations should be under taken to see the effect of trypanosomosis on hematological and serum biochemical parameters in small ruminants in a controlled experiment to assess the effect of different host and environmental factors on these physiological parameters.

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8. ANNEXES

Annex 1. Laboratory Materials and Procedures

RBC count

Materials

- Hemocytometer (improved Neubauer chamber)
- RBC pipettes that have red beads
- Light Microscope
- Hayem's solution
- Lancets
- Cotton swabs
- Alcohol
- Cover slip
- Venous blood from different domestic animals.

Methods

For RBC counting a dilution of 1 part blood to 200 Hayem's solution is necessary.

Haym's fluid contains:

- Sodium chloride (1 gm), to help prevent hemolysis,
- Sodium sulphate (5gm), to prevent rouleaux formation,
- Mercury chloride (0.5 gm), to prevents bacterial growth, and
- Distilled water 200 ml.

1. Identify a pipette with a red bead (used to count RBC only) and observe the markings stated on the pipette (0.5, 1, and 101). This pipette has a rubber attached on its one end (refer figures when possible).

2. Draw an-coagulated blood to 0.5 mark slowly by avoiding air bubbles to enter into the red cell pipette.

3. Wipe the outside part of the pipette clean and aspirate the Hayem‘s diluting fluid to the 101 mark and slowly mix the solutions (you made now 1:200 dilution). Taking blood to the 1.0 mark of the pipette and the diluent to 101 mark results in a dilution ratio of 1:100.

4. Discard the diluent found in the stem of the pipette and it is now ready to be applied or charged in the Neubauer‘s counting chamber (hemocytometer).

5. The counting chamber has microscopically ruled lines. At the center of the chamber there are 400 small squares. To observe these squares, cover the chamber with a cover sleep and identify them by using high magnification (40x objective, diaphragm opened, adjust the light intensity). RBC counts with a dilution of 1:200 require that the one found at center square and those found at four corner squares be counted (5 x 16 = 80). The depth of the chamber with the cover glass is 0.1mm. Then choose only for 80 small squares that are necessary to count RBCs (see the calculation below).

6. Bring the tip of the RBC pipette and attach it between the cover sleep and the chamber near to the groove. The diluent sample will directly flow into the ruled area by capillary suction. Take care that the diluent is completely filled and evenly distributed. Avoid overflow and air bubbles from being entrapped.

7. Start counting RBC‘s found at 80 small squares and multiply your results with 10,000. While counting, some RBC touches the line. Count RBCs touching the top and left hand line. Do not count those RBC that touch the lower and right-hand lines.

Materials:

- Microhematocrit tube, heparinized
- hematocrit centrifuge
- 70% alcohol
- Hematocrit reader
- Uncoagulated blood in test tubes
- Lancets and sealing clay

Methods: (Micro-hematocrit method)

- For a human blood, clean the tip of one of your left fingers with an alcohol swab. Pick up the lancet in your right hand and stab your clean left finger. Wait till a drop of blood flows out.
- For different species of animals, (Canine or Bovine) heparinized blood is already prepared and found in test tubes ready for use. The rest of the procedures are the same.

1. Keep the microhematocrit tube in a horizontal position so that blood from your fingertip or the test tubes is sucked to the required volume
2. Put the microhematocrit tube on the sealing clay in a perpendicular position.
3. Load the hematocrit tubes on the centrifuge and start centrifuging for 5 minutes at 3000 rotations per minute. This will separate the blood into cells and plasma.
4. After centrifugation, record the volume of the packed red cells and calculate the percent Hct values by using the above formula.

Note: If you are using the Hct scale found labeled on the centrifuge, then put the bottom (sealed end) of the packed RBC at 0% mark and read the Hct value at the junctional space between the buffy layer and the packed RBC's.

Hemoglobin

Materials:

The materials needed for Sahl's hemoglobinometer:

1. Hemoglobinometer pipette with a mark of 20 cubic mm.
2. Hemoglobinometer tube that is graduated on one side and in grams per 100 ml on the other side.
3. Glass rod, which acts as a stirrer.
4. Dropper, N/10 HCl, distilled water, and alcohol with pricking needle.

Methods:

Fill the graduated tube of the hemoglobinometer with 0.1N HCl up to the mark of 20. Then add blood into Shal's pipette till it is 20 cubic mm. Blow the blood from the pipette into the acid solution and mix. After about 2 to 3 minutes, continue adding distilled water drop by drop until the color matches with the standard. Then look for the reading in gram percent and record.

WBC count

Materials:

1. Uncoagulated blood, Microscope, and Hemacytometer (counting chamber).
2. Pipette (white bead with a white sucker) that has a marking of 0.5 and 11).
3. WBC diluent: 1 ml. Glacial acetic acid, and 1ml methylene blue, dilutes both with distilled water to 100 ml.

Methods:

The method of counting WBC is more or less similar to RBC counting in that it uses hemocytometer, cover slip, microscope and a pipette with a white bead. Exceptions are that the dilution is 1:20 and four large squares found at diagonals of the hematocytometer are used for counting. The WBC diluent consists of glacial acetic acid used to kill red blood cells. The dye methylene blue (crystal violet) stains the nuclei of white blood cells.

1. Aspirate blood with the pipette exactly to 0.5 marks by avoiding air bubbles to enter into the pipette.
2. Suck diluting fluid up to the mark of 11 by avoiding air bubbles to enter into the pipette (the dilution is now 1:20).
3. Mix the contents slowly and leave it for about a minute.
4. Place a cover slip on the hemacytometer and place a drop of the fluid at the side.
5. Then start-counting WBC's found at 4 corners using low magnification (10x). Leave those WBC touching the left and upper part of the lines.
6. Finally, multiply the total number of WBC counted by 50 to come to the total WBC's number.

Differential leukocyte count

Materials:

- Slides (2 slides for each student).
- Stains (either Wright's or Lehman's stain).
- Microscope (immersion oil).
- Distilled water.
- Alcohol, lancets, and
- Cotton swabs.

Methods:

1. Place a drop of blood near to the one end of a clean glass slide.
2. Bring another slide and by holding it at 45°, attach it to the drop of blood.
3. Then, push the slide slowly in forward direction to form a thin film of blood.
4. Dry the thin film of blood in the air and insert it into the stains.
5. Remove the slide from the stain; rinse it under running water, and air-dry it.
6. Put the dry smear on the microscope and add 1-drop of immersion oil on the smear.
7. By using oil-immersion objective, count 100 WBC's.
8. Finally, identify each type of leukocytes, and record your results as % of the total leukocyte count (see the colored atlas to help you identify the cell types

Red cell indices

Materials:
Methods applied to count RBC number, Hematocrit and, Hemoglobin concentration should be used.

Methods:

The following formulas depict method of calculating the blood indices.

$$\textbf{MCV*} = \frac{\text{Ht \% x 10}}{\text{RBC No. } (10^6/\mu l)}$$

*MCV indicates a change in the size of a single RBC.

- Normocytosis: means normal size,

- Microcytosis: indicates smaller size that is usually caused by iron deficiency and thus lower hemoglobin concentration.
- Macrocytosis: indicates larger size and is usually caused by Vit. B_{12} and folic acid deficiencies (DNA is inhibited from cell division).

MCH * $= \dfrac{\text{Hg (g/dl) x 10}}{\text{RBC No}(10^6/\mu l)}$

*MCH is mostly associated with hemoglobin concentration.

- Decreased MCH: causes hypocromic anemia (decreased hemoglobin level) because of a deficiency of iron in the diet.
- Increased MCH: may be caused during hemolysis where extracellular hemoglobin level increases. Hypercromic state is not usually manifested in clinical practices because hemoglobin has a constant level (33%) in a cell.

MCHC* $= \dfrac{\text{Hb (g/dl) x 100}}{\text{Hct (\%)}}$

*MCHC is the most accurate of the indices, because it does not require RBC counts. Decreased value indicates iron deficiency and reticulocytosis while increased value may be due to hemolysis.

Glucose test

Procedures

1. Bring the reagents and samples to room temperature
2. Pipette 1000µl of the reagent (glucose mono reagent) into the labeled tubes
3. Pipette 10µl the serum sample over the reagents in the labeled tubes
4. Mix and let the tubes stand for 5 minutes at 37^0c
5. Read the absorbance of the samples and the standard at 500nm of wave length against the reagent blank

AST test

Procedures

1. Bring the reagent and samples to room temperature
2. Pipette 1000µl of the reagent into the labeled tubes
3. Pipette 100µl of serum sample over the labeled tubes containing the reagent
4. Mix, incubate and read the absorbance after exactly 1, 2 and 3 minutes and then calculate delta absorbance at 340nm wave length

Total protein test

Procedures

1. Bring the reagents and the samples to room temperature
2. Pipette 1000µl of reagent R1 (potassium iodide, copper sulphate, sodium hydro oxide and potassium sodium tartarate) into labeled tubes
3. Pipette 20µl of the serum sample into the tubes containing R1
4. Mix, incubate for 5 minutes at 37^0c and read the absorbance at 546nm wave length

Triglyceride test

Procedures

1. Bring the reagents and the samples to room temperature
2. Pipette 1000µl of triglyceride mono reagent into labeled tubes
3. Pipette 10µl of serum sample in the tubes containing the reagent
4. Mix, incubate at 37 ^{0}c for 5 minutes at 546nm wave length and read the absorbance of sample against the reagent blank

Creatinine test

Procedures

1. Bring the reagents and the samples to room temperature
2. Mix equal volume of R1 (picric acid) and R2 (sodium hydro oxide)
3. Pipette 1000µl of the mixture in to labeled tubes
4. Pipette 100µl of serum sample to containing the mixed reagents
5. Mix, read A1 exactly after 20 seconds and A2 after 80 seconds at 492nm wave length and calculate delta absorbance

ALT/GPT test

Procedures

1. Bring the reagent and samples to room temperature
2. Mix 5 volume of R1 (tris buffer pH 7.8, L-Alanine and lactate dehydrogenase) and 1 volume of R2 ($NADH_2$ and 2-Oxoglutarate)
3. Pipette 1000µl of the mixed reagent into the labeled tubes
4. Pipette 100µl of serum sample over the labeled tubes containing the mixed reagents
5. Mix, incubate for 1 minute and read the absorbance after exactly 1, 2 and 3 minutes and then calculate delta absorbance at 340nm wave length

ALP test

Procedures

1. Bring the reagent and samples to room temperature
2. Mix 5 volume of R1 (Diethanolamine buffer, pH 9.8, Magnesium sulphate and detergents and stabilizers) and 1 volume of R2 (p-nitrophenylphosphate and stabilized liquid)
3. Pipette 1000µl of the mixed reagent into the labeled tubes
4. Pipette 20µl of the serum sample over mixed reagent into the labeled tubes
5. Mix and read the absorbance after exactly 1, 2 and 3 minutes and then calculate delta absorbance at 405nm wave length

Annex 2. Plates showing laboratory and field activities

Plate 1. Study animals during grazing

Plate 2. Host animal (Hippopotamus)

Plate 3. Deworming

Plate 4. Trap deployment for sampling tsetse flies

Plate 5. Blood sampling from jugular vein

Plate 6. Centrifugation for PCV determination

Plate 7. Differential count for WBC

Plate 8. Plasma separation

CURRICULUM VITAE

I. Personal information

Name	Tesfaye Mulatu Bereded
Nationality	Ethiopian
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Marital Status	Married
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II Educational Background

Elementary	Sire Hile Aba Mersa School (1972-1978)
Junior Secondary	Sire Junior Secondary School (1979-1980)
High School	Abyot Kirse High School (1981-1984)
Higher Education	Asmara University (1984-1988) and award granted B.Sc. degree in Biology

III Work Experiences

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DECLARATION

This thesis is my original work, has not been presented for a degree in any University and that all sources of material used for the thesis have been duly acknowledged.

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Signature________________________________

Date of submission ______________________

This thesis has been submitted for examination with our approval as University advisors.

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