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Therapeutic trial of diminazene aceturate and isomethamidium chloride in clinical cases of trypanosomiasis in donkeys, jawi district, north west ethiopia

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Abstract: A field clinical trial study was conducted with the aim of to assess the efficacy of Diminazine aceturate and Isomethamidium chloride and compare pathophysiological changes in hematological values of in trypanosomiasis donkeys from January to June, 2018 Jawi district, North West Ethiopia. Out of 61 suspected donkeys for Trypanosomisis and screened by using wet blood film, six of clinically infected donkeys were selected purposively and randomly allocated to three groups. Group I, II and III received treatment for diminazene aceturate and isomethamidum chloride and normal saline respectively. Hematological parameters (PCV and Hgb) and physical parametric changes (HR, Toc and BW) before and after treatment within and between groups were measure for 28 days. The data was analyzed by using STATA version 14 using paired t-test. The main clinical findings observed during the study period were weakness, fever, rough hair coat, enlarged superficial lymph nodes, pallor of the mucus membranes, lacrimation, enlarged superficial lymph node and weight loss in all trypanosoma infected donkeys. Out of 61 suspected donkeys for Trypanosomisis, six of them were confirmed to be infected with T. congolense. The mean rectal temperatures of group I treated with diminazine aceturate before and after treatment donkeys were 39.1^{o}C±0.42^{o}C and37.9^{o}C±0.14^{o}C respectively. The mean rectal temperatures of group II before and after treatment were 39.5^{o}C±0.63^{o}C, 38.4^{o}C±0.56^{o}C respectively and statistical insignificant ($p < 0.05$) decreased after treatment but There was statistical Significant difference ($P<0.05$) in mean of PCV in group I and group II before and after treatment. PCV improvement before and after treatment showed that there was significant difference ($p < 0.05$) in the means of the group I and II. The maximum PCV improvement was 40 % (Group I on week 1) and minimum was 7% (group II on week 3). Generally diminazene aceturate at dose of 7 mg/kg was the more effective in terms of improving PCV, as compare to isomethamidum chloride so that clinician better to use diminazene aceturate.

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1. Introduction

Trypanosomiasis is a diseases caused by unicellular protozoan parasites of the genus Trypanosoma and different species including *Trypanosoma congolense*, *Trypanosoma vivax, Trypanosoma brucei* and *Trypanosoma equiperdum* (Federica *et al.,* 2016). A cording to the study conducted by Geysen *et al*. (2003) stated that, Trypanosoma classified into three different types; *Trypanosomacongolense* savanna type, *Trypanosoma congolense* forest type and the *Trypanosoma congolense* Kilifi type. Most valuable domestic livestock (equids, bovines, ovines, caprines, camelids and suids) are susceptible to infection with one or more of these Trypanosoma species.

Lori *et al*. (2012) stated that Trypanosomiasis is transmitted through mechanical and vector, called Tsetse fly and particularly, *Trypanosoma brucei* and *Trypanosoma congolense* is largely dependent on tsetse flies for its transmission and also have the possibility of mechanical transmission with needles and other species of insects as it has been proved under experimental and field conditions by Sumba *et al*. (1998) and Desquesnes and Dia (2003). Trypanosomiasis is a wasting disease with a slow progressive loss of condition to the point of extreme emaciation, accompanied by enlargement of superficial lymph nodes, lacrimation, weakness, anemia, collapse and death of the animals (Ezeokonkwoa *et al*., 2010; Dagnachew *et al.,* 2005, Sharma *et al.,* 2000).

Trypanosomiasis can be treated by using different drugs such as Diminazene aceturate, Isometamidium chloride and Homidium chloride (Igoli *et al*., 2015). According to FAO (2001), AAT can be prevented through different strategies including

vector control, ecological control, and use of insecticides, sterile male technique and breeding of trypanotolerant breeds of animals in the area of Trypanosomiasis prevalent. Chemotherapy is more effective and accepted means preventing Trypanosomiasis as compared to different strategies.

Alirol *et al.* (2013) reported that Trypanosomiasis can lead to acute and/or chronic forms of wasting disease, causing high morbidity, mortality and infertility in the absence of treatment. Consequently, it affecting agricultural production and animal husbandry, and have a high economic and social impact in large areas of the tropics and subtropics where transmission occurs. According the study conducted by Alkhaldi *et al.* (2016). Africa has historically suffered the greatest burden of Trypanosomiasis in animals, but it is also spread to South America and South-East Asia, where unrestricted animal movements favor the spread the disease. In sub-Saharan Africa, *Trypanosoma congolense* is the most pathogenic trypanosome species infecting livestock (Stephen, 1986).

Trypanosomiasis is one of prevalently occurred disease of livestock in Ethiopia and cause huge economical loss through morbidity and mortality of animals. According to the study conducted by Afewerk *et al.* (2000) in Ethiopia, tsetse fly, the vector of Trypanosomiasis, is widely distributed particularly over lowland area of the country. The Southwest West and Northwest regions of Ethiopia accompanied huge numbers of animals raised under trypanosomosis risk in Africa (Abebe and Jobre, 1996; Afework *et al.,* 2000 and Sinshaw *et al*., 2006). Tsetse and non-tsetse transmitted trypanosomosis problem in the northwest regions of Ethiopia is also recently reported by Dagnachew *et al*. (2005).

According to FAO (1998) report, drug resistance develops by different species of Trypanosoma to one or more of the three-trypanocidal drugs (Diminazene aceturate, Isometamidium chloride and Homidium chloride) used in animals has been reported in many sub-Saharan Africa countries including Ethiopia. According to the report of NTTICC (1996) in Ethiopia, Trypanocidal drugs have been used for more than 40 years to control trypanosomosis in different domestic animals and the occurrence of drug resistant population of trypanosomes has also been confirmed by the previous studies reported in different regions of the country such as Diddessa and Angar valleys (Scott and Pegram, 1974), Benishangul Gumuz (Afework *et al,* 2000*)*, North Omo (Ademe and Abebe, 2000), Ghibe valley (Peregrine *et al.,* 1994), North west Amhara (Dagnachew *et al*., 2005).

The study conducted by Afework *et al* (2000) stated Diminazene aceturate and Isometamidium chloride are commonly used chemotherapy for Trypanosomiasis and among different species of trypanosomes, particularly *T. congolense* has been developed drug resistance in cattle in Ghibe valley and Benishangul Gumuz region, Ethiopia. Trypanocidal is also commonly used for the treatment of Trypanosomiasis in North West Ethiopia since trypanosomiasis in donkey is prevalent in jawi district. However, there is no well documented research on the development of drug resistance in the treatment of donkey against Trypanosomiasis in Jawi district.

Therefore, based on the above facts the current study undertaken based on following objectives.

- To assess the efficacy of diminazine aceturate and isometamidium chloride in the treatment of *typanosoma* infected donkeys from Jawi district, North West Ethiopia.
- To compare pathophysiological changes in hematological values of *trypanosoma* infected donkeys in the study area.

2. Literature review

2.1. Etiology

Trypanosomes are haemoprotozoal parasites which are cyclically transmitted by tsetse flies and affect man and economically important animals'. *T. conglolense, T.evansi, T.brucei and T equperdum* species which are commonly affect donkeys (Dagnachew, 2005).

2.2. Classification

Trypanosomes have been classified into taxonomic group based upon criteria's such as morphology, development in tsetse fly vector, preference for certain vertebrate host and molecular based analysis (Hoare, 1970).

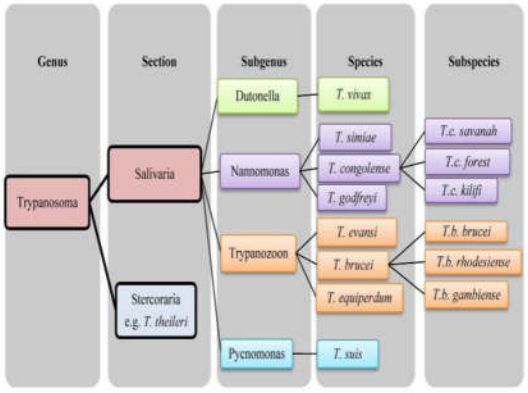


Figure 1: Schematic representation of the taxonomy of trypanosomes
Source (Gibson, 2003)

2. 3. Morphology

A sound knowledge of the basic features of the various trypanosomes enables the identification of each species and so the exact cause of the disease.

Trypanosomes are classified in the phylum Sarcomastigophora, the order Kinetoplastida and the family Trypanosomatidae (Bengaly, 2002). The trypanosome consists of a single cell varying in size from 8 to over 50 µm. There are distinct differences in appearance, shape and size between the various species of trypanosomes, allowing specific identification (FAO, 2006).

2.4. Locomotion

Trypanosomes move by the movement of their undulating membrane and free flagellum which acts kind of propeller. In fresh blood preparation almost all species of trypanosoma often just wiggle around without showing much forward progress except *T.vivax* which moves rapidly forward between the blood cells (FAO, 2006).

2.5. Life Cycle and Transmission

The life cycle of *trypanosoma* involves two phases (in mammalian host and in tsetse vectors) and four developments: the blood stream forms (BSF), the procyclic form (PF), epimastigot forms (EMF) and Meta cyclic forms (MCF) (Brett *et al.,* 2011). Some *trypanosoma* are reproducing by binary fission exchanging and recombination of genetic materials may take place in the tsetse fly between two trypanosomes, but unknown how frequently this occurs. The kinetoplast divided first. Then the second parabasal body develops, from which a second flagellum develops. The nucleus divides next, followed by the rest of the trypanosomes body duplicating all the structures present in the cytoplasm. The body divides into two daughter cells, beginning at anterior end the process is rapid and may result in vast population in the host within a short period of time (lori *et al.,* 2012).

The non-cyclical transmission of trypanosomes is aided by biting flies and thus, in the absence of *Glossina*, the transmission is maintained in the ecosystem. Biting flies, such as Tabanids (horse flies), *Stomoxys* and *Hippoboscids* transmit *T. evansi* mechanically through their mouthparts when they feed on more than one host within a short interval because the trypanosomes remain infective for only a short period. Cyclical transmission of trypanosomes undergo a cycle of development in the fly lasting between 8-35 days before infective metacyclic forare produced, and once infected the fly is capable of transmitting trypanosomes for the rest of its life (El-Sayed *et al.,* 2000.

2. 6. Pathogenesis

The earliest clinical sign of infection with *trypanosoma* in any host is the development at the fly bites of a chancre a cutaneous swelling in which the first trypanosomes multiply Bengaly *et al.* (2002). This initial replication increases the establishment of infection, while at this spot also the first interactions take place between the host immune system and the trypanosomes. After formation of a chancre, trypanosomes invade the blood stream, which is accompanied by pyrexia. The parasitaemia may remain high for 4 to 6 days after which it declines with remission of the temperature (Murray *et al*., 1988).

Anemia is a major component of the pathology of trypanosomosis and of African trypanosomosis, generally the degree of anemia might be considered as an indicator of the disease severity. The parasitaemia causes a large number of red blood cells (RBCs) to be removed from circulation by cells of the mononuclear phagocytic system (MPS) in the spleen, bone marrow, and haemal lymph nodes. The removal of a large number of RBCs leads to a fall in packed cell volume (PCV) to below 25% or even to as low as 10%. These results in affecting donkey with anaemia and it became dull, anorexic, listless, with ocular discharges, and loss of body condition (El-Sayed *et al.,* 2000).

In the late stages, anemia continues to be a major factor, with probably additional causes. However, irrespective of the cause of anemia the primary abnormality of function are the anoxic conditions created by the persistent anemia tissue anoxia, which results in a fall in tissue pH and vascular damage (Connor *et al.,* 2005). Following this are signs of dysfunction which appear in the various organs. An increase in cardiac output due to increases in stroke volume and heart rate and a decrease in circulation time are obvious manifestations (FAO, 2006)2.7. Clinical Signs

Donkey trypanosomiasis is characterized by pyrexia, progressive anemia, subcutaneous oedema, emaciation, ocular and nasal discharges and icterus (FAO, 2006). Locomotor ataxia is usually one of the earliest signs of the disease, and there is often obvious central nervous system involvement characterized by weakness, hyperexcitability and incoordination (Lori *et al.,* 2012).

2.8. Diagnosis

Trypanosoma infection can be diagnosed by clinical, parasitological, immunological and molecular methods but suspecting trypanosomiasis can be possible when an animal in an endemic area is anemic and in poor condition. Confirmation depends on the demonstration of the organism in blood or lymph node smears.

2.8.1. Clinical diagnosis

The disease is characterized by intermittent fever, anemia, lymphadenopathy, splenomegaly and cachexia often followed by death in untreated cases (Mulligan, 1970). Loss of condition will soon become obvious as first fat beneath the skin and then the muscles themselves are greatly reduced and the underlying bones become apparent. The skin often

loses its litheness because of dehydration, the eyes are sunken and at this stage the classical signs of anemia are obvious, the visible mucous membranes are pale and the blood is watery in appearance. Emaciation is associated with weakness and in the final stages results in inability to stand, and in pressure sores and ulceration of the skiover the bony prominences. There is very often an increased secretion of tears (Uilenberg, 1998).

2.8.2. Parasitological diagnosis

Parasite detection techniques are highly specific, but their sensitivity is relatively low. Due to this low sensitivity, the apparent parasitological prevalence of trypanosomosis is generally lower than the true parasitological prevalence. Moreover, in areas where trypanocidal drugs are used extensively, parasites may not be detected (Paris *et al*., 1982).

Direct examination techniques

Wet films of fresh blood, usually obtained from the ear vein, jugular vein or the tail constitute the simple, inexpensive and rapid method. Trypanosomes can be recognized by their movement among the red blood cells. Depending on the size and movement of the trypanosome a presumptive diagnosis can be made of the trypanosome species. The diagnostic sensitivity of the method is generally low but depends on the examiner's experience and the level of parasitaemia. Sensitivity can be improved significantly by lysing the RBCs before examination using a hemolytic agent such as sodium dodecyl sulfate (OIE, 2004). Thin and thick blood smear films are also simple and relatively inexpensive methods like wet blood films, but results are delayed because of the staining process. Trypanosomes are easily recognized by their general morphology, but may be damaged during the staining process. This may make it difficult to identify the species. Usually, both thin and thick smear is made from the same sample. Thick smears contain more blood than thin smears and, hence, have a higher diagnostic sensitivity. Thin smears on the other hand allow trypanosome species identification (OIE, 2004).

Parasite concentration techniques

Microhaematocrit centrifugation technique known as the Woo method (Woo, 1970) and buffy coat technique also called the Murray method (Murray *et al*., 1977) are commonly used concentration techniques. Identification of trypanosome species is difficult as the specific gravity of *T. congolense* is similar to that of RBCs, parasites are often found below the Buffy coat in the RBC layer. To improve the separation of RBCs and parasites, and increase the sensitivity for *T. congolense*, the specific gravity of RBCs can be increased by the addition of glycerol (Murray *et al.,* 1977). The plasma/white blood cell interface (Buffy coat) is examined by slowly rotating the tube. Trypanosome movement can first be detected at lower objective then increased (Desquesnes and Tresse, 1996).

The buffy coat technique represents another improved and widely used technique for the detection of trypanosomes where the Buffy coat and the uppermost layer of RBCs taken and extruded on to a clean microscope slide and examined for the presence of motile trypanosomes. The sensitivity of the buffy coat method can be improved by using the buffy coat double centrifugation technique. Unlike microhaematocrit centrifugation technique, the buffy coat technique has supplementary advantage that preparations can be fixed and stained for more accurate identification of species and for retention as a permanent record (Kratzer and Ondiek, 1989).

Animal inoculation

The sub inoculation of blood into rodents, usually mice or rats, is particularly useful in revealing sub-patent infections. The laboratory animals are injected intra-peritoneally with 0.25 ml (depending on the size) of freshly collected blood. They are bled three times a week for at least 2months. Collected blood is examined using the wet film method. Nevertheless, the method is not practical; it is expensive and diagnosis is not immediate. The method is highly sensitive in detecting *T. brucei* infections. However, some *T. congolense* strains are not easily transmitted and *T. vivax* rarely infects laboratory rodents. Also animal inoculation should be avoided as it raises serious animal welfare concerns (Schlater and Bossche, 2004).

2.8.3. Serological methods

Several antibody detection techniques have also been developed to detect *trypanosomal* antibodies for the diagnosis of animal trypanosomosis. Indirect fluorescent antibody test (IFAT) and the trypanosomal antibody-detection (ELISA) (Luckins, 1977; Hopkins *et al.*, 1998) are the methods of choice. ELISA using *T. congolense* precoated microtitre plates have been developed (Rebeski *et al.,* 2000). They detect immune responses to current and past infections and can, therefore, only provide a presumptive diagnosis of active infection. Sample collection and storage is made easy through the use of filter papers. All of these factors make the antibody ELISA a very useful test for large-scale surveys to determine the distribution of tsetse-transmitted trypanosomosis (Schlater and Bossche, 2004).

2.8.4. Molecular methods

A PCR method has been developed as a tool for the diagnosis of infections with African animal trypanosomes. Specific repetitive nuclear DNA sequences can be amplified for the three types of *T. congolense* (Masiga *et al.,* 1992; Desquesnes, 1997; Desquesnes and Davila, 2002). Unlike their prohibitive cost for routine use, PCR restriction

fragment length polymorphism (RFLP) assays have been recently developed that allow the identification of all *Trypanosoma* species as single or mixed infections using one single test (Delespaux *et al.,* 2003; Desquesnes *et al.,* 2001).

2.9. Treatment

2.9.1. Diminazene aceturate

Diminazene aceturate was started for the treatment of babesiosis and African trypanosomiasis in livestock in 1955. It belongs to the diamidine class of compounds a member of which (pentamidine) has also been used for human African trypanosoma (HAT) since the 1930s (Steverding, 2010). It was pursuing a structure-activity it synthesis from a compound belonging to a different class, Surfen at the time of its introduction in the 1930s, the best available agent against *T. congolense* infections that led to diminazene development (Although it was anti-*T. congolens*e activity in experimental rodents that initially group development, today's in vitro systems, where anti-parasite potency can be tested without confounding issues related to pharmacokinetic behavior in hosts, show that diminazene is substantially less potent against *T.congolense* than it is against *T. brucei* group trypanosomes (De Koning *et al.,* 2004). Diminazene is today the most commonly used drug in cattle, sheep and goats, due to its activity against both *T. congolense* and *T. vivax* and its relatively low toxic side effects (Reid, 2002).

The recommended therapeutic dose is 3.5 mg kg−1 body weight for African animal trypanosome (AAT) due to *T. congolense* and *T. vivax* (7 mg kg−1 may be recommended against resistant isolates) and 7 mg kg−1 is indicated for AAT due to *T. brucei* and for surra, administered by intramuscular or subcutaneous injection (Connor, 1992). The common practice of administering 3·5 mg kg−1 of the drug to treat *T. b. evansi* infections is considered an under dosing, and this miss use may have contributed to the emergence of resistant strains in South-East Asia (Desquesnes *et al*., 2013a). The fact that higher doses appear to be needed to treat *T. brucei* group trypanosomes, in spite of these parasites being more sensitive to the drug, probably relates to their wider tissue dispersal compared with *T. congolense* and *T. vivax*, underlining the key role of host pharmacokinetics.

Diminazene is only applied as a curative agent and is not used for prophylaxis, as it is rapidly metabolized and excreted (Peregrine and Mamman, 1993). After rapid absorption (the peak blood level is reached within 1hr of dosing), elimination follows a biphasic or triphasic behaviour depending on the animal species and formulation; elimination half life values following intramuscular administration varied from 11–19 h in sheep and goats, to 74to >200 h in cattle. Diminazene residues may persist for several weeks in the edible tissues of cattle and other food producing animals, especially in the liver and kidney, whereas the drug levels in milk peak at 6 hr and fall to below detection limits after 48 hr (FAO, 1990).

The trypanocidal mode of action of diminazene has not been completely elucidated. The compound binds the minor groove of the DNA at AT-rich sites (Wilson *et al*., 2008). In trypanosomes, the kDNA is a known target of the drug, and kDNA binding can cause inhibition of replication and kDNA loss possibly exacerbated by an inhibitory effect on mitochondrial type II topoisomerase (Portugal, 1994).

Chemically, diminazene is an aromatic diamidine made of two benzamidine moieties linked by a triazene bridge. Due to its charged nature, diminazene can only cross membranes via specific carriers and this has three important consequences: (a) the drug is not active on central nerves system (CNS) infections as it cannot cross the blood–brain barrier; (b) the compound is selectively toxic to trypanosomes, as they express transporters that specifically accumulate diminazene; and (c) trypanosomes may become resistant to the drug by losing these transporters or their activity. As mentioned above, diminazene uptake in *T. brucei* mainly occurs via an aminopurine transporter called P2, which is also implicated in the uptake of the related diamidine pentamidine and the melaminophenyl arsenical melarsoprol, two drugs licensed for HAT (De Koning, 2008).

Diminazene resistance is generally believed to be difficult to produce experimentally in *T. congolens*e (in contrast to *T. brucei*). High levels of resistance to the drug were obtained in mice infected with *T. b. evansi*, but only when using immunocompromised animals, a result which stresses the importance of the link between immunity and chemotherapy, as the efficacy of trypanocides appears to be reduced by immunosuppression, hence favouring development of resistance (Osman *et al*., 1992). In vitro experiments with *T. b. brucei* and *T. b. evansi* demonstrated that a shared mechanism of internalization accounts for the cross-resistance between diminazene and other diamidines as well as melaminophenyl arsenicals (melarsoprol and melarsomine) (Matovu *et al*., 2003).

9.2. Isometamidium chloride

Isometamidium chloride hydrochloride is a hybrid phenanthridine with amphiphilic and cationic properties, synthesized by coupling homidium with the diazotized p-aminobenzamide The veterinary formulations are typically a mixture of four phenanthridine compounds: isometamidium chloride hydrochloride [8-(3-mamidinophenyl-2-triazeno)-3-amino-5ethyl-6 phenylphenanthridinium chloride hydrochloride], the positional red isomer hydrochloride, the blue isomerphenylphenanthridinium chloride

hydrochloride, and the disubstituted compound (3-mamidinophenyltriazeno)-5-ethyl-6-phenylphenanthridinium chloride dihydrochloride]. A protocol for their individual purification from the mixture and a detailed structural analysis of each compound were described in a recent publication (Igoli *et al*., 2015). Moiety of diminazene, modified with the amidine group in Meta position. It has both curative and prophylactic properties and, since its launch in the 1960s, it has remained the only drug available for chemoprophylaxis of AAT, after quinapyramine was discontinued due to problems linked to toxicity and, particularly, the induction of multi-drug resistance (Holmes *et al.,* 1998).

Isometamidium is used mainly to treat and prevent *T. congolense* and *T. vivax* infections in livestock in Africa. Its activity against *T. brucei spp*. is less marked, but this drug can also be utilized against some *T. b. evansi* strains, although not when these have reached the CNS, as the compound does not cross the blood–brain barrier. The drug is administered to cattle at single doses of 0·25–1·0 mg kg−1 for cure, and at doses of 0·5–1 mg kg−1 for prophylaxis. The dosage for *T. b. evansi* infections is generally 1–2 mg kg−1, but in horses it is recommended not to exceed 0·5 mg kg−1 due to toxicity issues (Desquesnes *et al.,* 2013a).

Multiple intramuscular roots of administrations of isometamidium can cause severe fibrous lesions, hence damaging the carcass and meat quality from livestock. Intravenous administration has been successfully used to formal way agreement muscular damage, but it has been suggested that this could result in compromised prophylactic activity, due to the lack of a drug depository at the injection site. Isometamidium plasma concentrations reach their peak within 1 hr after administration and then fall relatively quickly during the first week post-treatment and thereafter more gradually (Eisler *et al*., 1994).

Three months after cattle had been injected, the circulating drug concentration was measured at 0·75 ng mL-1 This study showed that the serum concentration fits a bi-exponential model, with half-life of approximately 25 days for the second phase in cattle while another study indicated an elimination half-life of 9-19 days. In sheep and goats isometamidium appears to be eliminated more rapidly than in cattle (Wesongah *et al.,* 2004). Isometamidium may be used as part of a sanative pair with diminazene, the two drugs being used sequentially to minimize the risk of resistance development (Peregrine, 1994). Despite this recommendation, there are multiple reports of field isolates, from many African countries, indicating isometamidium resistance, particularly in *T. congolense* but also in *T. brucei* species and *T. vivax*, sometimes detailing cross-resistance with diminazene (Mamoudou *et al.*, 2008).

2.10. Detection of drug resistance

Several methods have been described to identify drug resistance in trypanosomes. At present, three types of technique are commonly used to identify drug resistance: tests in ruminants; tests in mice; and *in vitro* assays. None of these is, however, an ideal test and other tests are still in the Phase of development or validation (Peregrine, 1994).

2.10.1. Tests in ruminants

Tests in ruminants provide direct information from studies in ruminants using recommended doses of trypanocide. It is done by infecting a group of cattle or small ruminants with the isolate under investigation and later, when the animals are parasitaemic, treating them with various levels of trypanocide. The animals are then regularly monitored over a prolonged period (up to 100 days) to determine the effective dose and curative dose (Sones *et al.,* 1988). For these studies, the cattle or small ruminants must be kept in fly-proof accommodation or in a non-tsetse free area in order to eliminate the risk of reinfection during the study (sones *et al.,* 1989).

A useful indication of the level of resistance can be obtained from studies in ruminants (and mice) by recording the length of time between treatment and the detection of break through populations of trypanosomes. The advantages of studies in ruminants are that most trypanosome isolates of cattle are able to grow in these hosts and that the data obtained are directly applicable to the field. The disadvantages are the long duration (a follow-up of 100 days) is necessary to allow the detection of relapses) and the cost (purchase and maintenance of the animals are expensive). Furthermore, if only one isolate per animal is tested, it is usually impractical and too expensive to examine a large number of isolates (FAO, 1998).

2.10.2. Tests in mice

After expansion of an isolate in a donor mouse, groups of five or six mice are inoculated with trypanosomes. Twenty-four hours later, or at the first peak of parasitaemia, each group except the control group is treated with a range of drug doses. Thereafter, the mice should be monitored three times a week for 60 days. The advantage of the mouse assay is that it is cheaper than the test in cattle. There are several disadvantages, however: (i) most *T. vivax* isolates, and also some *T. congolense* isolates, do not grow in mice; (ii) although there is reasonable correlation between drug sensitivity data in mice and in cattle, higher doses of drug must be used in mice (normally ten times higher) in order to obtain comparable results to those obtained in cattle because of the vast difference in metabolic size; (iii) precise assessment of the degree of resistance needs a large number of mice per isolate,

which makes it a labour-intensive test - identification of a discriminatory dose, above which an isolate should be considered as resistant, could drastically reduce the number of mice and the amount of work to be carried out; and iv) it takes as long as60 days to evaluate the drug sensitivity of an isolate (FAO,1998).

.10.3. Trypanocidal drug ELISA

As an alternative to the tests mentioned above, the use of trypanocidal drug enzyme-linked immunosorbant assays (ELISAs) in combination with parasite detection tests has given promising results for the detection of resistant trypanosomes. A competitive ELISA which allowed the detection of small amounts of isometamidium in serum of cattle was first described by Whitelaw *et al* (1991). This technique was further improved and has been validated in cattle under experimental and field conditions (Eisler *et al.,* 1997). The test is both sensitive, detecting sub-nanogram (ng) concentrations, and specific. It allows the monitoring of drug levels overextended periods and the evaluation of factors influencing drug disappearance rates from the plasma (FAO, 1998). Observations showed that the presence of trypanosomes in animals with an Isometamidium chloride concentration of > 0.4 ng/ml suggests resistance the higher the drug level detected the greater the degree of resistance that could be inferred (Eisler *et al.,* 1993). Similar test for diminazene is in development (Murilla, 1996).

2.11. Control methods

For controlling of AAT, vector control, ecological, control, use of insecticides, sterile male technique and breeding of trypanotolerant animals are the major activities (FAO, 2001). Each of the available options for tsetse control or eradication has its own advantages and specific limitations. The strategies for using these options may vary considerably depending on the specific objective, technical and logistical feasibilities **(**FAO, 2001).

3. Materials And Methods

3.1. Description of the Study Area

The study was conducted in Jawi district of Amhara regional, North West Ethiopia. It is located approximately 600 km North West of Addis Ababa, the capital city of Ethiopia. The climate alternates with long summer rain fall (June-September) and a winter dry season (October-May) with mean annual rain fall of 1569 mm. The mean temperature varies between 16.680 ^{0}c to 37.60^{0}c and the altitude range from 648 to 1300 meter above sea level (NMSA, 2013). The land is covered by different vegetation types namely savanna grass land, forest, river and bush land. The study area is known by its tsetse fly infestation (Afework *et al.,* 2000).

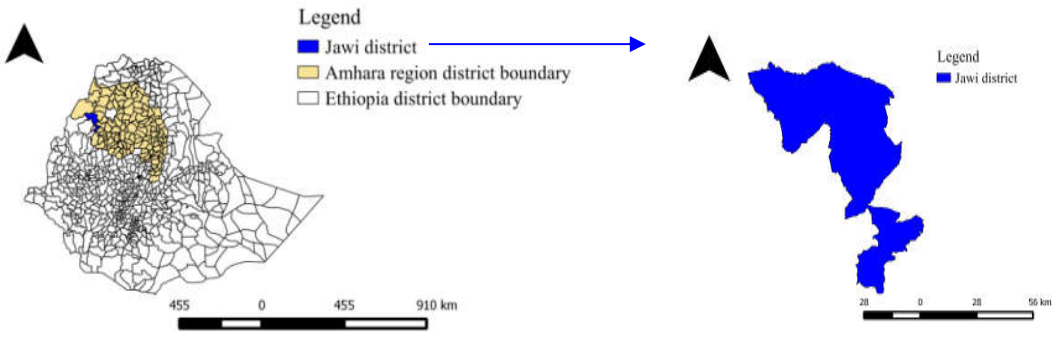


Figure 2: Map of study area

3.2. Study Animals

The study animals were local breed donkeys irrespective of sex and age those managed under different management systems, donkeys that came to district veterinary clinic with clinically suspected for Trypanosomiasis.

3.3. Study Design

Field clinical trial study design was conducted to compare the efficacy of Diminazene aceturate and Isometamidium chloride for the treatment of trypanosomiasis and to compare pathophysiological changes in hematological values of *Trypanosoma* infected donkeys in Jawi district, North West Ethiopia from January to Jun 2018.

3.3. Sampling Method

Purposive sampling method was employed to identify the *Trypansoma* infected donkey. Donkeys manifesting the clinical signs of Trypanosomiasis were selected purposively and thoroughly examined for the physical parameters. A blood sample was taken from ear vein and screened using wet blood film for parasite detection. Samples were also collected from those animals that were positive for *trypanosoma* for hematological examination.

3.4. Trial Treatment

The chemotherapy used in this study were Diminazene aceturate, at dose of 7mg/kg, given through intramuscular route of administration, stat and Isometamidium chloride, at a dose of 0.5mg/kg, given through intramuscular route of administration, stat.

3.5. Trial Procedures

Six positive donkeys were randomly grouped in to three groups, two donkeys for each group according to their admission to veterinary clinics, then one group taken as positive control and the other two groups treatment were administered randomly by lottery system (for group-I Diminazene aceturate and for group-II Isometamidium chloride).

To select the treatment randomly each group of treatment was designated by one specific number and this two different number of the two group treatments were multiplied two times to make 4 lotteries then the owner was select one of the lottery for his donkey treatment. Two positive control groups of donkeys were selected conveniently from the study area by convincing with the owner.

Drug regimens in each treatment group were-

Group. II. Diminazene aceturate 7 mg/ kg body wt IM for stat.

Group. II. Isometamediumchloride 0.5 mg/Kg IM for stat.

Isomethamidium chloride was given as a 1% solution, i.e. contents of the 1 g sachet dissolve in 100 ml sterile water. Administer the solution in deep intramuscular injection at 0.5 mg/kg live body weight (equal to 1 ml/20 kg (Bourdichon, 1998).

Group III-. Positive control –Normal saline

Post-Treatment Monitoring the experimental donkeys were monitored further for identified donkeys cure for 28 days during which blood samples were collected from all six infected donkeys groups as follows; once daily between day1 and day 7 post-treatment (PT), between day 8 and day 14 PT and day 15 and 21 PT and day 22 and 28 day PT. Clinical and hematological examination were made before and after treatment in each group and interpretation with conclusion was made on the basis of both findings.

3.4. Data Collection Methods.

3.4.1. Case history taking

A detailed history of feed intake, body condition, and hair coat and defecation status was recorded by preparing appropriate case recording sheet. Present, past, manage mental and environmental history was recorded according to OIE (2004) manual.

Clinical Examination: Clinical examination was undertaken according to Pascoe and Huntington, (2006). Vital signs (Body temperature, heart rate and respiratory rate) and detailed clinical examination were conducted and body weight was also estimated using heart girth meter (Melville *et al*., 2000).

Hematological examination: Five ml of blood was collected from jugular vein using Vacutainer tubes contain EDTA (Ethylene Diamine Tetra Acetic acid) from six Trypanosomiasis infected donkeys for hematological examination to determine Hemoglobin estimation (Hgb g/dl) and packed cell volume (PCV) according to Desquesnes (2002) method by using sahlihaemometer and microhaematocrit technique respectively.

Parasitological examination*:* After PCV was measured, then the capillary tube was broken 1mm below the buffy coat and 1cm above the buffy coat and express the content on the slide and cover with cover slip and examine under 40X objective lens using dark ground buffy coat techniques during study period (0-28 days) (Murray *et al*., 1983).

3.4. 1. Drug sensitivity tests protocol

For the drug resistance tests, after all of the field clinical trial 6 trypanosoma infected donkeys in each group showed parasitaemia, trypanocidal treatments were given according to the respective group as shown section 4.5 the treated donkeys were monitored for trypanosome parasitaemia by the wet smear and buffy coat technique one times in week for 28 consecutive days. If the parasite was not detected within 28 days after trypanocidal drug administration, the treatment was considered successful and the trypanosomas were well thought out sensitive to drug treatment.

3.6. Data Analysis

All data collected were checked for error, coded and entered in to Microsoft Excel spread sheet and analyzed using STATA software Version 14. The hematological and physical parametric change before and after treatment between treatment and positive control groups were analyzed by paired t- test. The mean value was set as mean standard deviation. For statistical significant ($P<0.05$) were considered as statistically significant. The mean PCV and mean body weight improvement and other parametric change after treatment were calculated using the following formulas (Argawa, 1996).

$$\text{Mean PCV Improvement} = 100 \times \frac{(\text{pcv2} - \text{pcv1})}{\text{pcv1}}$$

$$\text{Mean body weight improvement} = 100 \frac{(\text{bw2} - \text{bw1})}{\text{bw1}}$$

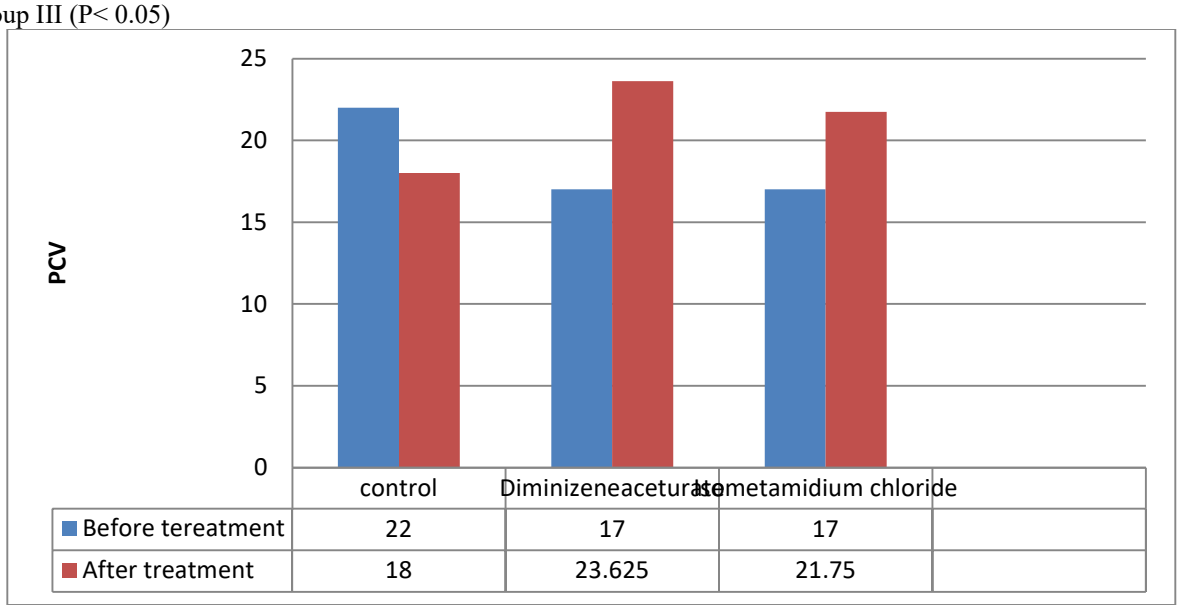
4. Result

4.1. Clinical findings

The main clinical findings observed during the study period were weakness, fever, rough hair coat, reduced feed intake, enlarged superficial lymph nodes, pallor of the mucus membranes, lacrimation, enlarged superficial lymph node and weight loss in all infected donkeys. Out 61of suspected donkeys for Trypanosomisis, 6 of them were confirmed with *T. congolense,* showing its movement was vibrating on

its position while one of its extremities is attached to the blood cell (OIE, 2004). The mean rectal temperatures of group I treated with diminazine aceturate before and after treatment donkeys were 39.1°C±0.42°C and 37.9°C±0.14°C respectively. The mean rectal temperatures of group II before and after treatment were 39.5°C±0.63°C, 38.4°C±0.56°C respectively. There was a statistically insignificant association between group I, group II. The mean of PCV, Hgb concentration, and heart rate were statistically significant in group I and II as compared group III (P< 0.05)

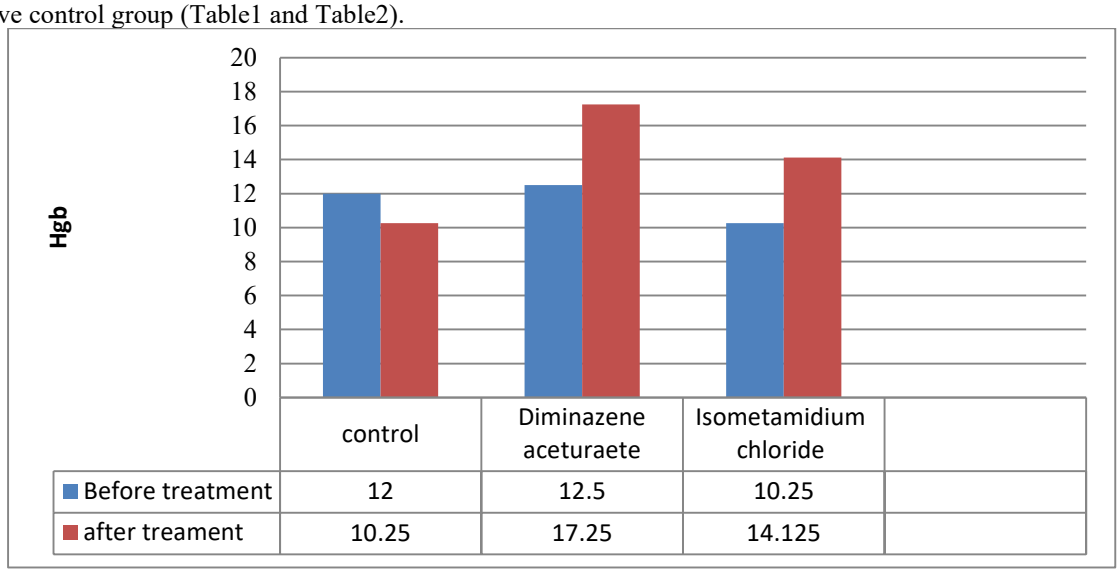
Infected donkeys temperature started rising before treatment with the appearance of parasitaemia and then fluctuated throughout after treatment. The highest mean temperature recorded was from group II on day 0 and the lowest temperature was recorded in group I on day 14 (table 1). The overall mean haematological changes, body weight estimation and temperature were significantly increased after treatment but the mean PCV in positive control group was decreased after treatment as shown in figure 3.



PCV=packed cell volume

Figure 3: The Mean PCV percentage measured before and after treatment.

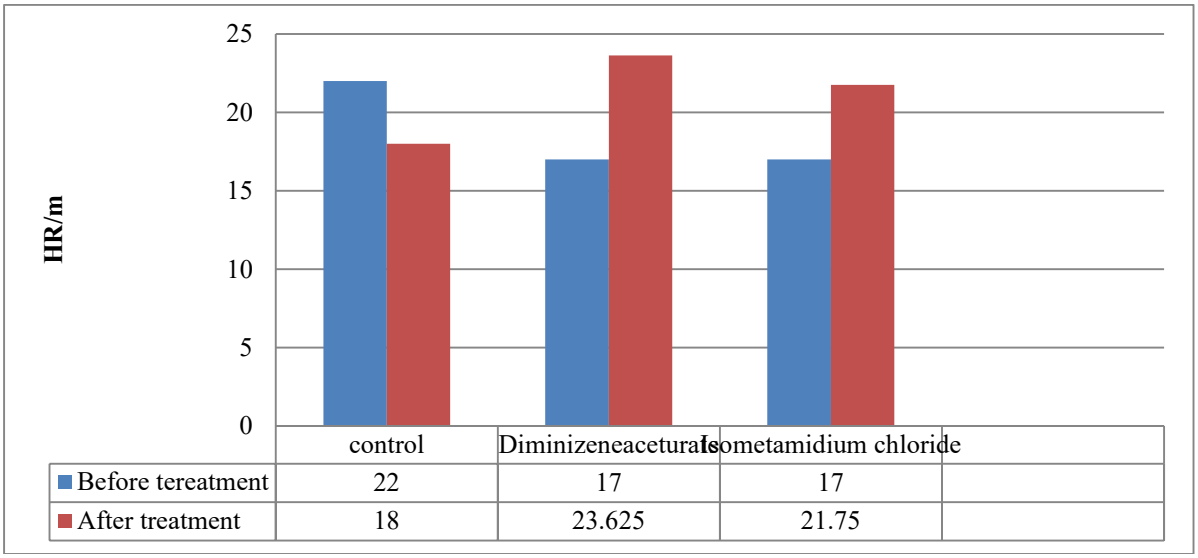
The mean Hgb in group I and II were significantly increased after treatment; however, it was decreased in positive control group (Table1 and Table2).



Hgb=Hemoglobin per desi litter

Figure 4: Mean of Hgb concentration measured before and after treatment.

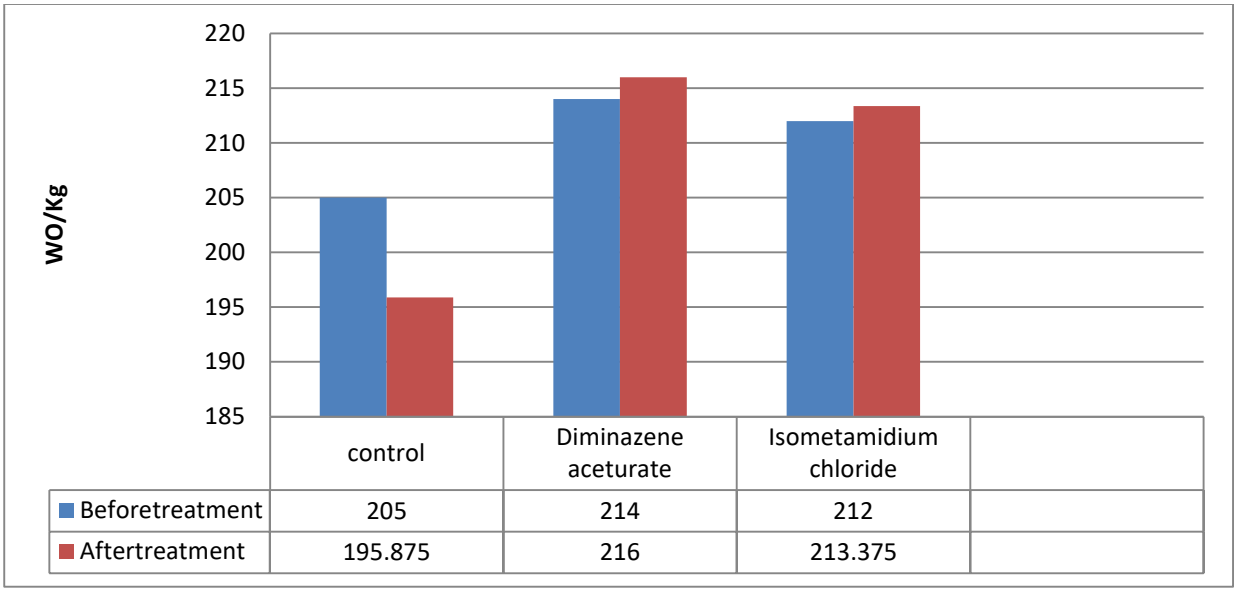
The mean HR in diminazene and Isometamidium treated infected groups were statistically significant and HR was decreased after treatment whereas the mean HR in positive control group was increased after treatment (figure 5).



HR/m= heart rate per minute

Figure 5: Mean of HR measurement before and after treatment

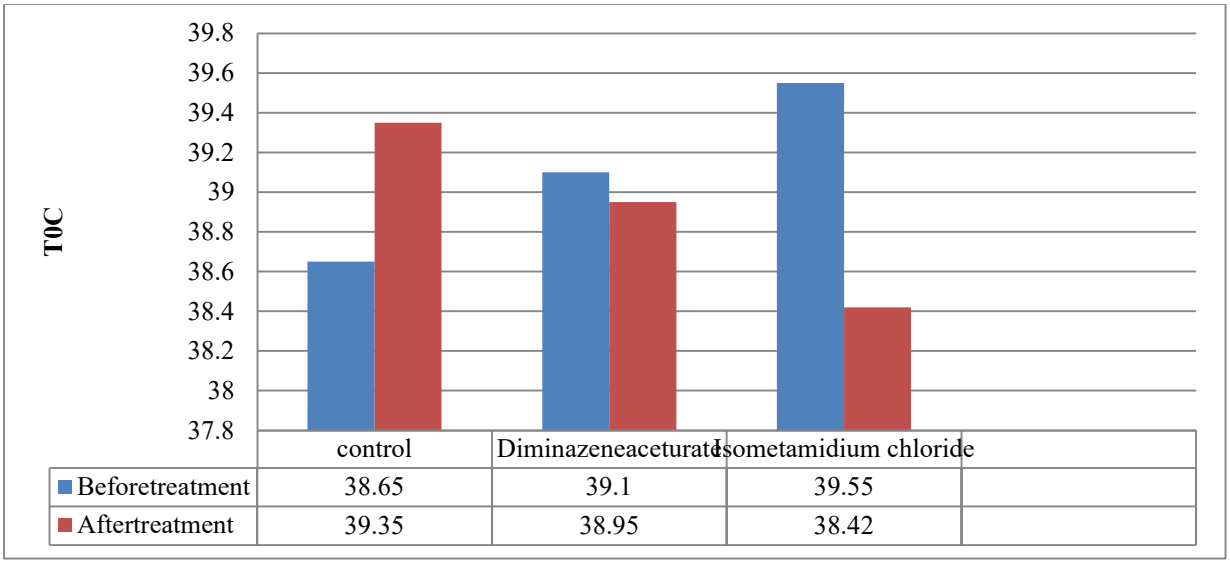
The mean body weight was significant increased after treatment in group I and group II but decreased in positive treatment group.



BW = Body weight per kilogram

Figure 6: Mean of BW measured before and after treatment

The mean T °C significantly decreased as shown (table1, table2) in group I and group II after treatment but increased in the positive control group during entire study period as shown in figure 7.



T^0c = temperature

Figure 7: The of mean T °c measurement before and after treatment

PCV, Hgb and BW change in pre-treatment and post treatment in group I was significantly increase after treatment but insignificantly decreased TEMP and PCV, Hgb and BW in control group was significantly decreased in but insignificantly decreased in TEMP and HR as shown the table 1.

PCV = packed cell volume, Hgb =hemoglobin, BW =body weight, HR=heart rate, TEMP = Temperature, control Table1

Parametric change in pre and post treatment in group II drug (Isometamediumchloride) was significantly increased after treatment but control group significantly decreased in PCV, Hgb, and BW but insignificantly increased TEMP and HR (Table 2).

Table 1: The parametric change in pre-treatment and post treatment in group II drug.

PCV = packed cell volume, Hgb =hemoglobin, BW =body weight, HR=heart rate, TEMP = Temperature, CO = control

The PCV values of almost all infected treated groups were below the standardized normal equine PCV value (22-38%) before treatment (Meredith and Robin, 2007). whereas treatment slight improvements of mean PCV values were observed.

Table 2: Mean PCV and percentage improvement during (0-28days)

	Group I			Group II			Group III		
Weeks	Mean	Imp	P -value	Mean	Imp	p-value	Mean	Imp	p- value
0	15			17			22		
1	21	0.4	0.0406	19	0.12	0.0232	20.5	-0.07	0.0125
2	24	0.14	0.0172	20.5	0.08	0.0074	18.25	- 0.11	0.0064
3	24.5	0.02	0.0177	22	0.07	0127	17	-0.07	0. 0172
4	26.5	0.08	0.0456	22.5	0.16	0.0032	15	-0.12	0.0030

Day 0 to 7: Statistical analysis using paired t-test on the PCV improvement before and after treatment showed that there was significant difference ($p < 0.05$) in the means of the two groups. The mean PCV improvement of animals in group I was higher than that of group II.

Day 0 to 28 –the means of the two treatment groups before and after treatments revealed that again there was significant difference ($p<0.05$. The maximum PCV improvement was 40 % (Group I on week 1) and minimum was 7% (group II on week 3) but the mean PCV in group III was decreased shown in table 3.

Imp= improvement, Group I= diminazine aceturate, Group II= Isometamidium, Group III = positive = control

Day 0 to 7: The Hgb improvement before and after treatment showed that there was significant difference ($p < 0.05$) in the means of the two groups.

Day 0 to 28– mean Hgb between groups in which group I had relatively higher Hgb than Group II. The maximum PCV improvement was 14% (Group I on week 1and 3) and minimum was 4% (group II on week1) but Hgb was reduced in the entire period of the study in group III.

Table 3: Mean HR and percentage improvement during the study (0-28days)

Imp= improvement, Group I= diminazine aceturate, Group II= Isometamidium, Group III = positive control

Day 0 to 7: The HR reduced before and after treatment showed that there was significant difference ($p < 0.05$) in the means of two groups. The mean HR reduced of animals in group I was higher than that of group II.

Day 0 to 28 -the means of the two treatment groups before and after treatments revealed that again there was significant difference ($p < 0.05$). There was a difference in mean HR between groups in which group I has relatively higher reduced HR than Group II. The maximum HR reduced was 19% (Group I on week 1) and the minimum reduced was 2% (group I on week4 and group II on week 2).

Table 4**:** Mean T ^{0}c and percentage improvement during the study period

Imp= improvement, Group I= diminazine aceturate, Group II= Isometamidium, Group III = positive control

Day 0 to 7: The T^{o}c reduction before and after treatment was observed from week 0 to week 2 and then improves from week 2 to week 3 then reduced from week 3 to week 4 that there was insignificant difference ($p > 0.05$) in the means of the two groups. The mean T^{0}c improvement of animals in group fluctuated throughout the study period.

Day 0 to 28- the mean T^{o}c of the two treatment groups before and after treatments revealed that again there was no significant difference ($p > 0.05$). But there was a difference in mean T^{o}c between groups in which group II had relatively higher T^{o}c than Group I. The maximum reduction was 5% (Group II on week 1) and the minimum improvement was 2% (group I on week3). The maximum improvement in control group III was 40% on week 3.

Table 5: Mean BW and percentage improvement during the study period.

Imp= improvement, Group I= diminazine aceturate, Group II= Isometamidium, Group III = positive control

Day 0 to 7: The BW improvement before and after treatment showed that there was significant difference ($p < 0.05$) in the means of the two groups.

Day 0 to 28 the means of BW in the two treatment groups before and after treatments revealed that again there was significant difference ($p<0.05$). But there is a difference in mean BW between groups in which group I had relatively higher BW than Group II. The maximum BW was improved by 0.7% (Group I on week 2 and the minimum BW was reduced by 0.1% (group II on week 4 and group II on week 3).

The mean body weight measurement of positive control group was significantly lower than treatment groups. The mean body weight measurements observed in the study was showed significant difference ($P< 0.05$) up to day 28 pi between infected treated groups.

5. Discussion

5.1. Clinical findings

According to current study the sign of *T.Congolense* infected donkeys were seen, weakness, fever, rough hair coat, enlarged superficial lymph nodes, lacrimation, weight loss, paleness of mucus membranes. This sign also similarly reported by (Hagos *et al*., 2014, Akpa *et al*., 2008).

According to the present study significant increase of the temperature in *T.congolense* infected donkeys dependent on the enhanced level of released pyrogens in the severely stressed donkeys Furthermore, loss of body weight during *T. congolense* infected donkeys due to mobilization of body energy reserves to deprivate essential nutrients for the synthesis of ATP in the anorexic donkeys. This study was frequently reported by several authors (Osaerio *et al*., 1998 and Pentreath, 1994).

5.2. Haematological findings

In the current study, changes in haematological parameters were marker of trypanosomiasis. The decrease in Hgb and PCV were observed trypanosomiasis infected donkeys due to decrease in the life span of erythrocytes and extensive erythrophagocytosis. Hemoglobin (Hgb) concentration (g/dl), (PCV) in affected control group was significantly lower range than the treatment group. These haematological findings were similarly reported by (Habila *et al.,* 2012**).**

According to the present study the improvement in PCV and Hgb readings after treatment was due to elimination of the sensitive species of trypanosomes from the animal body. However, the overall mean PCV and Hgb is below the physiological value. This may be due to blood sucking helminthiasis like haemonchosis, bunostomiasis and oesophagostomiasis, protozoal disease like babesiosis, anaplamosis and coccidiosis; and/or reduced response of the bone marrow due to exhaustion when the infection runs a chronic course. This finding also reported by Murry *et al. (*1977).

According to the current study the realistic reduction of PCV and Hgb concentration following infection indicates anaemia which is a cardinal feature of trypanosomosis in donkeys. This finding similarly reported by (Anosa *et al*., 1980). The reduction in mean PCV values might have a direct correlation with the decline in total RBC counts. The anemia is manifested by pallor of the mucous membrane of the eye. Many factors have been reported in the literature to be responsible for the reductions in Hgb concentrations and the subsequent PCV value (which

communally is termed as anemia) in trypanosomosis of donkeys according to the present study. Similar hematological findings were reported by (FAO, 2006, Ezeokonkwo *et al*., 2010, Murry *et al*., 1977).

In the current study indicate anemia associated trypanosomosis causes weakness, lethargy and lack of energy which ultimately reduce efficiency of working animals. The consequence of anaemia is one of the most typical signs trypanosome caused by *T. congolense* in susceptible donkeys. This sign was also similarly reported by Abebe (1991).

In the current study the use of trypanocidal drugs improved the body weight of animals in groups I filed clinical trial period by10% on week 2. Group II bodyweight improvement was 0% on week one group one, on week three, week four in group two, and non – treated group animals the maximum body weight reduced by1.3%, this emphasizing the reduced body weight trypanosomosis. Body weight improvement in group I had been improved that animals received due to the relative efficacy of diminazine aceturate (7 mg/kg) to cure infection in the study area. The loss of weight in-group, II may be attributed to the number of relapses experienced. The mean body weight gain of animals' in-group I was higher than II groups. This result was similarly reported by FAO, (2000).

According to the current study, heart rate in the late stages, anemia continues to be a major factor, with probably additional causes. However, irrespective of the cause of anemia the primary abnormality of function are the anoxic conditions created by the persistent anemia tissue anoxia, which results in a fall in tissue pH and vascular damage. This was also reported by (Connor *et al.,* 2005). Following this were signs of dysfunction which appear in the various organs. An increase in cardiac output due to increases in stroke volume and heart rate and a decrease in circulation time are obvious manifestations but reduced cardiac output after treatment. Similarly finding was reported by FAO, (2000).

In the current study, rectal temperature in the natural infected donkeys developed fever throughout the *T. congolense* infection. The infection causes an increase in body temperature and a decline in the PCV in the early or acute phase. Afterwards, when the infection enters its chronic phase, the PCV remains relatively low but stable temperature but fast recovery to normal after administration trypanocidal drug. This change was similar to report by Van den Bossche, (2005).

6. Conclusion And Recommendations

Out of 61 suspected donkeys for Trypanosomisis and screened by using wet blood film, six of them were confirmed having infected with *T. congolense*. The present study was concluded that the efficacy of diminazene aceturate was more effective than Isometamidium chloride analyzing hematological and physical parameter changes. Hgb, PCV and body weight were significantly ($p< 0.05$) increased during post treatment but heart rate and temperature were insignificantly decreased during the treatment of diminazene aceturate. In isometamidium chloride group heart rate and temperature were significantly ($p< 0.05$) decreased, however, Hgb, PCV, and body weight were significantly ($p< 0.05$) decreased in positive control group. Heart rate and temperature were insignificantly ($p> 0.05$) increased in positive control group. The maximum PCV improvement was 40 % (Group I on week 1) and minimum was 7% (group II on week 3).

Therefore based on the above conclusion the following recommendations are forwarded.

- Diminazene aceturate is more effective for the treatment of trypanosoma infected donkeys in the study area so that clinician better use this drug for the treatment of trypanosoma infected donkeys.
- Trpanosomiasis which is caused *by T. Congolese endemic* from jawi district so that people use trypanocidal drugs along with vector control strategies.
- Further experimental study should be conduct to cheek the efficacy of diminazene aceturate and isometamidium chloride in the study area.

References

1. Abebe, G. and Jobre, Y. (996): Trypanosomosis: A threat to cattle production in Ethiopia. *RevuMédcal. Véternaria,* 147: 987- 902.
2. Ademe, M. and Abebe, G. (2000): Field Study on drug resistance trypanosomes of bovine in kindokoyshawereda southern Ethiopia. *Bulletin Animal health and production in Africa*. 48: 131-138.
3. Afework, Y., Clausen, P.H., Abebe, G., Tilahun, G. and Mehlitz, D. (2000): Multiple-drug resistant *Trypanosoma congolense* populations in village cattle of Metekel district, northwest Ethiopia. *Acta tropical veterinaria*, 76: 231-238.
4. Argawa, B.L. (1996): Basic statistics, 3rd edition. New Age International (P) Limited Publishers.
5. Bengaly, Z., Sidibe, I., Ganaba, R., Desquesnes, M., Boly, H. and Sawadogo, L. (2002): Comparative pathogen city of 3 genetically distinct types of *Trypanosoma congolense* in cattle: clinical observations and haematological changes. *Veterinary Parasitology,* 108: 1–19.
6. Biryomumaisho, S., Katunguka-Rwakishaya, E. and Rubaire-Akiiki, C. (2003): Serum biochemical changes in experimental *Trypanosoma congolense* and *Trypanosoma brucei* infection in Small East Africa goats. *Veterinarski Arhiv,* 73 (3): 167–180.

7. Bourdichon, A. (1998): Report on the use of the trypanocidal drug 'trypan'. *J. Protozool. Res*. 8, 258-262.
8. Brett, A., Eyfored, T., Derek, s., Binca, L., Hertz-flower, C., Jhon, Edonelson, N., Conner, R., and van den Bossche, p. (2011): African animal Trypanosomes. JAW Coetzer, RC Testing, infection Diseases of Livestock, 2nd edition., volume-1,.
9. Brett, A., Eyford, T. S., Derek, S., Bianca, L., Hertz-Fowler, C., John, E., Donelson, N., Connor, R. J, and van den Bossche, P. (2011): African animal Trypanosomes. In JAW Coetzer, RC Tustin, Infection Diseases of Livestock, 2nd edition, volume- 1.
10. Connor, R. J. (1992): The diagnosis, treatment and prevention of animal trypanosomiasis under field conditions. *In Programme for the Control of African Animal Trypanosomiasis and Related Development: Ecological and Technical Aspects*. FAO Animal Production and Health Paper No. 100. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.
11. Connor, R. J, van den Bossche, P., (2005): African animal Trypanosomes. In JAW Coetzer, RC Tustin, Infection Diseases of Livestock, 2nd ed., Volume 1, Oxford University Press, Cape Town, Pp.251-296.
12. Dagnachew, S., Sangwan, AK. and Abebe, G. (2005): Epidemiology of bovine trypanosomosis in the Abay (Blue Nile) basin areas of Northwest Ethiopia. *Revue d'élevageetdemedecinevétérinairedes pays tropicaux*, 58:151–7.
13. De Koning, H. P. (2008): Ever-increasing complexities of diamidine and arsenical cross-resistance in African trypanosomes. *Trends in Parasitology* 24: 345–349.
14. De Koning, H. P., Anderson, L. F., Stewart, M., Burchmore, R. J., Wallace, L. J. and Barrett, M. P. (2004): The trypanocide diminazene aceturate is accumulated predominantly through the TbAT1 purine transporter: additional insights on diamidine resistance in African trypanosomes. Antimicrobial. *Agents and Chemotherapy* 48, 1515–1519.
15. Desquesnes, M. and Davila, A. (2002): Applications of PCR-based tools for detection and identification of animal trypanosomes; a review and perspectives. *Veterinary Parasitology,* 109: 213-231.
16. Desquesnes, M. and Dia, M. L. (2003): Mechanical transmission of *Trypanosoma congolense* in bovine by the African tabanid Atylotusagrestis. *Experimental Parasitology,* 105:226-231.
17. Desquesnes, M. and Tresse, L. (1996): Evaluation de la sensibilité du test de Woo pour la détectionde *Trypanosoma vivax*. *Journal of Tropical Livestock Science,* 49: 315-321.
18. Desquesnes, M., Dargantes, A., Lai, D. H., Lun, Z. R., Holzmuller, P. and Jittapalapong, S. (2013a): Trypanosoma evansi and surra: a review and perspectives on transmission, epidemiology and control, impact, and zoonotic aspects. *BioMed Research International* 2013, 321237.
19. Desquesnes, M., McLaughlin, G., Zoungrana, A. and Davila, A. M. (2001): Detection and identification of *Trypanosoma* of African livestock through a single PCR based on internal transcribed spacer 1 of rDNA. *International Journal of Parasitology,* 31:610-614.
20. Eisler, M. C., Arowolo, R. O., Gault, E. A., Moloo, S. K., Holmes, P. H. and Peregrine, A. S. (1994): Isometamidium concentrations in the Federica Giordani and others 1884sera of Boran cattle: correlation with prophylaxis against tsetse-transmitted Trypanosoma congolense. *Acta Tropica* 56, 39–50.
21. Eisler, M. C., Gault, E. A., Moloo, S. K., Holmes, P. H. and Peregrine, A. S. (1997): Concentration of isometamidium in sera of bovine challenged with drug-resistant *Trypanosoma congolense*. *Acta Tropica,*63: 89-100.
22. Eisler, M. C., J brandt, B., Sinyangwe, L. and Geerts, S. (2001): Standardized test in mice and cattle for the detection of drug resistance in tsetse transmited trypanosoms of African domestic cattle. *Veterinary Parasitology,* 97:171-182.
23. El-Sayed, N. M., Hegde, P., Quackenbush, J., Melville, S. E. and Donelson, J. E., (2000): The Africantrypanosome genome. *International Journal of Parasitology*, 30: 329-345.
24. Ezeokonkwoa, C. R., Ezeha, I. O., Onunkwob, J. I. Obia, Onyenwea, I. W. and Agu, W. E. P. O. (2010): Comparative haematological study of single and mixed infections of mongrel dogs with *Trypanosoma congolense* and *Trypanosoma brucei* b *brucei*. *Veterinary Parasitology,* 173: 48–54.
25. FAO (1990): *Residues of Some Veterinary Drugs in Animals and Foods*. FAO Food and Nutrition apper No. 41/2. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.
26. FAO, (2006): Corprate Document Repository. A field 13 Whitelaw, D. D., P. R. Gardinareand M. Murray, 1988. Guide for diagnosis treatment and prevention of African animal trypanosomosis. Food and agricultural organization of united nation available on www.fao.org/DOCREP/2006
27. FAO. (1998): Drug management and parasite resistance in bovine trypanosomosis. *ISBN 92-5-104185-7*. Rome, Italy.
28. FAO. (2006): Corporate Document Repository: A field 13. Southern Africa, Cape Town, pp. 251-296.
29. Federica, G. Liam, J. Morrison, G. Rowan. Harry, P. De Koning and Michael, P. Barrett. (2016): The animal trypanosomiasis and their chemotherapy: a review *Parasitology,* 143, 1862–1889.
30. Food and Agricultural Organization of the United Nations (FAO) (2000): A field guide for the diagnosis, treatment and prevention of African

animal trypanosomosis, 2nd edition. FAO, Rome, Italy.
31. Geysen, D., Delespaux, V. and Geerts, S. (2003): PCR-RFLP using Ssu-rDNA amplification as an easy method for species-specific diagnosis of *Trypanosoma* species in bovine. *Veterinary Parasitology,* 110: 171-180.
32. Gibson, W. (2003): Resolution of the species problem in African trypanosomes. International *Journal of Parasitology*, 37: 829 838.
33. Hoare, C. (1970): The trypanosomes of mammals, Azoological monograph. Black well scientific publication, oxyford, England, Pp.1-746.
34. Hopkins, J. S., Chitambo, H., Machila, N., Luckins, A. G., Rae, P. F., Van den Bossche, P. and Eisler, M. C. (1998): Adaptation and validation of the antibody trapping ELISA using dried blood spots on filter paper, for epidemiological surveys of tsetse transmitted trypanosomosis in bovine. *Preventive Veterinary Medicine,* 37: 91-99.
35. Igoli, J. O., Blackburn, G., Gray, A. I., Sutcliffe, O. B., Watson, D. G., Euerby, M. R. and Skellern, G. G. (2015): Chromatographic and spectroscopic analysis of the components present in the phenanthridinium trypanocidal agent isometamidium. *Analytical and Bioanalytical Chemistry* 407, 1171–1180.
36. Kratzer, R. D. and Ondiek, F. O. (1989): The buffy coat double centrifugation technique, an improved method for the diagnosis of African trypanosomosis. Nairobi: OAU/IBAR (Organization of African Unity/Interafrican Bureau for Animal Resources). International Scientific Committee for Trypanomonosis Research and Control (ISCTRC), Mombassa, Kenya, April 1989.
37. Lori, p., Simon, C., Vanessa, F., Mick, B. and Wendy, G. (2012): The life cycle of *Trypanosoma conglolense* in tsetse fly, 5:109,.
38. Luckins, A. G. (1977): Detection of antibodies in trypanosome infected bovine by means of a microplate enzyme-linked immune-sorbent assay. *Tropical Animal Health and Production,* 9: 53-62.
39. Mamoudou, A., Delespaux, V., Chepnda, V., Hachimou, Z., Andrikaye, J. P., Zoli, A. and Geerts, S. (2008): Assessment of the occurrence of trypanocidal drug resistance in trypanosomes of naturally in fected cattle in the Adamaoua region of Cameroon using the standard mouse test and molecular tools. *Acta Tropica* 106, 115–118.
40. Matovu, E., Stewart, M. L., Geiser, F., Brun, R., Maser, P., Wallace, L. J., Burchmore, R. J., Enyaru, J. C., Barrett, M. P., Kaminsky, R., See beck, T. and de Koning, H. P. (2003): Mechanisms of arsenical and diamidine uptake and resistance in *Trypanosoma brucei. Eukaryotic Cell* 2, 1003–1008.
41. Mulligan, H. W: (1970): The African Trypanosomiasis. Pp. 950 (Mulligan, H. W. edition.) London: George Allen and Unwin Ltd.
42. Murray, M., Murray, P. K. and McIntyre, W. I. M. (1977): An improved parasitological technique for the diagnosis of African trypanosomosis. *Transactions of the Royal Society of Tropical Medicine and Hygene*.71: 325-326.
43. Murray, M; P. K and Mcintyre W. I. M. (1988): An improved parasitological technique for the diagnosis of African trypanosomosis. *Trans. R. Soc. Trop Med Hyg* 71: 325-326.
44. Naessens, J. (2006): Bovine trypanotolerance: a natural ability to prevent severe anemia and haemophagocytic syndrome. *International Journal of Parasitology,* 36: 521-528.
45. NMSA (National Meteorological Services Agency), (2013): Monthly report on temperature and Rainfall distribution for Awi Zone, Regional Metrological Office, Bair Dar, Ethiopia.
46. NTTCC (1996): Annual Report. Ministry of agriculture, National Tsetse and Trypanosomosis investigation and control centre (NTTCC) Bedelle, Illubabor, Etiopiapp.29. OAU/STRC: 29 September to 3October, r1997.
47. Nwoha, R. I. O. and Anene, B. M. (2016): Symptoms and response to treatment with diminazene.
48. OIE. (2004): Tsetse-transmitted trypanosomosis. In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. World Organization for Animal Health. 5 The edition. Available on*http://www.oie.int/eng/OIE/organisation/en_LR.htm*, *Parasitology*, 110: 171-180.
49. Osaerio, S., Goossens, B., Jeffcoate, I. and Holmes P. (1998): Effects of *Trypanosoma congolense* and nutritional supplements in Djallonké ewes on live weight during pregnancy, postpartum weight, haematology parameters and lamb performance. *Research of Veterinary Science*.65: 65–9.
50. Osman, A. S., Jennings, F. W. and Holmes, P. H. (1992): The rapid development of drug-resistance by Trypanosoma evansi in immunosuppressed mice. *Acta Tropica* 50, 249257.
51. Paris. J., Murray, MPK., Mc-Oimba, F. (1982): A comparative evaluation of the parasitological techniques currently available for the diagnosis of African animal trypanosomosis in cattle. *ActaTropical,*39: 307–16.
52. Pentreath, V. W. (1999): Cytokines and blood–brain barrier in human experimental African trypanosomiasis. In: Dumas, M., Bouteille, B., Buguet, A. (Eds.), Progress in Human African Trypanosomiasis, Sleeping Sickness. *Springer-Verlag, Paris.* Pp. 105–117.
53. Pentreath, V. W. and Kennedy, P. G. E., (2004): Pathogenesis of human African Trypanosomiasis. In: Maudlin, I., Homes, P. H., Miles, M. A. (editions), The Trypanosomiasis. CAB International, UK, Pp. 283–301.

54. Peregrine, A. S. (1994): Chemotherapy and delivery systems: haemoparasites. *Veterinary Parasitology* 54, 223–248.
55. Peregrine, A. S. and Mamman, M. (1993): Pharmacology of diminazene: a review. *Acta Tropica* 54, 185–203.
56. Portugal, J. (1994). Berenil acts as a poison of eukaryotic topoisomerase II. *FEBS Letters* 344, 136–138.
57. Rebeski, D. E., Winger, E. M., Okoro, H., Kowalik, S., Burger, H. J., Walters, D. E., Robinson, M. M., Dwinger, R. H. and Crowther, J. R. (2000): Detection of *Trypanosoma congolense* antibodies with indirect ELISAs using antigen-precoatedmicrotitre plates. *Veterinary arasitology,* 89:187- 198.
58. Reid, S. A. (2002). Trypanosoma evansi control and containment in Australasia. *Trends in Parasitology* 18, 219–224.
59. Schlater, J. and Bossche, V. D. (2004): Trypanosomosis: International des Epizooties Manual of diagnostic tests and vaccines for terrestrial animals, 5th edition. In: URL: http://www.oie.int/Eng/Normes/Mmanual/Asummry.html.
60. Sharma, DK., Chauhanb, PPS., Saxenac, VK. And Agrawal, RD. (2000): Haematological changes in experimental trypanosomiasis in Barbari goats. *Small Rum Research*, 38:145–9.
61. Stephen, L. E. (1986): Trypanosomiasis, a veterinary perspective. Pergamon Press, Oxford, UK, pp. 551.
62. Steverding, D. (2010). The development of drugs for treatment of sleeping sickness: a historical review. *Parasites & Vectors*3, 15.
63. Sumba, A. L., Mihok, S. and Oyieke, F. A. (1998): Mechanical transmission of *Trypanosoma evansi* and *T.congolense* by *Stomoxysniger* and *S. taeniatus* in a laboratory mouse model. *Medical and Veterinary Entomology,* 12: 417-422.
64. Uilenberg, G. (1998): Basic morphology of trypanosomes: in a Field Guide for the Diagnosis, Treatment and Prevention of African Animal Trypanosomosis. Food and Agriculture organization of the united nations rome.
65. Urquhart, G. M. (1974): Immunization against trypanosomiasis. Paper presented at the 3rd International Congress of Parasitology, Munich.
66. Van den Bossche, P., Shumba, W. and Makhambera, P. (2000): The distribution and epidemiology of bovine trypanosomosis in Malawi. *Veterinary Parasitology,* 88:163-176.
67. Wesongah, J. O., Jones, T. W., Kibugu, J. K. and Murilla, G. A. (2004): A comparative study of the pharmacokinetics of isometamidium chloride in sheep and goats. *Small Ruminant Research* 53, 9–14.
68. Whitelaw, D. D., P. R. Gardiner and Murray, M. (1988): Guide for the diagnosis, treatment and prevention of African animal trypanosomosis. Food and Agriculture Organization of the United Nations. Available on: *www. fao. org/DOCREP/2006*.
69. Whiteside, E. F. (1960): Recent work in Kenya on the control of drug-resistant bovine trypanosomosis. In: Proceedings of the 8th Meeting of the International Scientific Council for Trypanosomosis *Research and Control, Jos, Nigeria*, Pp.141–154.
70. Wilson, W. D., Tanious, F. A., Mathis, A., Tevis, D., Hall, J. E. and Boykin, D. W. (2008): Ant parasitic compounds that target DNA. *Biochimie* 90, 999–1014.
71. Woo p. t. k. (1970): the haematocrit centrifugation technique for the diagnosis of African trypanosomiasis. *Canadian journal of zoology*, 47: 921-923.

9. Annex

Annex 1: Wet blood film (MAFA, 1986)

Procedure

- A drop of blood was placed on the center of a microscope slide Covered it with a cover slip and observe under a microscope (40× objective).

Annex 2: The packed cell volume determination (Murray, 1977)

Procedure:

- Approximately ¾ of the capillary tubes were filled with the well-mixed whole blood.
- Excess blood outside the tube was wiped out carefully by using cotton after firmly closed the top of the capillary tube with index finger to prevent spillage of the blood.
- The vacant end of the tube is sealed with sealing clay sealant made for this purpose.
- The capillary tubes are placed in the microhaematocrit centrifuge with the sealed end toward the periphery and centrifuged for five minutes at full speed of 12,000rpm; duplicate tubes are placed opposite each other for balance.
- Using Hawksleymicrohaematocrit reader the readings were made in percent; the bottom Sealed end of the capillary tube put at 0% mark and the top plasma end was adjusted at 100 % mark.
- Then the PCV value was read at the junction space between the buffy layer and the Packed RBC. Results were recorded to the nearest whole number.

Annex 3: Haemoglobin concentration determination (Murray, 1977)

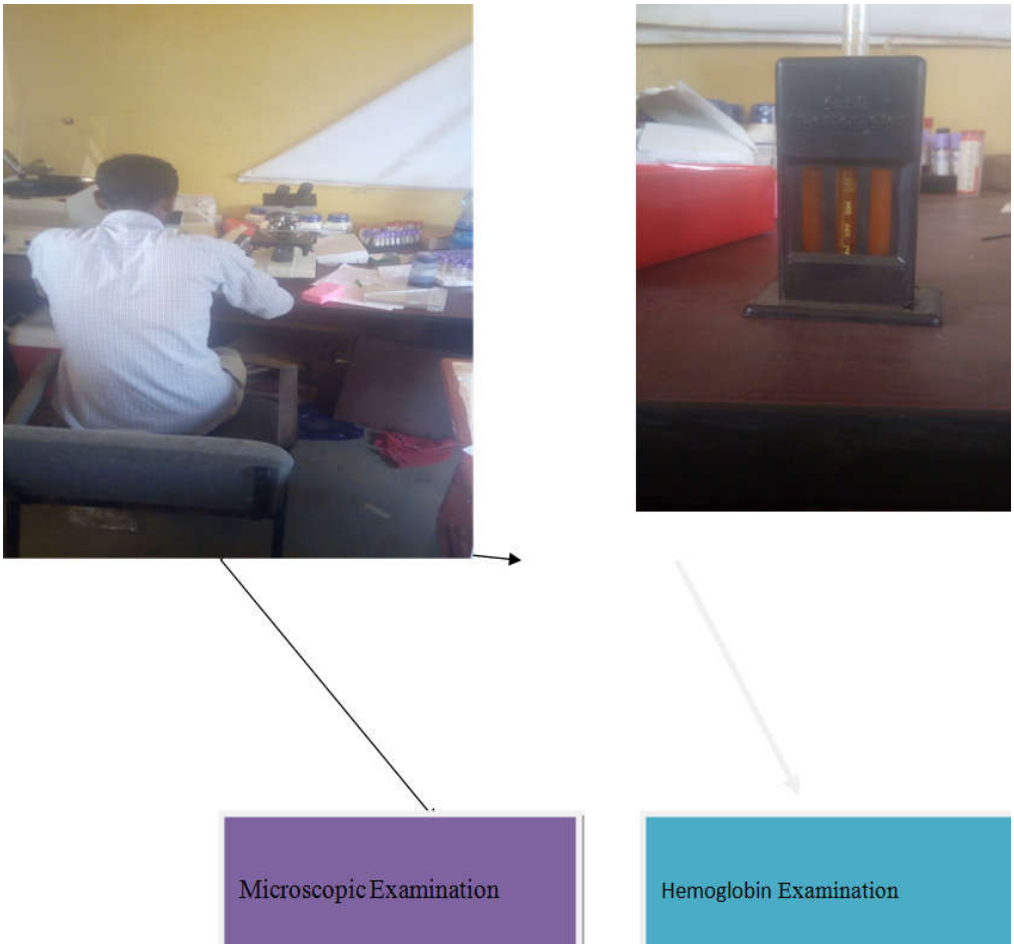
Procedure*:*

- The graduated tube of the haemoglobin meter was filled to 20 marks with 1% HCl.
- The micropipette tip was attached to the eppendorf micropipette and 20 µl of blood was

measured and the excess blood was wiped from outside of the tip with Gauze and blood was added into the graduated tube.

- One drop of distilled water was added to the graduated tube and mixed with glass stirring rod and adding of drops of distilled water and stirring was continued until the colours of the solution in the graduated tube matched with the colour of the glass standard on the Haemoglobin meter.
- The upper level of the solution in graduated cylinder was read and determined ashaemoglobin value in g/dl.

Annex 4: Pictures taking during sample taking and microscopic examination



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