Thesis Ref. No _____

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF PARASITOLOGY AND PATHOLOGY

BOVINE TRYPANOSOMOSIS: PARASITOLOGICAL PREVALENCE, VECTORIAL DENSITY AND TRYPANOCIDAL DRUG UTILIZATION PRACTICES IN TSETSE SUPPRESSION AND NON- SUPPRESSION AREAS OF SOUTH OMO ZONE, ETHIOPIA

MVSc THESIS

BY

TEGEGN TESFAYE TEGA

ADVISOR: PROFESSOR HAGOS ASHENAFI

JUNE, 2019

BISHOFTU, ETHIOPIA

BOVINE TRYPANOSOMOSIS: PARASITOLOGICAL PREVALENCE, VECTORIAL DENSITY AND TRYPANOCIDAL DRUG UTILIZATION PRACTICES IN TSETSE SUPPRESSION AND NON- SUPPRESSION AREAS OF SOUTH OMO ZONE, ETHIOPIA

A thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Veterinary Science in Veterinary Parasitology

BY

TEGEGN TESFAYE TEGA

ADVISOR: PROFESSOR HAGOS ASHENAFI

JUNE, 2019

BISHOFTU, ETHIOPIA

Addis Ababa University
College of Veterinary Medicine and Agriculture
Department of Veterinary Parasitology and Pathology

BOVINE TRYPANOSOMOSIS: PARASITOLOGICAL PREVALENCE, VECTORIAL DENSITY AND TRYPANOCIDAL DRUG UTILIZATION PRACTICE IN TSETSE SUPPRESSION AND NON-SUPPRESSION AREAS OF SOUTH OMO ZONE, ETHIOPIA

Submitted by:

Tegegn Tesfaye	_____	20-06-2019
Student	Signature	Date

Approved for submittal to thesis assessment committee:

Professor Hagos Ashenafi	_____	_____
Academic Advisor	Signature	Date
Professor Yacob Hailu	_____	_____
Department Head	Signature	Date

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Veterinary Parasitology and Pathology

As members of the Examining Board of the final MVSc open defense, we certify that we have read and evaluated the thesis prepared by Tegegn Tesfaye Tega, entitled: **Bovine Trypanosomosis: Parasitological Prevalence, Vectorial Density and Trypanocidal Drug Utilization Practice in Tsetse Suppression and Non-suppression Areas of South Omo Zone, Ethiopia** and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Veterinary Science in Veterinary Parasitology.

Professor Yacob Hailu	_____	_____
Chairman	Signature	Date
Dr. Zewdu Seyoum (Associate Professor)	_____	_____
External Examiner	Signature	Date
Dr. Dinka Ayana (DVM, MVSc, Associate Prof.)	_____	_____
Internal Examiner	Signature	Date
Professor Hagos Ashenafi	_____	_____
Academic Advisor	Signature	Date
Professor Yacob Hailu	_____	_____
Department Head	Signature	Date

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my work that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in the partial fulfillment of the requirements for MVSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and deposited at the University/College library to be made available to borrow under the rules of the library. It is hardly forbidden to submit for any other institution anywhere for the award of any academic degree, diploma or certificate. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgement of source made. Request for permission for extended quotation from the production of this manuscript in whole or in part may be granted by the head of major advisor or department or the dean of the college when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however permission must be obtained from the author.

Name: Tegegn Tesfaye

Signature: _____

Date of submission: _____

AKNOWLEDGEMENTS

First of all I would like to thank and praise the Almighty God for the eternal activities and the strength he give me in every moment of my life.

My special gratitude, appreciation and thoughtful thanks go to my academic advisor, Professor Hagos Ashenafi for his intellectual guidance, devotion of time on critical correction of this paper and generally for his fatherly approach and advice. I would like to thank Southern Agricultural Research Institute (SARI) and Jinka Agricultural Research Center (JARC) for its financial, logistic and other supports. Furthermore, my heartfelt thanks extend to my friend, Dr. Tekle Olbamo for his intellectual guidance and other support he provided me during my thesis work.

I would like to appreciate all Jinka regional laboratory field and Parasitology laboratory experts for their trustworthiness during my laboratory activities and field works in such a harsh environment. My special recognition to Dr. Abayneh Leza (Head, Jinka Regional Veterinary Laboratory) for his logistic support by allowing me to use his laboratory's vehicle during my field works. My staff member, Mr. Aschenaki Abate (Animal health expert at Jinka agricultural research center) would also be appreciated for his unobstructed support on field sample collection and local language translation during questionnaire survey.

The last but not the least, this paper would not be successful without the moral contribution from my whole family members. I know no words can express their deepest love to me but simply I would like to say thanks for their unrestricted desire for my success from childhood to this level.

TABLE OF CONTENTS

CONTENTS **PAGES**

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXES

LIST OF ABBREVIATIONS

AAT	African Animal Trypanosomosis
BCT	Buffy Coat Technique
CAHWs	Community Animal Health Workers
CSA	Centeral Statistical Authority
ETB	Ethiopian Birr
F/T/D	Flies per Traps per Day
FEWSNET	Famine Early Warning Systems Network
HAT	Human African Trypanosomosis
HCT	Haematocrit Centrifugation Technique
KAP	Knowledge Attitude and Practice
MoA	Mechanism of Action
MPS	Mononuclear Phagocytic System
NMSA	National Metreology Service Agency
PCV	Packed Cell Volume
SAD	Stationary Attractive Device
SAT	Sequential Aerosol Technique
SIT	Sterile Insect Technique
SNNPRS	Southern Nations Nationalities and Peoples Region State
SOFED	South Omo Zone Finance and Economic development
SOZLFD	South Omo Zone Livestock and Fishery Department
STEP	Southern Tsetse Eradication program
TDR	Trypanocidal Drug Resistance
VSG	Variable Surface Glycoprotein

ABSTRACT

Questionnaire survey to assess knowledge and attitude of trypanocidal drug utilization practices, entomological survey to know apparent cyclical vector density and other mechanical vectors responsible for trypanosome infection transmission and parasitological study to elucidate the prevalence of bovine trypanosome was conducted in tsetse suppression areas and tsetse non-suppression areas of South Omo Zone, Southern Ethiopia from November 2018- May 2019. SPSS version 20 and R- software packages were used to analyze collected field data. For questionnaire survey, 124 cattle owners from tsetse suppression areas and 60 cattle owners from tsetse non-suppression areas were interviewed about their knowledge, attitude and practice (KAP) of trypanocidal drug utilization and constraints of trypanosome infection in the area. Blood samples from 1284 local breed cattle; 642 samples during dry season (344 from tsetse suppression areas and 298 from tsetse non-suppression areas) and 642 during wet season (344 from tsetse suppression areas and 298 from tsetse non-suppression areas) were parasitologically examined by using buffy coat technique and thin blood smear method. For entomological survey, 96 NGU traps were deployed (64 traps in tsetse suppression areas, 32 traps in tsetse non-suppression areas) in suspected Glossina multiplication habitats. The overall prevalence of bovine trypanosome infection in South Omo zone was found to be 11.05% (142/1284). The overall seasonal prevalence of bovine trypanosome infection in South Omo zone was 14.33% (92/642) and 7.78% (50/642) for dry and wet seasons, respectively. There was a statistically significant difference ($P < 0.05$) in disease prevalence between the two seasons. During dry season, only sex and the grazing system were significantly associated with trypanosome infection occurrence but in wet season none of the risk factors were statistically significant. Multiple logistic regression analysis of trypanosome infection prevalence indicated that sex, body condition score (BCS), grazing system and season were significantly associated ($P<0.05$) with the prevalence of the disease in tsetse suppression areas. However, only sex and season for tsetse non-suppression areas were significantly associated with the prevalence of trypanosome infection. *Trypanosoma congolense (T. congolense)* was the dominant trypanosome species; 80% and 71.64% respectively from tsetse suppression areas and for tsetse non-suppression areas followed by *Trypanosoma vivax (T. vivax)*. Overall pooled mean packed cell volume (PCV) indicated parasitaemic animals (23.57±3.13) had significantly lower PCV than aparasitaemic animals (27.80±4.95) and animals examined during dry season

(26.22±4.37) had lower mean PCV than animals examined during wet season with significant association. Similarly, parasitaemic animals from tsetse suppression areas (PCV= 23.76± 3.07) and tsetse non-suppression areas (PCV= 23.37±3.21) had significantly lower mean PCV than their aparasitaemic counterpart (tsetse suppression areas, PCV= 27.73±5.07 and tsetse non-suppression areas, PCV= 27.88±4.82). However, mean PCV of animals infected with *T. congolense* (23.59±3.22) was not statistically different ($P > 0.05$) from those animals infected with *T. vivax* (23.26±3.31). It was also indicated that the probability of anaemic animals to be parasitaemic was significantly higher ($P < 0.05$) than non-anaemic animals in both tsetse suppression areas and tsetse non-suppression areas. Entomological survey result revealed that 2.64 F/T/D and 2.03 F/T/D respectively from tsetse suppression areas and tsetse non-suppression areas during dry season and 0.42 F/T/D and 0.56 F/T/D during the wet season. *Glossina pallidipes (G. pallidipes)* was the only cyclical vector identified from the area along with numerous mechanical vectors of genus *Tabanus, Stomoxys* and *Haematopota*. Higher dependency of cattle owners on trypanocidal drugs to treat their sick animals and limited trypanocidal drug availability in the veterinary pharmaceutical market in conjunction with unsustainable and less participatory vector control activities may intensify the threat of the disease in the area. Furthermore, upsetting current prevalence report of the disease in such area with frequent trypanocidal drug usage and drug injection by unskilled herdsmen and owners report on trypanocidal drug treatment failure may point out the issue of trypanocidal drug resistance in the area. Therefore integrated and safe control and prevention effort should be engaged to uphold cattle production and productivity in the area.

Key words: *Trypanosomosis, Bovine, Prevalence, PCV, Vector survey, Trypanocidal Drugs, Tsetse suppression, South Omo Zone, Ethiopia*

1. INTRODUCTION

Trypanosomosis is a complex debilitating and often fatal disease, caused by species of unicellular parasite; Trypanosoma which is found in the blood and tissues of vertebrate including livestock, wild life and people. The disease can be transmitted between the hosts mainly by tsetse flies cyclically, or by other biting haematophagous flies mechanically and also by coutis (Claes *et al.*, 2005). Trypanosome infections in livestock are known by their common names as nagana, surra and dourine which are caused by different species of trypanosome like *T. congolense, T. vivax, T. brucie, T. evansi and T. equiperdium*. With its striking features of the dramatic suppression of the immune responses, the trypanosome infection might result in a high susceptibility of the host to opportunistic infections which further complicate the pathological sequel of this disease (Namangala, 2011; Desquesnes *et al*., 2013).

The fight against trypanosomosis is managed by using trypanotolerant host, by controlling of the vector and parasite by using different approaches or a combination of three. However, in poor rural communities, which are mostly affected by the disease, control is mainly relying on the use of trypanocidal drugs. The main drugs used by livestock keepers are isometamidium chloride (ISM), diminazine aceturate (DA) and ethidium bromide. About 35 million doses of drugs are used in Sub-Saharan Africa each year, costing livestock producers and consumers an estimated 1340 million USD each year. If lost potential in livestock and crop production are considered, then trypanosomosis is costing Africa an estimated 5 billion USD per year but still about 50–70 million animals are at risk of contracting the disease (Claes *et al*., 2005; Chitanga *et al*., 2011; Melaku and Birasa, 2013).

Since most of trypanocidal drugs have been in use for more than half a century, they can cause the appearance of the drug resistant strain of trypanosomes (Cross, 2001; Cossic *et al*., 2017). Furthermore, due to privatization of veterinary services in most parts of Africa, farmers have easy access to all available trypanocidal and this has resulted in rampant misuse and under-dosage of the medications, actions which have been blamed for the emergence of trypanocidal drug resistance (Tekle *et al*., 2018). Since there is no indication that new products will become available in the near future, it is of utmost importance that measures are taken to avoid or delay the development of

resistance and to maintain the efficacy of the currently available drugs (Shiferaw *et al*., 2015; Tsegaye *etal*., 2015).

In Ethiopia, bovine trypanosomosis is widely distributed in Western and Southwestern part of the country. About 180,000-220,000 km^2 of agriculturally suitable lands were infested with *Glossina* species which is responsible for cyclical transmission of the disease. It is estimated that some 10 to 14 million heads of cattle in Ethiopia and an equivalent number of small ruminants together with a significant number of equines and camels are exposed to the risk of trypanosomosis (Dumesa and Demessie, 2015). Six species of trypanosomes have been recorded in Ethiopia and the most important trypanosomes in terms of economic loss are the tsetse transmitted species: *T. congolense, T. vivax* and *T. b. brucie*. Annual estimated losses for Ethiopia as a result of trypanosomosis is roughly $200 million, in terms of mortality and morbidity losses in livestock (excluding utilization of fertile land for crop and livestock production) and the costs included in controlling the disease (Dumesa and Demessie, 2015; Shiferaw *et al*., 2015; Tekle *et al*., 2018).

South Omo zone is among the prominent pastoral areas of Ethiopia and possesses huge livestock resources. Despite the presence of huge livestock population, the production and productivity as well as economic yield from livestock sector is very low due to different ailments affecting the livestock population; among which trypanosomosis is the primary one. Although there is scarce data on vector densities, parasitological prevalence and trypanocidal drug resistance of bovine trypanosomosis in the area, it is suspected that South Omo zone is one of endemic area which shares favorable conditions for the occurrence of bovine trypanosome infection and its vectors.

Due to increasing population growth and plantation of different development projects such as Omo Kuraz sugar factory in the zone, previously unsettlement areas were occupied by pastoralists and agro-pastoralists from different areas of the zone as well as from other neighboring zones. This occupation of bare land by residents of the zone creates new habitat and increased interaction between the host, the parasite and its vectors because pastoralists and agro-pastoralists occupy the new area with their animals. The presence of conserved area for wild life (Mago National Park) makes the condition more realistic as pastoralists and agro-pastoralists allow their animals to graze in and around this conserved area especially during the dry season.

Mago National Park which is 2,162 square kilometers (835 square miles) in area, ranging in altitude from 450 to 2,528 meters (1,476 to 8,292 feet), with temperatures swing between 14° and 41°C and rainfall, which falls from March to May and October to December of being 480 mm on average is situated in the zone and borders most of the districts in the zone (Gedech and Guangul, 2017). The movement of pastoralists and agro-pastoralists to this conserved area makes the interaction (epidemiological triangle) very close because there are reservoir wild life (buffalo, lesser kudu, greater kudu, Lewel's hartebeest's grant's gazelle) and suitable tsetse fly habitant in and around this national park (Gedech and Guangul, 2017).

Unbalanced demand and supply of trypanocidal to cattle owners at veterinary clinic to treat their diseased animals; exposing owners to purchase poor quality trypanocidal from smugglers (personal contact with South Omo zone pastoral and agro-pastoral department head). This in turn could results in the development of trypanocidal drug failure in the zone. Although there is *Glossina* vector suppression program conducting in selected districts of the zone by Southern Tsetse Eradication Project (STEP) (Kebede *et al*., 2017), all districts suitable for vector multiplication were not included under the program. Active reinvasion ability of tsetse fly to eradicated area may become the main obstacle for the successes of the suppression campaign in those selected areas in addition to the non-sustained control efforts by the project. Less community participation in control strategies driven by the project rise a question on successfulness of tsetse fly control activities since the local communities have a key role on these vector control activities.

Therefore, the objectives of this study are:

- To elucidate the prevalence of bovine trypanosome infection in tsetse-suppressing and non-suppressing areas.
- To assess the vectorial densities and its seasonal distribution in tsetse-suppressing and non-suppressing areas.
- To identify risk factors contributing to the occurrence of the trypanosome infection.
- To assess the knowledge, attitudes and practices of trypanocidal drug utilization of livestock owners for further trypanocidal drug resistance study.

2. LITERATURE REVIEW

2.1.General Features of African Animal Trypanosomosis (AAT)

2.1.1. Epidemiology

There are three types of African animal trypanosomosis (AAT), namely nagana, surra and dourine. Nagana affects ruminants (cattle, goats, sheep, camels as well as dogs and pigs) and horses. It is said to be derived from a Zulu word meaning to be depressed or unfit. Nagana is caused by *T. congolense, T. vivax, T. uniforme, T. simiae* as well as *T. b. brucie* and tsetse flies are responsible vectors for the cyclic transmission of the disease in these domesticated animals. Additionally, African mammals may also harbor non-pathogenic trypanosomes namely *T. theileri* and *T. ingens* commonly found in both domestic and wild animals (Biryomumaisho *et al*., 2013).

Surra which is caused by *T. evansi* is widely distributed in Africa, Asia, the Middle East and South America. *T. evansi* is transmitted mechanically by bloodsucking insects, from the genera *Tabanus* and *Stomoxys* as well as vampire bats such as *Desmodus rotondus*. It causes a disease in camels, horses, cattle, pigs, buffaloes, and dogs. In Southeast Asia, *T. evansi* infection is a disease of economic importance since it affects the health of buffalo, cattle, and swine. Lastly dourine is a venereally transmitted disease caused by *T. equiperdium* that commonly affects equines and has a wider geographical range as compared to the other two diseases (Taylor and Authié, 2004; Hagos, 2010).

2.1.2. Life cycle

Tsetse transmitted trypanosomosis**:** Different trypanosome species develop in different organs within the tsetse fly. Inside the flies, parasites undergo a number of developmental stages starting from the midgut migrating to the mouthparts. Transmission of both African animal trypanosomosis (AAT) and human African trypanosomosis (HAT) starts when a susceptible mammalian host is bitten by an infected fly vector therefore injecting metacyclic trypomastigotes form of the parasite together with its saliva during a blood meal. Subsequently parasites multiply locally by binary fusion at the site of the bite before migrating to the blood stream and lymphatic system of the

mammalian host. The parasites will then migrate to other organs including the CNS and at this stage they occur in two forms of trypomastigotes, firstly as a long slender form that can reproduce by asexual division and secondly a non-replicating, short stumpy form (Chappuis *et al*., 2005). The ratio of the long slender form to the short stumpy form of trypomastigotes varies with each wave of parasitaemia and regularly more stumpy form of trypomastigotes are observed later in the infection. This is because the stumpy forms play a dual functional role by limiting the parasitaemia wave in the infected mammalian host and pre- adaptation for effective transmission to vector tsetse fly (Wiser, 2011).

When trypomastigotes are sucked up during a blood meal, they migrate to the midgut of the tsetse fly vector. In the fly's midgut the trypanosome parasites are most likely to encounter different consequences whereby, the slender forms are rapidly killed by proteases. Stumpy forms survive and differentiate to procyclic trypomastigotes (Roditi and Lehane, 2008). This differentiation is characterized by changes in the expression of the surface proteins as well as changes in metabolism. This is then accompanied by loss of the surface coat and replacement of the variant surface protein (VSG) with another membrane surface protein called procyclin (Roditi and Lehane, 2008; Wiser, 2011).

Proteases are abundant in the fly posterior midgut, and provide at least one of the natural triggers; however, additional signals such as cold shock may also contribute to differentiation *in vivo*. These additional signals reduce the trypanosome parasites to low concentrations of citrate or cis-aconitate (Roditi and Lehane, 2008). In addition, starvation may also contribute to decreased immune gene expression as a result leading to an increase in susceptibility of the nutritionally stressed tsetse flies in developing a trypanosome infection (Akoda *et al*., 2009).

For a trypanosome parasite to complete its life stages it must colonize the salivary glands and generate metacyclic trypomastigotes that are infectious to mammals. The migratory forms found in the proventriculus include long trypomastigotes that replicate their nuclear DNA and shift the position of the kinetoplast to give rise to long epimastigotes. Subsequently, the long epimastigotes then undergo an asymmetric division and in doing so, generating short epimastigotes that are alleged to be the parasitic form colonizing the salivary glands. Under optimum conditions many as half of the flies with a midgut infection will give rise to infected salivary glands (Akoda *et al*.,

2009). These epimastigotes will then multiply in the salivary glands to produce infective metacyclic trypomastigotes that will be transmitted to a mammalian host during the next blood meal (Roditi and Lehane, 2008).

Inside the mammalian host these metacyclic trypomastigotes transform into bloodstream trypomastigotes. The bloodstream trypomastigotes will also migrate to spinal fluid and lymph whereby they will again multiply by binary fusion. Re-infection to other cells will result due to a high number of trypomastigotes in the blood and spinal fluid. These trypomastigotes will then transform into slender and stumpy forms inside the host. In the host the slender form trypomastigotes will cause acute symptoms and the stumpy form parasite will be ingested by the vector flies during another blood meal and the cycle will be repeated all over again (Roditi and Lehane, 2008; Akoda *et al*., 2009). But this morphological change from the long thin slender forms, through the intermediate, to the short stumpy is obviously associated with *T. b .brucie* not with other species.

The life cycle of *T. vivax* is an exception in its mode of transmission in the tsetse fly vector. All the life cycle stages (trypomastigotes, epimastigotes and the infective metatrypomastigotes) are formed in the proboscis. *Trypanosoma vivax* differs from other Salivaria trypanosomes by its elongated and granular bloodstream form with a large kinetoplast and a centrally placed nucleus. In addition, *T. vivax* has been reported to be congenitally transmitted from mother to fetus during pregnancy via the placenta or when bleeding occurs during birth (Uilenberg, 2011). Moreover, (Stevens *etal*., 2004) suggested that all these features enable this species to better adapt to development in its host than any other Salivaria species.

Non-tsetse-transmitted trypanosomes**:** Not all trypanosome parasites are cyclically transmitted by tsetse flies, and some are mechanically transmitted by contaminative transmission through faeces of other haematophagous arthropods during their blood meal. The geographic distribution of these non-tsetse trypanosome parasites is more widespread ranging from Africa, Asia, and Central as well as South America respectively and they include all members of Stercoraria and a few Salivaria parasites. From the Stercoraria group, in Central and South America *T. cruzi* and *T. rangeli* (*Schizotrypanum*) are transmitted to humans and animals by triatomine insects and in human *T.*

cruzi is responsible for a disease known as Chagas disease. Secondly is *T. lewisi* (*Herpetosoma*) which parasitic to rats is solely transmitted by rat fleas worldwide (Stevens *et al*., 2004).

The cosmopolitan *T. theileri* (*Megatrypanum*) is transmitted by various haematophagous arthropods ranging from insects to arachnids. In Africa, *T. theileri* is mechanically transmitted by tsetse flies (*Glossina*), black (*Chrysops*), horse (*Tabanus*) and stable (*Stomoxys*) flies from the families Tabanidae and Muscidae respectively (Ponte-Sucre, 2016). Furthermore, *Hyalomma anatolicum* is responsible for transmitting *T. theileri* in North Africa, southern Europe, Middle East, Russia, and China as well as in India. However, *T. theileri* is not pathogenic to both animals and humans mainly because, it doesn't possess a VSG gene which makes it exposed to antibodies released by the immune system (Stevens et al., 2004).

Lastly, *T. evansi* and *T. equiperdium* which belong to the subgenus *Trypanozoon* are responsible for surra in dogs, livestock as well as horses and dourine in camels and equines. Both parasites are morphologically similar and they are both widely distributed in Africa, Asia and South America (Elhaig *et al*., 2013). *T. evansi* however, is mechanically transmitted by blood sucking insects such as *Tabanus, Stomoxys, Lypersoia* and *Haematopota* (Taylor and Authié, 2004). Dourine, which is caused by *T. equiperdium* on the other hand, is transmitted during coitus and it is only lethal in equines as they are the only known hosts (Stevens *et al.*, 2004; Taylor and Authié, 2004).

2.1.3. Pathogenesis and virulence

Initial replication of metacyclic trypanosomes begins at the site of inoculation, inducing a swelling and a sore called 'chancre'. This 'chancre' disappears after a few days (3 to 15 days) when the trypanosomes spread to the lymph nodes and blood and continue to replicate (Singh and Singla, 2013). After an infection has become established, the B-lymphocytes of the mammalian host produce anti-VSG antibodies (IgG) that lyse a large number of the trypanosomes and result in the development of immune complexes. These are removed by phagocytes and presented to lymphoid cells. The attachment of antigen-antibody complexes to red blood cell membranes contribute to the damage and lysis of the erythrocytes.

Moreover, the lysis of the trypanosomes release many harmful substances in the extracellular environment, such as haemolysins and enzymes (proteases, phospholipases and neuraminidases, etc), which directly damage the host's erythrocytes membranes. The elimination of large numbers of damaged erythrocytes from the blood circulation occurs soon after the beginning of the parasitaemia, by cells of the mononuclear phagocytic system (MPS), and produces a decrease in the Packed Cell Volume (PCV). Some trypanotolerant cattle (e.g. Baoule, N'dama) control the parasitaemia quite effectively (earlier and higher antibody responses to the first peak of parasitaemia) when compared with similarly infected Zebu cattle (Swanepoel and Coetzer, 2004). The PCV values of infected trypanotolerant animals are definitely higher than that of susceptible ones when they are well maintained.

Anemia is largely attributable to an increased rate of erythrophagocytosis in the early phase of infection. Nevertheless, there is no single cause of the anemia in AAT. Indeed, as a response to the trypanosomal infections both IgM and IgG antibodies are produced. While the IgM appear to be directed mainly against VSG antigens, IgGs are oriented against both the somatic trypanosomal antigens and the host's own cells. Anti-erythrocyte antibodies contribute to the anemia of AAT and it is likely that a similar mechanism contributes to leucopenia (Matthews *etal*., 2015).

However, anti-VSG antibodies do not clear the infection as the trypanosome produces many different surface-coat glycoprotein (10^7Variable Antigen Types or VAT) and change their surface glycoprotein to evade the host immune response (Baral, 2010). Thus, there is a persistent fluctuating parasitaemia that results in continuing cycles of trypanosome replications, antibody production, immune complex development, and changing of surface-coat glycoprotein. Furthermore, one of the most significant and complicating factors in the pathogenesis of AAT is the immunosuppression that occurs following infection by these parasites. This marked immunosuppression lowers the host's resistance to other infections and thus results in secondary diseases, which greatly complicate both the clinical and pathological feature of AAT (Tabel *etal*., 2008).

It has been suggested that immunosuppression is mediated by both the macrophages and the T cells (Tabel *et al*., 2008). As a result of this, antigenic variation and immunosuppression, conventional vaccination strategies against AAT are not effective (Baral, 2010; Magez *et al*., 2010). Recently, alternatives approaches were explored based on camelid antibodies (e.g. use of Nanobodies®)

(Magez and Radwanska, 2009; Baral, 2010;). Promising results were obtained using low molecular weight VSG-specific trypanolytic nanobodies that impede endocytosis (Stijlemans *et al*., 2011).

Significant tissue lesions are observed in AAT and depend on the species of trypanosome. Considered to be mainly intravascular parasites, *T. congolense* and to a lesser extent *T. vivax*, cause changes in the endothelium of capillaries, and thus indirectly provoke damage to adjacent tissues. The severity of endothelial damages also depends on the host-parasite interaction. Indeed, in *T. congolense* infections a generalized dilatation of capillary beds is observed, which alters the hemodynamic. In contrast, *T. vivax* infections commonly cause disseminated intravascular coagulation (Swanepoel and Coetzer, 2004).

2.1.4. Clinical signs

Due to the fact that simultaneous infections with more than one trypanosome species and/or with other haemoparasites (Babesia spp., Theileria spp., Anaplasma spp., and Ehrlichia spp.) are very common, it is difficult to attribute the clinical signs to a given parasite. Moreover, the disease may have acute, chronic or subclinical forms complicating its recognition. Nonetheless, the major clinical sign observed in AAT is anemia, followed invariably by an intermittent fever, weight loss, and roughness of the hair coat and whimpering (Taylor and Authié, 2004).

The severity of the clinical response depends on the species and the breed of the affected animal but also on the trypanosome species, the size of the inoculums and the virulence of the infecting trypanosomes. For example, *T. b. brucie* is very well tolerated by cattle but is rapidly life threatening for horses and dogs (Vitouley, 2014). Even within a same species virulence might vary a lot as is the case for *T. congolense* (Bengaly *et al*., 2002; Masumu *et al*., 2006).

2.1.5. Diagnosis of trypanosomosis

Clinical diagnosis: Due to the absence of any pathognomonic clinical sign for AAT, the clinical diagnosis is somewhat difficult. Nonetheless, anemia, pyrexia, weight loss, roughness of the hair coat, hypertrophy of peripheral lymph nodes, abortion, reduced milk yield and, in absence of treatment, death are often observed in cattle affected by the acute form of the disease (Masumu *et*

al., 2006). Whatever the clinical forms of evolution of AAT (acute, sub acute and chronic forms), it is essential to confirm the presence of the parasite in blood or lymph node smears by using the following parasitological tests.

Direct microscopic examination: The examination of a drop of fresh blood between slide and cover slip can be of great use in the field to demonstrate the parasitaemia of animals under observation or treatment or to determine the health status of a herd throughout the seasons. Using a light microscope at 400x magnification, the species of trypanosomes can be determined by examining their size and movement patterns. *T. congolense* appears short (size of about 8 to 24µm) with a poorly developed undulating membrane; the free flagellum is absent or very brief and the trypanosome is stuck on erythrocytes and moves slowly. In contrast, *T. vivax* quickly crosses the field of the microscope; it is a larger trypanosome measuring about 18 to 31µm with a free flagellum and a less developed undulating membrane. *T. brucie* has a well-developed undulating membrane and moves freely but slower than *T. vivax* and often describes little circles. Microscopic examination of fresh blood films is simple and inexpensive but lacks sensitivity, with a detection limit of about 10^4trypanosomes /ml of blood (Uilenberg and Boyt, 1998).

Thin or thick blood smears fixed in methanol or acetone and colored with May- Grünwald-Giemsa as well as stained lymph node smears (Uilenberg and Boyt, 1998) are mostly used for accurate trypanosome identification and are found to be more sensitive than the fresh blood film. However, none of these techniques is sensitive enough to detect the low parasitaemia usually observed in the field when compared to concentration techniques.

Concentration techniques: The Haematocrit Centrifugation Technique (HCT) is based on the centrifugation of microhaematocrit capillary tubes containing the blood sample. Afterwards, the buffy coat/plasma junction is observed under a microscope at 250x magnification in a special Woo chamber, allowing the suppression of the refraction of the light on the capillary tube. The Buffy Coat Technique (BCT) is a variant of the HCT. The capillary tubes are cut at the level of the buffy coat/plasma interface. The buffy coat is extruded on a slide, covered with a cover slip and examined under the microscope at 400x magnification with dark ground or phase contrast illumination. In addition to be more sensitive, these two techniques have also the advantage of measuring the Packed Cell Volume (PCV) or haematocrit.

Another available method that is not commonly used for animals in field conditions is the miniature-Anion Exchange Centrifugation Technique (m-AECT). This technique using miniature anion-exchange columns for the separation of trypanosomes from erythrocytes prior to concentration by centrifugation has recently been improved for sleeping sickness diagnosis and staging (Büscher *et al*., 2009). Due to the different electric charges of the trypanosomes and the erythrocyte surface, the erythrocytes get captured in the column whereas the trypanosomes are eluted.

Serological diagnosis: Several antibody-detecting tests are used for the diagnosis of trypanosomal infection. These tests only confirm the contact between the host and the parasite without specifying whether the infection is still active or has been cleared (Uilenberg and Boyt, 1998). The most commonly used tests in cattle are the Indirect Fluorescent Antibody Test (IFAT), the antibody Enzyme-Linked Immunosorbent Assay (Ab-ELISA) and the Card Agglutination Test for *T. evansi* (CATT/*T. evansi*).

In the IFAT, the antigen is constituted of a blood smear containing fixed trypanosomes. The primary Abs are detected by commercially available secondary antibodies conjugated with a fluorophore (like the fluorescent isothiocyanate or FITC). Despite the fact that antigen production is easy, this test has the disadvantage of not being sufficiently species specific. Cross reactivity between species are frequently observed. Moreover, the commercial conjugates are expensive and only few numbers of sera can be examined in a given period as the method is rather labor intensive (Uilenberg and Boyt, 1998).

The principle of the Ab-ELISA is quite similar to IFAT. However, when compared to IFAT, the indirect ELISA proved to be more sensitive (Eisler *et al*., 2004). The test is more useful for epidemiological purposes than for individual routine diagnosis, and in areas with a low AAT prevalence. When the fly challenge is high the method is of limited interest as serological prevalence can then reach 80 – 90% (Van den Bossche *et al*., 2000). The prevalence of anti-trypanosomal antibodies is a sensitive indicator of the impact of tsetse control operations on disease challenge and for confirming the disease-free status of animals in areas from which tsetse has been eliminated (Van den Bossche *et al*., 2000). Serology can then be performed on the new born calves which should not have been in contact with trypanosomes if the eradication was successful.

The CATT/*T. evansi* test is a direct card agglutination test for the detection of antibodies to 'surra' i.e. *T. evansi* infection in blood, serum or plasma of various animals species such as, camels and water buffaloes. It consists of mixing a drop of whole blood or serum on a plastic card with fixed and stained trypanosomes as antigen, and the test is positive when the antigen agglutinates. This test is easy to carry out in the field, although its specificity and sensitivity need to be enhanced. More recently, a highly specific and sensitive ELISA (ELISA/rrISG75) using a recombinant non-variable antigen was developed for diagnosis of 'surra' in camels (Desquesnes *et al*., 2013).

2.1.6. Control of trypanosomosis

The control of AAT includes the use of trypanotolerant cattle, vector control and the use of trypanocidal drugs or any combination of the methods according to the specific conditions of an area. The choice of a strategy will depend mainly on the tsetse fly challenge, the susceptibility of the host, the presence/absence and type of drug resistance (simple or multiple) and the breeding system (transhumant, sedentary not confined, sedentary confined).

Breeding trypanotolerant cattle (*Bos taurus*) is an interesting alternative for the control of AAT. When properly housed and fed, these cattle can maintain good productivity levels in tsetse infested areas. They include short-horned cattle represented by the breeds *Baoulé*, *Sumba*, *Muturu* of savannah, *Lagune*, bred in Côte d'Ivoire, Benin, Togo, Ghana, Nigeria, Burkina Faso and Northern Cameroon and the long-horned cattle living in Southern Senegal, Mali, northern Côte d'Ivoire, Guinea, Gambia, Liberia, Sierra Leone, Bissau Guinea, Burkina Faso. Long-horned cattle are represented by the breed *N'dama*. Sheko cattle of Ethiopia also stand out as the most trypanotolerant animals; they rarely get infected by trypanosomes, and have good PCV, production and reproduction (Mekuriaw and Kebede, 2015). Due to their small size, farmers are somewhat reluctant to breed trypanotolerant breeds. This has led farmers to cross trypanotolerant cattle with Zebu to increase the size of the animals and the milk yield. The obtained crossbreds are partially trypanotolerant and represented by the breeds *Borgou*in Benin and Togo, *Méré*in Guinea, Burkina Faso, Côte d'Ivoire, *Bambara* in Mali and *Djakoré*in Senegal and Gambia (Stein, 2011).

Fly populations can be down-regulated by chemical control including the use of insecticides by ground application, aerial spraying, and impregnation of traps / screens or spraying / pour-on

application on the back line of host animals, spraying the belly and legs of cattle. This last method is cheap and easy to implement and take advantage of the specific tropism of haematophagous flies for the lower parts of the animals (Bouyer *et al*., 2009). When the tsetse fly population is decreased by 95% or more in isolated areas that cannot be reinvaded, eradication can be achieved by the Sterile Insect Technique (SIT) (release of sterile males). This technique is based on the fact that female flies only accept one single mating. If this mating is performed by a sterile male, this female will never produce any offspring.

Methods such as deforestation and elimination of the game animals that were used in early eradication campaigns, have been abandoned because of obvious environmental and ethical reasons (Hargrove, 2003). Paradoxically, encroachment has the same effect and large areas are in this way freed from the burden of tsetse flies allowing agriculture and cattle breeding. However, livestock keepers often lead their animals to less degraded environments where grass and trees are still available and tsetse flies as well.

Currently available trypanocidal drugs for use in domestic livestock are: homidium salts (Ethidium-Novidium), diminazine aceturate (Berenil), isometamidium (Samorin, Trypamidium), quinapyramine sulfate (Antrycide), suramin sodium and melarsomine (Cymelarsan). The use of quinapyramine in cattle is discontinued due to its capacity to induce multi-drug resistance (Holmes *et al.*, 2004). Major Trypanocidal in use today and their mechanism of action is described on section below.

2.1.7. Major Trypanocidal drugs and their mode of action (MoA)

Due to lack of interest by pharmaceutical industry to invest in to research and development of anti-trypanosomal drugs, there has been a major stimulus for intensive research into the few existing drugs; and in the recent past, considerable body of knowledge has emerged on a number of important aspects, such as drug disposition, mechanism of action, resistance and toxicity. The three antitrypanosomal compounds up on which treatment and prophylaxis of cattle trypanosomosis currently depends are isometamidium chloride, homidium chloride or bromide and diminazine aceturate. Whereas, quinapyramine, surramine and melarsomine are primarily used as therapeutic drugs for infection caused by T. evansi in equidae, camels and buffaloes. Quinapyramine is also

used for prophylactic purpose although it was banned from the market due to its cross resistance to major trypanocidal drugs used now a day (Melaku and Birasa, 2013; Giordani *et al*., 2016).

Diminazine aceturate: DIM is very active, stable and easy to use and has very low toxicity. These advantages make it a practical and risk free trypanocidal at least for cattle. Diminazine solution can only be kept for two to three days. It is injected subcutaneously in cattle (slight local reactions possible) or intramuscularly (very rapid absorption) at a dose of 3.5 mg/kg live weight for treating *T. vivax* and *T. congolense* infections. Infections due to *T. brucie* can be treated in horse and cattle with the dose of 7mg/kg (Tsegaye *et al*., 2015).

Diminazine binds to trypanosomal kinetoplast DNA. This binding does not occur by interaction but via specific interaction with sites rich in adenine-thymine (A-T) base pairs. Non-interactive binding of diminazine to DNA with strong affinity to A-T base pair regions, has similarly demonstrated in vitro, using DNA obtained from various sources. Such studies have shown that the molecule binds with higher affinity to 5'-AATT-3' than to 5'-TTAA-3' regions of DNA. Through this specific interaction in trypanosomes, diminazine inhibits synthesis of RNA primers, resulting in accumulation of replicating intermediates, thereby inhibiting kDNA replication (Wilkinson and Kelly, 2014; Tsegaye *et al.*, 2015).

Other studies also shown that diminazine specifically inhibits mitochondrial type II topoisomerase in viable trypanosomes. Thus, inhibition of DNA replication may also occur via this intercalation. The rate of excretion of the different compounds is known to affect their activity. Diminazine, which is rapidly excreted, is used only for its therapeutic effect. Dimidines accumulate in the liver for months, like wise in the kidney and the adrenal glands respectively (Tsegaye *et al*., 2015).

Isometamidium (ISM): Isometamidium is a phenanthridine aromatic amidine with a narrow therapeutic index which has been marketed for both a prophylactic and a therapeutic trypanocidal agent. Isometamidium chloride is used as curatively at lower dosage rates and prophylactically at higher dosage rates. It is usually prepared as red powder easily soluble in water (Melaku and Birasa, 2013). It is used in a one or two percent aqueous solution and administered by deep intramuscular injection at the rate of 0.25-1mg/kg, depend on drugs resistant risk. Strain of trypanosomes resistant to isometamidium and other phenanthridine appear frequently, but they remain susceptible to

diminazine aceturate. It is given to the animal at dose rate of 0.51mg/kg and it will be protected for two to four months depending on the extent infections risk. Dromedaries appear to be more sensitive to this drug than other animals (Melaku and Birasa, 2013; Wilkinson and Kelly, 2014; Shiferaw *et al.*, 2015).

The primary mode of action currently considered to account for the molecular mechanisms of antitrypanosomal activity of phenanthridinium drugs is blockade of nucleic acid synthesis through intercalation between DNA base pairs, inhibition of RNA polymerase, DNA polymerase and incorporation of nucleic acid precursors into DNA and RNA. Other biochemical reactions that may account partly to their effects include modulation of glycoprotein biosynthesis, lipid metabolism, membrane transport and selective cleavage of kinetoplast DNA minicircles (Shiferaw *et al*., 2015).

The mechanism that is considered primary is blockade of nucleic acid synthesis, which does not explain the basis of their selective toxicity. However, there are a number of biochemical peculiarities that have been demonstrated in trypanosomes that appear to be candidate targets for drug modulation, and that might explain the basis of selective toxicity. Generally it inhibits DNA synthesis in a similar manner as diminazine aceturate, it modifies the mitochondrial membrane, it modifies the glycoprotein structure in surface of the endoplasmic reticulum and Isometamidium is slowly excreted, and is the most effective prophylactic compound currently available (Cross, 2001; Babokhov *et al*., 2013; Cossic *et al*., 2017).

Homidium salts: Homidium salts are effective against *T. vivax* infections in cattle but less so against *T. congolense* and *T. brucie*. Their limited and protective activity in cattle depends on severity challenge and may last three to five weeks. Homidium resistant trypanosome can be controlled by diminazene or isometamidium. Novidium, which is a mixture of homidium chloride and bromide, has the same action as ethidium and is used in the same way, but it, is soluble in cold water. It can also be used in *T. brucie* infections in dogs at the rate of 3-5mg/kg (Mungube *et al.*, 2012; Tsegaye *et al*., 2015).

Disruption of genome function has long been believed to underlie its trypanocidal effects. Indeed, it was found that homidium blocks both kinetoplast and nuclear DNA replication in *T. brucie* by distorting and changing the double helix topology. The inhibition of minicircles replication and,

consequently, loss of the kinetoplast network, was found to be the primary killing mechanism at low doses (0·02 μg mL−1), but at higher doses homidium was also shown to affect nuclear DNA, which could account for its ability to kill dyskinetoplastic trypanosomes. The reason for the initial targeting of the kinetoplast over the nucleus is believed to be the result of the preferential accumulation of lipophilic cations (such as homidium) in the mitochondrion, as shown with other experimental trypanocidal (Mungube *et al*., 2012; Alsford *et al*., 2013; Tihon *et al*., 2017).

Suramin sodium: Suramin sodium is a symmetrical polyanionic sulfonated naphthylamine. It is the oldest trypanocidal still in use, having been introduced in 1921 for the treatment of surra in camels. Suramin is also the standard treatment for equine trypanosomosis (T. brucie spp.), being more effective than diminazine and less toxic than quinapyramine. The current treatment for camels and horses is 10 mg kg−1, administered intravenously. Intramuscular administration is avoided as it causes intense local irritation. Suramin has further been used for cure and prophylaxis of onchocerciasis and other microfilarial infections. Because of its large molecular size and highly anionic nature, suramin does not cross the blood–brain barrier (BBB) (Alsford *et al*., 2012).

The drug was proposed to enter trypanosomes via receptor-mediated uptake bound to LDL and to accumulate in the lysosome. This hypothesis, however, looked doubtful after it was demonstrated that in *T. brucie* (procyclic form at least) suramin and LDL uptake are not coupled. A definitive mode of action for the compound has not been determined. Fairlamb and Bowman proposed that suramin curbs glycolytic ATP production in *T. brucie* by inhibiting glycerol-3-phospate oxidase and NAD+-dependent glycerol-3-phosphate dehydrogenase. However, being highly charged, suramin binds many enzymes when assayed and a multitude of putative targets have been proposed, including 6-phosphogluconate dehydrogenase, of the pentose phosphate pathway, of which it is a competitive inhibitor (Allen *et al*., 2003; Hung *et al*., 2004).

2.1.8. Trypanocidal Drug Resistance (TDR)

Trypanocidal drug resistance (TDR) is defined as the decreased or absence of sensitivity of trypanosome strains to standard quality trypanocidal drugs at the dose recommended by the manufacturer and administered according to the good veterinary practice. It is still unclear if TDR is spreading from resistant genotypes existing in wild trypanosome populations that are selected by

drug pressure or if it is the drug pressure that is inducing mutations having resistance as a consequence. The fact that it is possible to induce TDR *in vitro* by gradual exposure to the drug tends to indicate that acquisition of TDR is a reality (Vitouley, 2014).

However, the isolation of drug resistant trypanosomes from wildlife that were never in contact with the drug (Chitanga *et al*., 2011) suggests that TDR is existing without any drug pressure and was existing before the discovery of the drugs. A combination of both processes could be considered when we observe the gradation of TDR varying from a slight decrease to a complete loss of sensitivity to the drug (i.e. that the host is killed by the drug before the parasite). This gradation could be explained by the co-existence of different mechanisms of TDR, which are adding their effects to achieve a certain level of resistance (Vitouley, 2014).

Resistance to major trypanocidal drugs systematically occurs within approximately ten years following the introduction of trypanocidal to the market. With the trypanocidal drugs, such as isometamidium chloride (ISM), the homidium salts and diminazene aceturate, which were introduced during the 1950s; the first reports of acquired resistance were published during the 1960s. Quinapyramine was marketed earlier, but was withdrawn in 1976 because of resistance and toxicity problems. It was later reintroduced for use in camels and horses and may still be used in error in cattle in some locations (Taylor *et al*., 2007; Vitouley, 2014).

The resistance developed by trypanosome strains to trypanocidal drugs available on the market could either be cross or multidrug resistance. When the trypanosome is resistant to more than one drug, it is considered as multidrug resistant. In this case, different resistance mechanisms are acquired / selected independently through exposure to different drugs. This is the case for example resistance developed by trypanosome strains for DA and ISM (Tihon *et al.*, 2017).

Cross resistance is a resistance to a particular drug that often results in resistance to another drug, usually from a similar chemical class, to which the trypanosome may not have been exposed. Here, a single mechanism is responsible for resistance to more than one drug. This is the case for Quinapyramine (table 1) that causes resistance to DA and ISM (Tihon *et al.*, 2017).

Table 1. Cross-resistance between trypanocidal drugs

	Cross resistant to									
	At curative dose					**At increased dose**				
Trypanosome resistant to	QP	HM	PB	ISM	DA	QP	HM	PB	ISM	DA
QP	+	+	+	+	+	+	+	±	-	-
HM	+	+	+	+	-	+	+	+	-	-
PB	+	+	+	+	-	+	+	+	-	-
ISM	+	+	+	+	-	+	+	+	-	-
DA	+	-	-	-	+	+	-	-	-	+

QP= Quinapyramine, HM= homidium bromide, PB= Pyrithidium bromide, ISM= Isometamidium, DA= Diminazine aceturate, + = resistant, - = not resistant, ±= some strains resistant. Source: (Melaku and Birasa, 2013)

For this reason it has been removed from the market in the seventies. Unfortunately, this drug is currently again available in Africa originating from Asian markets where the drug is allowed. Quinapyramine should be strictly restricted to the treatment of horses and camels infected with *T. evansi* (Alsford *et al.,* 2013). Against this widespread use of chemotherapy for control of trypanosomosis, trypanocidal drug resistance has been reported from different parts of Sub-Saharan African countries (Table 2).

Table 2: Trypanocidal resistant trypanosome species in Sub- Saharan African countries

Country	**Trypanosoma species**	**No of isolates**		**% of Resistant isolates**	**Resistance to**	**Reference**
		Examined	**Resistant**			
Kenya	Tc	7	2	29	ISM	(Gray *et al*., 1993)
Nigeria	Tv	19	12	63	DA, H, ISM	(Saror *et al*., 1979)
Nigeria	Tb	12	2	17	DA, ISM	(Kalu, 1995)
			1	8	ISM	
Sudan	Tc, Tv, Tb	12	5	42	H	(Peregrine, 1994)
Uganda	Tb	36	1	3	DA, ISM	(Matovu *et al*., 1997)
Zimbabwe	Tc	14	6	43	DA	(Joshua *et al*., 1995)

DA= Diminazine, H=Homidium bromide, ISM= Isometamidium, Tc= *T. congolense*, Tb= *T. brucie*, Tv= *T. vivax*

Table 3. Summary of multiple and single trypanocidal drug resistance reports from Ethiopia.

Study Area		Drugs Tested	Species of parasites	References
Oromia Regional State	Gibe Valley	ISM & DA	Tc	(Moti *et al*., 2012)
	Upper Didessa	ISM	Tc, Tb &Tv	(Tewelde *et al*., 2004)
	Gibe Valley	ISM & DA	Tc	(Chaka and Abebe, 2003)
	Bedelle	ISM &DA	Tc	(Chaka and Abebe, 2003)
	Gibe Valley	ISM, DA &H	Tc	(Mulugeta *et al*., 1997)
Benshangul Gumuz	Metekel	ISM & DA	Tc	(Afewerk *et al*., 2000)
SNNPRS	Soddo	ISM & DA	Tc	(Chaka and Abebe, 2003)
	Arbaminch	ISM & DA	Tc	(Chaka and Abebe, 2003)
	Omo Valley	ISM & DA	Tc	(Ademe, 1998)
Tigray and Afar region	Tigray and Afar	ISM and Homidium	*T. evansi*	(Mekonnen *et al*., 2018)

Tc= *T. congolense*, Tb= *T. brucie*, Tv= *T. vivax*

From Ethiopia, as summarized on table 3 above, multiple drug resistant *T. congolense* populations to DA and ISM were reported in naturally infected cattle of Metekel district (Afewerk *et al*., 2000). *T. congolense* populations resistant to diminazene aceturate (Berenil®), isometamidium chloride (Samorin®) and homidium chloride (Novidium®) were also reported in cattle from Ghibe (Mulugeta *et al*., 1997). *T. congolense* isolates resistant to DA and ISM were found in Ghibe, Bedelle and Soddo (Chaka and Abebe, 2003). Similarly, (Tewelde *et al*., 2004) reported isometamidium resistance in cattle from upper Didessa valley of Western Ethiopia. All of these reports employed either a mice experiment model or block treatment in field conditions for the diagnosis of trypanocidal drug resistance.

To help people to survive in tsetse infested and trypanosome endemic marginal areas, Ethiopian government have been successfully implemented trypanosomosis control activities: (i) in the Ghibe valley using 'pour-on' insecticides (Leak *et al*., 1996; Rowlands *et al*., 1999), (ii) in Didessa valley using community based odour-baited, insecticide impregnated target/trap technology as part of the

Eastern African Regional Program and, finally (iii) in the Southern Rift valley, the eradication of the flies using the sterile insect technique (SIT) (Getachew, 2005). However, due to the lack of natural barriers and to the non-sustained control efforts, flies reinvade the tsetse-cleared territories. And also the way chemotherapy and chemoprophylaxis is performed in the field and its efficacy depends on multiple factors such as farming type, herd size and structure, breed, knowledge of the disease, availability of veterinary services and drugs and legislation about drug administration.

2.2. Glossina Vectors and its Control Strategies

Glossina species(tsetse flies) can be ranked among the world's most destructive pests and are the vectors of the causative agents for sleeping sickness in humans and African Animal Trypanosomosis (AAT) or Nagana in livestock (Vreysen *et al*., 2000). Based on both their morphological and ecological specifics, there are about 31 tsetse species and subspecies divided into three subgenera or groups, namely the subgenus Austenina (fusca group), the subgenus Nemorhina (palpalis group) and the subgenus *Glossina* (morsitans group) (Cecchi *et al*., 2008)

Fusca groups are also known as forest flies or forest loving species as they are typically found in humid forest except for *G. longipennis s*and *G. brevipalpis.* As forest isn't particularly suited for livestock breeding, their medical or veterinary importance is generally low except for *G. brevipalpis and G. longipennis* which have been implicated as significant vectors of animal trypanosomosis. *G.longipennis* lives in the arid and semi-arid savannas of East Africa while *G. brevipalpis* lives in the more humid parts of wooded savannas of Central, South and East Africa. This species can fly over relatively long distances and therefore is also observed in open grassland. Flies of this sub-genus are larger and also more primitive flies which feed on small mammals in forested areas (Cecchi *et al*., 2008).

Sub-genus palpalis groups which are mostly of reverine species as they found in close association with local patches of dense vegetation along the banks of rivers and lakes in arid country and also in dense, wet, heavily forested equatorial rain forest of West and Central Africa. They need a relatively high atmospheric humidity and shade although *G. tachinoides* can support great climatic variations and is found from arid zones in Niger to humid degraded forest in Nigeria. There are 9 species and sub-species in this group and found in gallery forests (e.g. *G. p. gambiensis* and *G. tachinoides*),

near lakes and river systems, flowing either into the Atlantic Ocean or in the Mediterranean (e.g. *G. f. fuscipes* and *G. p. palpalis*), or in rain forest (e.g. *G. p. palpalis*, *G. calliginea* and *G. pallicera*). The best conditions for "palpalis" flies are a temperature of approximately 25°C and an atmospheric humidity of 80 to 85%. These "reverine" tsetse flies cope better with increased human occupation of the land and environmental changes than the "savannah" flies (Cecchi *et al*., 2008; FAO, 2014).

Morsitans group include the most effective vector of sleeping sickness, *G. morsitans* and animal trypanosomosis *G. pallidipes, G. m. sub morsitans* and *G. austeni*. It is closely associated with open woodland and wooded savannah with *Brachystegia* (miombo) or *Colophospermum* (mopane) trees in East and Central Africa (e.g. *G.m.morsitans* and *G. pallidipes*) or *Isoberlinia* (doko) trees in West Africa (e.g. *G.m.morsitans*) (De Deken, 2012). These savannah tsetse flies penetrate during the rainy season in arid areas, while during the dry season they concentrate in dense vegetation along drainage lines or in better watered woodlands. The flies of the morsitans group are usually very sensitive to agricultural development, degradation of natural habitats and reduced wildlife density (especially *G. swynnertoni, G. pallidipes* and *G. longipalpis*), although they will feed readily on domestic livestock when available (Cecchi *et al*., 2008).

Current vector control interventions involve the use of insecticides either through sequential aerosol spraying technique (SAT); ground spraying; insecticide treated animals - live baits; Stationery Attractive Device (SAD) (traps and targets), and the sterile insect technique (SIT) (Shaw *et al*., 2013; Percoma *et al*., 2018). Insect control strategies based on chemical and biological control have had some notable successes but in many cases control has not been sustainable in the long term. This can be attributed to many reasons, including insecticide resistance, re-invasion, environmental damage and poor control program implementation (Shaw *et al*., 2013; Percoma *et al*., 2018). Moreover, some of the interventions conducted in the past such as bush clearing (tsetse habitat destruction) or elimination of wild animals (tsetse reservoir hosts) have been discarded for ecological and environmental concerns. Although it is effective in complete elimination of residual tsetse population from eradication area, it is not feasible to use modern tsetse elimination methods such as SIT in resource scarce countries due to its high cost of establishment and it is also not suitable for continuous control or suppression of fly population (Bourn *et al.*, 2005; Shaw *et al*., 2013).

2.2.1. Chemical Control

When chemical agents are used to combat tsetse flies, it must be taken into account that tsetse flies spend about 50% of their lifespan under the ground as pupae. Therefore, either insecticide must be used, which remain active for at least the maximal pupal period, or repeated treatments with a short-acting product must be foreseen. Another issue is the possible reinvasion by flies coming from untreated areas. This requires the creation of barriers (natural or chemical) between the infested and control zones which constitute an unbridgeable gap for the tsetse fly. In case of artificial barriers, the larger the control zone surface, the smaller the proportion of barrier costs in the total costs (De Deken, 2012).

Ground spraying method: Preferably during the dry season, a residual insecticide is applied to the resting and refuge sites of the flies. Spraying is discriminating (only certain types of vegetation are treated, generally 7 to 15% of the total surface) and selective (only the parts of trees and bushes are treated where the tsetse flies rest during the day). Persistent insecticides such as dieldrin (2% at 15 to 60 kg a.i. /km2), deltamethrine (w.p.) 0.1% (1 to 3 kg/km²) and alpha-cypermethrin were effective for this purpose and less harmful to the environment, but these products are more expensive. Ground spraying may be used to create chemical barriers. Because of environmental concerns and organizational difficulties this method is nowadays seldom used (De Deken, 2012; FAO, 2014).

Insecticide target screens or impregnated traps: This method of reduction of the population of adult tsetse flies make use of systems to capture flies (traps or screens, which act as traps because they are treated with "Temo-o-cid" glue) or to kill flies with persistent insecticides applied to screens or traps. Target screens consist of a blue, black or blue and black cloth screen that has been sprayed with insecticide (De Deken, 2012).

Insecticide treated hosts: In livestock breeding areas tsetse flies can be controlled by dipping the cattle regularly (e.g. every two weeks) in a solution of deltamethrine (Decatix 5% SC), applying up to 20 times/year a pour-on insecticide such as cylence 1% and renegade 1.5%or spraying the animals every two weeks with 0.005% of deltamethrine using a portable compressed air sprayer. Such treatment frequencies are too expensive for most African cattle owners and may be decreased

when the area is not subjected to invasion (Bourn *et al*., 2005; Vale *et al*., 2015). Treatment of only a part of the herd is cost beneficial as the percentage of tsetse fly feeds from an animal is correlated to its live weight (Torr *et al.*, 2007). However, depending on the region and its local customs the benefits of this particular strategy may vary heavily as some pastoral groups in Africa graze their adult cattle often separately from the calves (e.g. Massai) while small stock of the Shona in Zimbabwe graze all their livestock together (De Deken, 2012).

2.2.2. Genetic control (SIT)

This method of control aims to alter the reproductive potential of the vector or its vectorial competence. SIT considers primarily the eradication of isolated populations of tsetse flies. It involves the breeding of thousands of tsetse flies of each subspecies present in the area and the male tsetse are then sterilized using gamma (or X-) radiation and released at regular intervals, thus swamping the population with males that are unable to fertilize females successfully (Munhenga *et al*., 2011).

Glossina austeni has been successfully eradicated from Zanzibar using this method (Vreysen *etal*., 2000). During this campaign 5.5 million sterile males were released in total. Before flooding the area with the sterile males the local wild tsetse population must be largely reduced by other control methods. It is estimated that a proportion of 10 to 30 sterile males per fertile male is needed in order to assure that most couplings with the few remaining wild females will occur with sterile males and, since most female tsetse fly does copulate only once with a male, the population will be eradicated (De Deken, 2012).

Sterilization by irradiation is possible for adult male or female tsetse as well as for pupae. Before being released the sterile males are nourished with blood from animals treated with trypanocidal in order to decrease as such as possible the chances of transmission by the sterile flies (Byamungu *et al*., 2011). For reverine tsetse fly species it seems easier for the sterilized, released male to find wild flies of the opposite sex than for savannah species.

Genetic control via transgenic tsetse fly symbionts: Current research on genetic control of tsetse flies involves the development of transgenic symbionts of the tsetse fly (Aksoy and Rio, 2005). The

3 symbionts of the tsetse fly (*Sodalis glossinidius*, *Wigglesworthia glossinidi*um and a strain of *Wolbachia*) are all maternally transmitted to progeny. The research aims to alter the genome of the endosymbiont, *Sodalis glossinidius*, so that the symbionts express trypanolytic substances into the fly. *Wolbachia* will be used to introduce cytoplasmic incompatibility in the natural tsetse population. Cytoplasmic incompatibility is achieved when wild-type females are mated with males infected by a *Wolbachia* strain that is non-existent in the female (Abd-Alla *et al*., 2013). The intracellular *Wolbachia* will then cause embryonic mortality. When both methods are combined it must, in theory, be possible to replace the wild-type, trypanosome-susceptible tsetse population with the engineered, trypanosome-refractory line (Abd-Alla *et al*., 2013).

The sterile insect technique via chemical sterilization: Mass breeding followed by gamma ray sterilization of males could possibly be replaced by the direct sterilization of the males or/and females in the field thanks to systems of traps or screens allowing a sufficient contact between the insect and a sterilizing chemical product (e.g. bisazir, tepa, hempa) (Munhenga *et al*., 2011). As these products are relatively dangerous and toxic to mammals, they may be substituted by analogues of the juvenile hormone (e.g. pyriproxyfen) (Glare and Callaghan, 1999; Hargrove and Langley, 1993). An amount of 0.02 μg of pyriproxyfen applied on a tsetse fly female inhibits any hatching of pupae by this female. This juvenile hormone mimics the chitin synthesis inhibitor, triflumuron, and may provide a safe way to effectively auto sterilize female tsetse flies. These methods may eliminate the need for the costly artificially maintained tsetse fly colonies (Hargrove and Langley, 1993).

2.3. Tsetse Vector Suppression Activities in South Omo Zone

Glossina vector suppression activities in South Omo zone was started in 2013 by Southern Tsetse Eradication Program (STEP) based on initial entomological survey conducted on 2012 by the project. According to the initial survey, the vector density of South Omo zone was 51.5 F/T/D, (5,099 Glossina species, dominantly of *G. pallidipes* and few undefined species) by deploying 33 NGU trap in selected areas of the zone (STEP, 2012). After this initial report, five districts (Debub Ari, Benatsemay, Male, Selamago and Hammer) that were highly suspected to be infested by the vector were selected and vector suppression activity was started by using deltamethrine (1%) pour-

on technique. At the beginning there were other suppression methods such as aerial spray, traps and targets. However, there was no sustainable use of these methods. Thus only deltamethrine pour-on was implemented to suppress the vector in South Omo zone.

According to STEP project, starting from initial suppression year, most of pour-on suppression activities were done at the start of rainy season during which there was high fly population in the area. Dry season pour-on activity was also done on selected PAs close to wild life conservation areas. Accordingly, 2,853 liters of deltamethrine pour-on for 49,273 animals from 62 PAs during dry season and 13,087 liters of deltamethrine pour-on for 592,297 animals from 253 PAs were participated in suppression activity until 2018. But due to logistic problems, no suppression activity was conducted in 2015 and also the initial proposed numbers of districts were minimized from five districts to three districts. Accordingly, Debub Ari, Benatsemay, Male and partially Selamago districts were included in the vector suppression campaign that had been conducted in the zone by using deltamethrine pour-on technique. Between each terms of suppression, vector density survey works and rarely of parasitological prevalence survey were conducted. Among the survey results, the record of STEP indicated 28.7 F/T/D (dry season, 2013), 24.10 F/T/D (dry season, 2014), 2.35 F/T/D (wet season, 2014), 10.3 F/T/D (dry season, 2016), 14.3 F/T/D (wet season, 2016), 4.3 F/T/D (wet season, 2017) and 3.7 F/T/D (dry season, 2018) vector densities during the survey (STEP, 2018).

3. MATERIALS AND METHODS

3.1. Study Area Description

South Omo zone is situated in the Southern Nations, Nationalities and Peoples Region (SNNPR) of Ethiopia. The zone is bordered on the South by Kenya, on the Southwest by the Ilemi Triangle, on the West by Bench Maji, on the Northwest by Keficho Shekicho, on the North by Semien Omo, on the Northeast by the Dirashe and Konso Special Districts, and on the East by the Oromia Region. The administrative center of South Omo zone (Jinka city) is located at 750 km and 525 km from South of Addis Ababa and Hawassa respectively (SOZLFD, 2018).

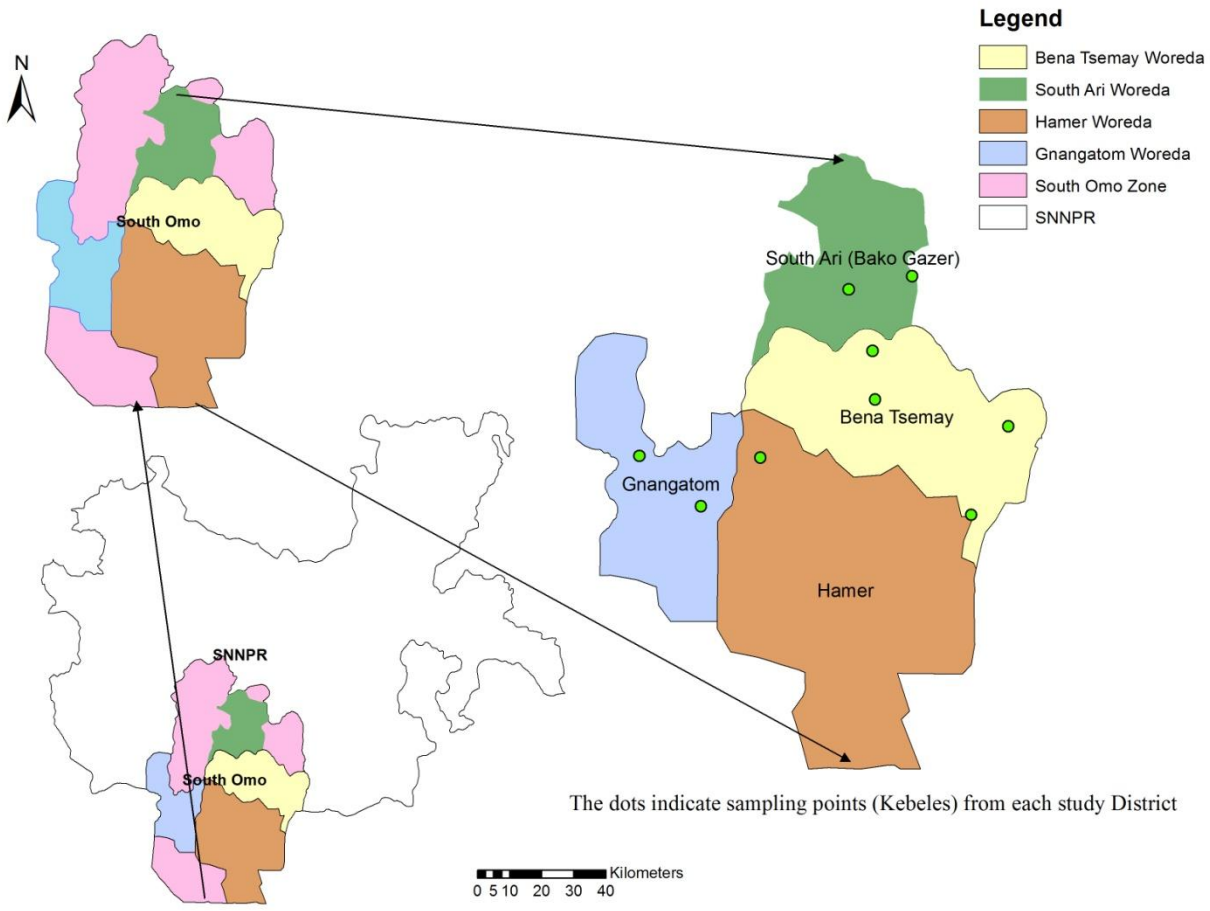


Figure 1. Map showing study zone on regional map (left bottom) and four study districts (right)

According to Central Statistical Agency (CSA) population projections for the year 2016, more than 700,000 people were projected to be living in the zone (CSA, 2013). This zone has area coverage of 24,249 km2. Similarly, CSA (2017) agricultural sample survey indicated the total cattle, sheep and goat population of South Omo zone (in million) as 1.75, 1.55 and 2.88 respectively. Specific study district's livestock and human population was indicated on table 2 below. There are eight (8) districts representing South Omo zone which have diverse ethnic groups; out of 56 ethnic groups found in the SNNPR, 16 ethnic groups were from South Omo zone.

The zone is located between 4^0 43' North to 6^0 46' North latitude & 35^0 79' East to 36^0 06' East longitude, and found from 376 m to 3,500 m. a. s. l. with mean annual rainfall ranges from 400 mm to 1,600 mm. It has a diverse agro-ecological zones ranging from hot arid to the tropical humid with average temperature ranging from minimum of 10.1^0C to maximum 27.5^0C (SOFED, 2012). In the study area, rain is erratic and usually bimodal occurring from September to December and from March to May (NMSA, 2005; FEWSNET, 2012).

Table 4. Human and livestock population of study districts

Study districts	Human population	Livestock population	References
Debub Ari	253,810	232,718	
Benatsemay	70,418	564,042	(CSA, 2017; SOZLFD, 2018)
Hammer	79,419	717,362	
Gnangatom	23,250	685,506	
Total	426,897	2,199,628	

Mixed crop-livestock farming is the main source of livelihoods in agro-pastoral districts of the zone (Debub Ari, Semen Ari, Male and few areas of Benatsemay district) where maize, sorghum and legumes are the major subsistence crops, and onion, cabbage and 'korerma' are the cash crops. Other crops include root crops (yams, sweet and Irish potatoes, cassava, enset, "godere"); fruit trees and small-scale horticulture (vegetables) are also available in these agro-pastoral districts. Out of eight districts of the zone, four districts namely Hammer, Dasenech, Gnangatom and Selamago are occupied largely by pastoralists even though there are few pastoralists practicing farming near Omo

River after the river get out from the farming land following heavy rain which is locally known as ‘Omo shish’. The livelihood of these pastoral districts depends on livestock rearing which are mainly local breeds of both large and small ruminants.

Since South Omo zone is characterized by districts having three different agro-ecological conditions as mentioned above, each selected districts were defined according to their specific agro-ecological, agricultural and production system. Accordingly, out of eight districts, Semen Ari district is classified as ‘Dega’. The whole Debub Ari district and very small parts of Benatsemay and Male districts are classified as ‘Woina Dega’ and the remaining districts i.e. Hammer, Selamago, Gnangatom and Dasenech districts and most parts of Male and Benatsemay districts are classified as ‘Bereha’ or arid areas and totally inhabited by pastoral communities.

Regarding livestock health infrastructures, the zone have seven (7) veterinary clinics, six of which are found in six different districts under the zone and one on capital city of the zone. The zone also have 86 animal health posts, 9 veterinarians, 301 animal health experts (154, animal health assistants, 126, animal health technicians and 21, Bachelor of veterinary science graduates (BVSc) and 364 community animal health workers (CAHWs). According to SOZLFD (2018), there are fifteen (15) veterinary drug shops situated on different districts under the zone.

3.2. Study Animals and their Management Practice

Zebu cattle breeds which are traditionally reared by agro-pastoralists and pastoralists were used as study animals. The four study districts, Debub Ari, Benatsemay, Hammer and Gnangatom were characterized by having cattle populations which are local breeds with low productivity except very few cross breeds of large ruminants with exotic breeds which are crossed bred by respective district’s livestock and fishery department. All cattle population in four districts was managed under pastoral and agro-pastoral extensive management system with very few supplementations given to milking cows and draught oxen especially in agro-pastoral districts (Debub Ari and Benatsemay). In agro-pastoral districts, cattle are grazed away from farms during the cropping season (rainy season), but allowed to graze on crop residues (not the whole herd) after harvesting with their dung directly fertilizing fields in readiness for the next cropping season and all animals were watered at river or at community bore holes when seasonal rivers dry out. But in pastoral districts (Hammer and

Gnangatom), all types of cattle were allowed to graze on extensive pastoral range land which are shared by animals from different districts of the zone and sometimes become source conflict between pastoralists.

The traditional housing system of animals in agro-pastoral districts which is constructed from locally available woody materials gives protection from extreme weather condition and from different predators at night time. But in most parts of the zone, the animals were kept under large trees such as mango, avocados and coffee trees near the owner's house during the night time. There is also crash system (especially in pastoral districts of the zone) of housing made from locally available materials (Annex 7) with no roof to protect animals from rain or sun light but it simply protects animals from going out during night time. Because of free watering system at river, leech is the main water born parasite affecting cattle during dry season. Shortage of animal feeds and water are main problem facing livestock owners especially during dry season so that animals were travel very long distance for search of feed and water during these seasons. To tackle this water shortage, pastoralists and agro-pastoralists of the zone also use community bore holes which is locally known as 'chirosh' which is bore at large river after the river was dry out (Annex 7).

3.3. Study Design and Methods of Sampling

Repeated cross-sectional survey was undertaken from January 2019- March 2019 (for dry season) and from November 2018- December 2018 and April 2019- May 2019 (for wet season) to establish the levels of tsetse fly, trypanosome prevalence and trypanocidal drug utilization practices. Representative study districts and PAs were selected purposively based on ease of access and sake of convenience. From selected PAs, representative livestock owners were selected by simple random method by using their lists from PAs administration. Herds owned by livestock owners were stratified based on their sex, age, BCS and grazing system to include these factors in to the study. From selected strata (herd), the study subjects were systematically drawn by taking every selected animal by calculating the interval between 1^{st} and 2^{nd} and then n^{th} selected animal depending on the size of each herd chosen. Systematical drawing of the cattle was needed because agro-pastoralists/pastoralists have no practice of giving identification except cultural tattooing, iron brand or ear cutting for their animal which is not feasible for selecting study subjects.

3.4. Sample Size Determination

From tsetse suppressing districts, sample size was determined by assuming an estimated prevalence of trypanosomosis as 18.195% which is average prevalence of previous report of (Muktar *et al*., 2016; Senait and Asnake, 2016) and a desired accuracy level of 5% at the 95% confidence level. Accordingly, the following formula was used to determine the sample size (Dohoo *et al*., 2003).

$$n = \frac{Z^2_{\alpha}. P. (1-P)}{e^2}$$

Where:

$Z\alpha = Z_{0.05} = 1.96$ (the value of Z_{α} required for confidence=95%)

e = the precision of the estimate (allowable error or margin of error) equal to 1/20 the normal approximation of sample size for the binomial distribution confidence interval.

Applying the above formula, a total of 229 animals would have constituted the optimal estimated sample size for parasite prevalence study of the zone. However, to increase the precision of the study result and to see the effect of vector suppression, the sample was increased by 50% and hence a total of 344 animals were sampled from tsetse suppressing districts.

Sample size for tsetse non-suppressing districts (Hammer and Gnangatom) was calculated separately from that of tsetse suppressing area by taking previous prevalence report of 26.3% (Belete, 2017). Thus the sample size for tsetse non-suppressing study areas was found to be 298 animals by applying the same formula used above. Study subjects were taken proportionally from four purposively selected districts (two districts from tsetse suppressing and two districts from non-tsetse suppressing) to accommodate the required sample size based on the number of cattle population of each district and the above calculated sample sizes.

Cattle of different age sex, body condition, herd size and grazing system were considered for the parasitological survey on both tsetse-suppression and non-suppression districts. Age of the study animal were determined by dentition according to (Pace and Wakeman, 2003). Body condition of all animals were determined according to (Nichlson and Butherworth, 1986) body condition scoring for zebu cattle and classified as poor, medium and good.

The sample size for trypanocidal drug utilization practice survey was calculated by the formula given below according to online survey sample size calculation from the website.

$$\frac{\frac{z^2 * p(1-p)}{e^2}}{1 + \left[\frac{z^2 * p(1-p)}{e^2 N}\right]}$$

Source: (https://www.surveymonkey.com/mp/sample-size-calculator).

Where:

N= total population size of study districts which was obtained from (CSA, 2007)

e= margin of error (equal to 0.05 or 1/20)

z= z-score indicating the number of standard deviations a given proportion is away from the mean whose value is equal to 1.96 for 95% confidence level p=percentage of my sample that picks a particular answer.

Accordingly 124 and 60 agro-pastoralists/pastoralists respectively from tsetse suppressing and tsetse non-suppressing districts were participated in questionnaire survey.

3.5. Sample Collection and Laboratory Technique

3.5.1. Parasitological prevalence study

Blood samples were obtained by puncturing of the marginal ear vein with a lanced and collected directly into two capillary tubes, which has been treated with anti-coagulant sealed one end with crystal seal (figure 2). The capillary tubes were placed in micro haematocrit centrifuge. Following centrifugation at 12,000 rpm for five minutes (OIE, 2017), each tube was then placed in haematocrit reader (figure 2) and expressed the reading as a percentage of packed red cells to the total volume (PCV) of whole blood. The level of anemia was determined by taking cut-off PCV value of 24, i.e.

animals with PCV value of less than 24 (< 24) as anaemic and those animals with PCV of ≥ 24 as non- anaemic (OIE, 2017).

Buffy coat technique: After centrifugation, the capillary tube was cut 1 mm below the buffy coat to include the upper most layers of the red blood cells and 3 mm above to include the plasma. The content of capillary tube was expressed on to slide, homogenized onto a clean glass slide and covered with cover slip. The slide was then examined under 40x objective and 10xeyepieces for movement of parasites and the species identification. Species identification of the parasite was conducted by observing the motility of the parasite which was described in (OIE, 2017) as *T. vivax* (extremely active, traverses the whole field very quickly, pausing occasionally), *T. congolense* (sluggish, adheres to red blood cells by its anterior extremity), *T. brucie* (rapid movement in confined areas) and *T. theileri* (tends to rotate or stay immobile as if stuck to the glass).

Thin blood smear: A small drop of blood from marginal ear vein was applied to a clean slide and spread by using another clean slide at angle of 45º. The smear was dried by blowing it in the air and fixed for 2 minutes in absolute methanol. Then it was flood with Giemsa stain (1:10 solution) for 30 minutes, drain and wash of excess stain using distilled water, allowed it to dry by standing upright on the rack and finally it was examined under the microscope (100X) oil immersion objective lens and the trypanosome species were identified based on their morphological characteristics (OIE, 2017).

3.5.2. Entomological survey and their identification

A repeated cross-sectional entomological survey was conducted to establish tsetse fly densities and its variation during rainy and dry season in the study area. In both season, a total of 96 NGU traps (64 traps in tsetse suppressing districts and 32 traps in tsetse non-suppressing districts), 4-6 traps per PA were deployed at an interval of about 100-200 meter in purposively selected and suspected tsetse habitats along rivers and streams, human settlements, forests, plantations and major watering points of the study districts (figure 3). Trap deployment sites were selected to represent all habitats that could be related to fly multiplication, behavior, feeding and other related aspects (Shereni *et al*., 2016). All trap deployment sites (PAs) were geo-referenced and their respective altitude above sea level was also recorded (Annex 4).

Figure 2. Animals congregated for bleeding (upper left), bleeding of animals (upper right) Micro-haematocrit tube after centrifugation (lower left) and PCV reading (lower right)

In the case of South Omo zone, most traps were deployed around Mago national park which is mostly grass land and tree vegetation bordered by most of the selected study districts. Although the park policy restricts the grazing of animals in and around the park, animals from the study districts (mostly from Debub Ari, Benatsemay and Hammer) used to graze in and around the park especially during dry season. From Benatsemay and Hammer district, two study PAs (one PA from each) were selected purposively from around Woito river; the river which is the source of water for most of cattle from both districts during dry season which in turn represent major watering point of the districts. From Gnangatom district, PAs bordering Omo River was selected purposively for trap deployment to include major watering points of the district.

Figure 3. NGU Trap deployment at different entomological survey sites

For rainy season tsetse survey, traps were deployed in rainy seasons which range from September to December and from April to May and for dry season tsetse survey the traps were deployed starting from January to March which is dry season in South Omo zone (NMSA, 2005; FEWSNET, 2012). This schedule was also applied for seasonal trypanosome prevalence study in the study area. The traps were emptied 48 hours later and flies counted, identified, and separated into their respective species and sex by using a modified entomological key described in (Brunhes*et al*., 1994) (Annex 7).

Sex identification of *Glossina* species was made by observing structure called hypopygium at the ventral side of the posterior abdomen a tumefaction that is in fact the folded male terminalia (De Deken, 2012). The tsetse fly density was calculated by number of flies caught per traps per day according to (Shereni *et al*., 2016).Tsetse fly species were identified based on morphological characteristics. Other mechanical vectors responsible for the trypanosomosis transmission such as *Tabanus, Stomoxys and Haematopota* flies were identified to their genus level based on their morphological characteristics (Desta *et al*., 2013; Hoppenheit *et al*., 2014).

3.5.3. Trypanocidal drug utilization practice survey

A structured questionnaire was administered to herd owners to collect data on problems of trypanosomosis and trypanocidal drug utilization. Prior to dissemination of questionnaire to respective participants, the questionnaire was first translated in to Amharic version and then to local

language. Information from experts as well as recorded data at study zonal and district level was also used. The entire questionnaire used in this study is annexed at the end of this manuscript (Annex 1).

3.6. Data Analysis

Data recorded during entomological survey, blood sample collection, parasitological examination and PCV measurement as well as questionnaire responses were entered into Excel Spread Sheets to create a database and were imported to SPSS version 20 for descriptive analysis. R-software package was used to analyze the association of different risk factors with the prevalence of trypanosome infection. Tsetse catches were summarized for species at district and PA level as the number of tsetse caught per trap per day. Trypanosome prevalence was estimated at district, sex, age, BCS, season and tsetse suppression status of selected study areas as the proportion of trypanosome positive cattle in the population at risk by using univariate analysis through simple logistic regression tests to determine associations between trypanosome infection prevalence and each of study risk factors. Multiple logistic regression analysis was performed to see the effect of combined risk factors on the prevalence of trypanosome infection. Poisson regression analysis followed by negative binomial regression analysis was used to see the effect of different risk factors on tsetse vector count. Student's t-test was employed to compare the mean PCV of the parasitaemic animals with that of the aparasitaemic animals. Data generated from questionnaire survey was analyzed using descriptive statistics like means, frequencies and percentages for different parameters. All tests were done at the 5% global significance level.

4. RESULTS

4.1. Parasitological Prevalence

Overall pooled prevalence of trypanosomosis in South Omo zone was 11.05% (142/1284). There was statistically significant difference (P <0.05) in trypanosome infection prevalence among two study seasons. Accordingly, the prevalence in dry season (14.33%) was higher than wet season (7.78%) (Table 5).

Table 5. Overall bovine trypanosome infection prevalence in South Omo zone

Parameters	**Category**	**No of animal examined**	**No of +ve**	**%**	**X^2-value**	**p-value**	**95% CI**
Season	Dry	642	92	14.33%	13.96	0.000*	0.34-0.72
	Wet	642	50	7.78			
	Overall	**1284**	**142**	**11.05%**			

A total of 344 and 298 animals were examined during dry season from tsetse-suppressing and tsetse non-suppressing study areas respectively. The overall parasitological prevalence was found to be 13.66% and 15.10% for tsetse suppressing and non-suppressing areas respectively. Similarly, the wet season parasitological prevalence was 8.13% and 7.38% for tsetse suppressing and non-suppressing areas respectively. Within seasonal prevalence of the disease was not statistically significant (P > 0.05) between tsetse suppressing and non-suppressing areas. However, there was statistically significant difference (P <0.05) between prevalence of trypanosome infection and season. Accordingly, the prevalence of trypanosome infection was significantly higher in dry than wet season for both tsetse suppressing and non-suppressing areas (Table 6).

Table 6. Among Seasonal prevalence of bovine trypanosome infection in tsetse suppressing and non- suppressing areas

Parameters	**Group**	**animal examined**	**No of +ve**	**Prevalence**	**X^2-value**	**p-value**	**95% CI**
Suppression status							
TSA	Dry	344	47	13.66%	5.40	0.020*	0.33-0.91
	Wet	344	28	8.13%			
TNSA	Dry	298	45	15.10%	8.896	0.003*	0.25-0.75
	Wet	298	22	7.38%			
Overall		**1284**	**142**	**11.05%**			

*"significant", TSA= Tsetse Suppression Area, TNSA= Tsetse Non-Suppression Area

Table 7. Overall dry season prevalence of bovine trypanosomosis in South Omo zone

Risk factors	**Category**	**No of animal examined**	**No of +ve (%)**	**X^2-value**	**p-value**	**95% CI**
District	Debub Ari	150	24(16%)		0.26	0.76-2.63
	Benatsemay	194	23(11.85%)			1*
	Gnangatom	146	22(15.06%)	1.43	0.38	0.70-2.47
	Hammer	152	23(15.13%)		0.37	0.70-2.47
Age	< 2 years	194	29(14.94%)			1*
	>2 years	448	63(14.06%)	0.08	0.76	0.58-1.51
Sex	Male	284	32(11.26%)	3.89	0.04*	0.39- 0.99
	Female	358	60(16.75%)			1*
BCS	Poor	402	59(14.67%)		0.17	0.81-4.91
	Medium	170	27(15.88%)	2.26	0.14	0.84-5.60
	Good	70	6(8.57%)			1*
Grazing system	Communal	612	84(13.72%)			
	Tethering	30	8(26.66%)	3.91	0.04*	0.92-5.11

Overall dry season prevalence of trypanosome infection revealed sex and grazing system to be significantly associated (P <0.05) with the occurrence of the disease (Table 7). In the case of wet season, the overall prevalence of trypanosome infection indicated that none of the risk factors were significantly linked with its occurrence (Table 8).

Table 8. Overall wet season prevalence of bovine trypanosome infection in South Omo zone

Risk factors	**Category**	**No of animal examined**	**No of +ve (%)**	**X^2-value**	**p-value**	**95% CI**
Districts	Debub Ari	150	15 (10%)		0.27	0.71-3.40
	Benatsemay	194	13 (6.70%)	1.68		
	Gnangatom	146	12 (8.21%)		0.59	0.54-2.83
	Hammer	152	10 (6.57%)		0.96	0.40-2.29
Age	< 2 years	175	12 (6.85%)	0.29		
	>2 years	467	38 (8.13%)		0.59	0.63-2.45
Sex	Male	266	21 (7.89%)	0.007	0.93	0.56- 1.83
	Female	376	29 (7.71%)			
BCS	Poor	339	34 (10.02%)		0.23	0.75-4.68
	Medium	204	10 (4.90%)	5.14	0.67	0.28-2.40
	Good	99	6 (6.06%)			
Grazing system	Communal	562	41 (7.29%)	1.52		
	Tethering	80	9 (11.25%)		0.22	0.70-3.31

The proportion of *T. congolense* (80%) was higher than *T. vivax* (20%) in tsetse suppression area throughout the entire risk factors (Table 9). Although there was higher disease prevalence in Debub Ari district (13.0%) than Benatsemay district (9.27%) of tsetse suppression area, the variation in the prevalence was not statistically significant (P>0.05) (Table 10). The odds of occurrence of trypanosome infection in Debub Ari was higher by the factor of 1.46 (CI: 0.90-2.36) and 1.26 (CI: 0.76-2.11) than Benatsemay district respectively in simple and multiple logistic regression. The prevalence of trypanosome infection in tsetse suppression area was not significantly associated (P>0.05) among age groups.

Table 9. Prevalence of trypanosome infection among different risk factors and proportion of identified trypanosome species in tsetse suppression area

Parameters	**No of animal examined**	**No of +ve**	**Prevalence**	***T. congolense* (%)**	***T. vivax* (%)**
Districts					
Debub Ari	300	39	13.0%	10%	3%
Benatsemay	388	36	9.27%	7.73%	1.54%
Age groups					
< 2 years	183	19	10.38%	9.83%	0.54%
> 2 years	505	56	11.08%	8.31%	2.77%
Sex groups					
Male	296	21	7.09%	6.08%	1.01%
Female	392	54	13.77%	10.71%	3.06%
BCS					
Poor	367	52	14.16%	10.62%	3.54
Medium	223	19	8.52%	8.07%	0.44%
Good	98	4	4.08%	3.06%	1.02%
Grazing system					
Communal	578	58	10.03%	8.30%	1.73%
Tethering	110	17	15.45%	10.90%	4.54%

There was statistically significant difference (P< 0.05) in disease prevalence observed among sex, body condition scores and season tsetse suppression areas. Female (13.77%) were more infected than male (7.09%) (OR= 0.47, CI: 0.27-0.79), poor body conditioned animals were 3.87 times more infected than good body conditioned and mediums were 2.18 times more infected than good body conditioned animals in tsetse suppression areas when the risk factors act individually (simple logistic regression analysis). Trypanosome infection prevalence on animals grazing on communal range land (10.03%) and those animals tethered on individual pastoralist's/agro-pastoralist's own

farming lands (15.43%) (OR= 1.63, CI: 0.89-2.88) was not statistically significant in univariate analysis (Table 11).

On multiple logistic regression analysis, in addition to the above statistically significant risk factors (variable), one additional risk factor which is the grazing system of the study animals become statistically significant. Accordingly, the prevalence of trypanosome infection in male was lower by a factor of 0.48 (CI: 0.27-0.83) than its prevalence in female animals. Similarly the incidence of trypanosome infection in poor and medium BCS animal was higher by a factor of 3.25 (CI: 1.26-11.09) and 2.07 (CI: 0.74-7.37) respectively than good body conditioned animals (Table 10).

Table 10. Simple and Multiple logistic regression analysis trypanosome infection prevalence and its association with different risk factors in tsetse suppression areas of South Omo zone

Risk factors	**Category**	**No of animal examined**	**No (%) +ve**	**Simple logistic regression**			**Multiple logistic regression**		
				OR	**OR 95% CI**	**P-Value**	**OR**	**OR 95% CI**	**P-Value**
District	Debub Ari	300	13.0%	1.46	0.90-2.36	0.12	1.26	0.76-2.11	0.35
	Benatsemay	388	9.27%	1*			**1***		
Age	>2 years	505	11.08%	1.07	0.63-1.90	0.79	0.99	0.57-1.79	0.98
	< 2 years	183	10.38%	1*			1*		
Sex	Male	296	7.09%	0.47	0.27-0.79	0.006	0.48	0.27-0.83	0.01
	Female	392	13.77%	1*			1*		
BCS	Poor	367	14.16%	3.87	1.53-13.06	0.01	3.25	1.26-11.09	0.02
	Medium	223	8.52%	2.18	0.79-7.70	0.16	2.07	0.74-7.37	0.20
	Good	98	4.08%	1*			1*		
Grazing	Communal	578	10.03%	1*			1*		
	Tethering	110	15.45%	1.63	0.89-2.88	0.09	2.07	1.06-3.92	0.027
Season	Dry	344	13.66%	1*			1*		
	Wet	344	8.13%	0.55	0.33-0.91	0.02	0.52	0.30-0.87	0.015

Note: OR= Odds Ratio, CI= Confidence Interval, 1*= Reference

Finally multiple logistic regression analysis of grazing system and season of tsetse suppression areas shown that the occurrence of trypanosome infection in tethering animals was 2.07 times significantly higher ($P < 0.05$) than those animals graze on communal range land and the disease prevalence in wet season was lower by a factor of 0.52 than its prevalence in dry season and the difference was statistically significant (Table 10).

The result of trypanosome species from tsetse non-suppression areas also indicated that *T. congolense* (71.64%) was the dominant species in the area followed by *T. vivax* (28.35%). There was no mixed infection report from the area (Table 11). There was no statistically significant difference in species distribution among two study areas. Simple and multiple logistic regression result displayed on table 12 indicated the association of each of risk factor (one variable) as well as their commutative effect (all variables together) in the occurrence of trypanosome infection in the study area.

Table 11. Prevalence of bovine trypanosome infection and proportion of trypanosome species in tsetse non-suppression areas, South Omo zone

Parameters	**No of animal examined**	**No of +ve**	**Prevalence**	***T. congolense* (%)**	***T. vivax* (%)**
Districts					
Gnangatom	292	34	11.64%	7.53%	3.76%
Hammer	304	33	10.85%	8.55%	2.63%
Age groups					
< 2 years	180	16	8.88%	7.22%	2.22%
> 2 years	416	51	12.25%	8.41%	3.60%
Sex groups					
Male	238	18	7.56%	5.04%	2.52%
Female	358	49	13.68%	10.05%	3.63%
BCS					
Poor	374	41	10.96%	8.55%	2.13%
Medium	151	18	11.92%	8.60	4.97%
Good	71	8	11.26%	4.22%	7.04%
Season					
Dry	298	46	15.43%	11.40%	4.02%
Wet	298	22	7.38%	4.69%	2.34%

In tsetse non-suppression areas of South Omo Zone, there was no statistically significant difference in the prevalence of trypanosome infection among selected study districts, age groups and body condition scores. However, there was high prevalence of trypanosome infection on animals of > 2 years of age (12.25%) than animals aged < 2 years (8.88%) (Table 12). This condition holds true in both simple and multiple logistic regression analysis. On the other hand, the season and sex groups declared statistically significant difference (P <0.05) on disease prevalence. There was higher trypanosome infection occurrence during dry season (15.43%) than wet season (7.38%) and on female (13.68% than male animals (7.56%).

Table 12. Simple and Multiple logistic regression analysis trypanosome infection prevalence and its association with different risk factors in tsetse non-suppressing of South Omo zone

Risk factors	Category	No of animal examined	No (%) +ve	Simple logistic regression			Multiple logistic regression		
				OR	OR 95% CI	P-Value	OR	OR 95% CI	P-Value
District	Gnangatom	292	11.64%	1*			1*		
	Hammer	304	10.85%	0.92	0.55-1.53	0.76	0.93	0.55-1.56	0.78
Age	>2 years	416	12.25%	1.43	0.80-2.66	0.23	1.58	0.88-2.99	0.13
	< 2 years	180	8.88%	1*			1*		
Sex	Male	238	7.56%	0.51	0.28-0.89	0.02*	0.48	0.27-0.85	0.01*
	Female	358	13.68%	1*			1*		
BCS	Poor	374	10.96%	0.96	0.45-2.31	0.94	0.82	0.38-2.01	0.65
	Medium	151	11.92%	1.06	0.45-2.71	0.88	0.92	0.38-2.39	0.86
	Good	71	11.26%	1*			1*		
Season	Wet	298	7.38%	0.44	0.25-0.75	0.003*	0.41	0.23-0.70	0.001*
	Dry	298	15.43%	1*			1*		

Note: OR= Odds Ratio, CI= Confidence Interval, 1*= Reference

The odd of occurrence of trypanosome infection was increased by a factor of 1.43 (CI: 0.80-2.66) when the animal age was > 2 years as compared with animal of ages below two years (simple logistic regression) but its occurrence was increased by a factor of 1.58 (CI: 0.88-2.99) when all risk factors act together. In the case of animal sex, occurrence of trypanosome infection in male animal was 0.51 and 0.48 times less than female animals with statistically significant difference ($p < 0.05$) in simple and multiple logistic regression analysis (Table 12). The prevalence of trypanosome infection among the season was statistically significant as the odd of occurrence of the disease in wet season was less by a factor of 0.44 (CI: 0.25-0.75) and 0.41 (CI: 0.23-0.70) than dry season in simple and multiple logistic regression analysis respectively.

4.2. Hematological Findings

Trypanosome species specific mean PCV-value indicated that animals infected with *T. congolense* had mean PCV which was statistically not different from those animals infected with *T. vivax*. Parasitaemic animals were found to have lower mean PCV than aparasitaemic animals and such difference in mean PCV value was statistically significant ($p < 0.05$) for those animals examined from both tsetse suppressing and tsetse non-suppressing areas (table 13).

Table 13. Mean PCV-value of parasitaemic and aparasitaemic and *T. congolense* infected and *T. vivax* infected animals from tsetse suppressing and non-suppressing study areas, South Omo zone

Parameters	**Category**	**Mean PCV (%)**	**SD**	**P-value**	**95% CI**	
					Lower	Upper
Tsetse suppressing areas	Parasitaemic	23.76	3.07			
	Aparasitaemic	27.73	5.07	0.000*	-5.15	-2.80
Tsetse non-suppressing area	Parasitaemic	23.37	3.21			
	Aparasitaemic	27.88	4.82	0.000*	-5.70	-3.32
Trypanosome species	*T. congolense*	23.59	3.22	0.61	-0.97	1.62
	T. vivax	23.26	3.31			

SD= Standard deviation

When samples from both study sites (tsetse suppressing and tsetse non-suppressing) and both seasons (dry and wet) were pooled together, overall mean PCV was significantly lower in parasitaemic animals than aparasitaemic animals ($P < 0.05$). Similarly, animals sampled during wet season had significantly higher ($P < 0.05$) mean PCV value than those animals sampled during dry season regardless of infection status of the animal and suppression status of the study sites. But the pooled overall mean PCV value of animals sampled from tsetse suppressing sites were not statistically different ($P > 0.05$) from those animals sampled from tsetse non-suppressing sites (Table 14).

Table 14. Overall mean PCV-value of pooled samples from tsetse suppressing and non-suppressing sites of South Omo zone

Parameters	**Group**	**No examined**	**Mean PCV (%)**	**SD**	**P- value**
Infection status	Positive	142	23.57	3.13	
	Negative	1142	27.80	4.95	0.000*
Sampling season	Dry	642	26.22	4.37	
	Wet	642	28.45	5.27	0.000*
Suppression status	tsetse suppressing	688	27.30	5.04	
	tsetse non- suppressing	596	27.38	4.88	0.77
Grazing system	Communal	1174	27.26	4.93	
	Tethering	110	28.12	5.28	0.08

SD= Standard deviation

There was statistically significant association ($P < 0.05$) between infection status and anaemia. That means anaemic animals were highly parasitaemic (to be infected) than non-anaemic animals and vice-versa in both tsetse-suppressing and tsetse non-suppressing study sites (Table 15).

Table 15. Analysis indicating association between anaemia and parasitaemia in study sites

Parameters	**Groups**	**No of animal examined**	**No of +ve**	**% of infection**	**X^2-value**	**P-value**	**95% CI**	
							LB	UB
TSA	Anaemic	137	36	26.3%				
	Non-anaemic	551	39	7.10%	41.64	0.000*	0.12	0.35
TNSA	Anaemic	140	35	25%				
	Non-anaemic	456	32	7.01%	34.71	0.000*	0.13	0.38

LB= Lower Bound, UB= Upper Bound

4.3. Entomological Survey Results

A total of 64 traps (32 traps in dry and 32 traps in wet season) were deployed in suspected main tsetse multiplication areas of the tsetse- suppression districts from South Omo Zone. The total number of tsetse fly caught during the dry season in tsetse suppressing areas were 165; out of which 22 and 147 were males and females respectively with an average apparent density of 2.64 F/T/D and male to female ratio of 1 male to 6.68 females. Total catch of *Stomoxys, Tabanus* and *Haematopota* flies were 205, 34 and 50 respectively during dry season (Table 16). In the wet season, a total of 27 tsetse flies (4 males and 23 females, sex ratio =1:5.57) were caught with apparent densities of 0.42 and *Stomoxys, Tabanus* and *Haematopota* caught of 155, 54 and 33 respectively from tsetse suppressing areas (Table 16).

Glossina pallidipes were the only tsetse species identified in the area as all *Glossina* species caught during both seasons were *Glossina pallidipes* under stereo-microscope by using tsetse identification keys. Among the tsetse suppressing sites, highest tsetse caught (9.2 F/T/D) was from Kure PAs of South Ari district which is situated near Mago national park and the lowest caught (0.5 F/T/D) was from Enchete PA of Benatsemay district from around Woito River. Among the tsetse-suppressing districts, the tsetse caught was higher in South Ari district (4.53 F/T/D) than Benatsemay district (0.97 F/T/D) during the dry season. In tsetse suppressing areas of the zone, there were six times increments (2.64/0.42) in tsetse density during dry season as compared with rainy season.

Table 16. Dry and wet season flies caught from tsetse suppressing areas of South Omo zone

Parameter	**Trapping sites**	**No of traps deployed**	**No of flies caught**	**Tsetse caught**				**F/T/D**	**Biting flies**		
				Total no	*Gp*	M	F		Sto	Tab	Hae
Dry season Tsetse suppressing sites	Kure	5	165	92	92	14	78	9.2	60	7	6
	Tembel	5	66	16	16	2	14	1.6	37	9	4
	Chelegod	5	94	28	28	4	24	2.8	46	5	15
	Goldiya	5	49	16	16	-	16	1.6	23	2	8
	Diziaman	6	49	11	11	2	9	0.91	20	8	10
	Enchete	6	35	6	6	-	6	0.5	19	3	7
	Total	32	364	169	169	22	147	**2.64**	205	34	50
Wet season Tsetse suppressing sites	Kure	6	46	9	9	1	8	0.75	24	13	10
	Tembel	5	48	9	9	-	9	0.9	32	8	3
	Chelegod	5	64	5	5	2	3	0.5	40	7	12
	Goldiya	5	18	0	0	0	0	0	18	10	-
	Diziaman	5	29	0	0	0	0	0	25	4	-
	Enchete	6	35	4	4	1	3	0.33	16	12	8
	Total	32	240	27	27	4	23	**0.42**	155	54	33

Gp= *Glossina pallidipes*, M= Male, F= Female, Sto= *Stomoxys*, Tab= *Tabanus*, Hae= *Haematopota*

In selected tsetse non-suppressing areas (Hammer and Gnangatom), a total of 32 traps (16 traps in each season) were deployed for 48 hours to caught *Glossina* and other mechanical vectors during both seasons. Apparent density of 2.03 and 0.56 F/T/D respectively in dry and wet season were recorded in the areas with identified *Glossina* species of *G. pallidipes* only. The sex ratio of tsetse non-suppression areas was 1 male to 3.33 females and 1 male to 5 females, respectively for dry and wet season. Other mechanical vectors caught in the areas were 56, 8 and 7 *Stomoxys, Tabanus* and *Haematopota*, respectively (Table 17).

Table 17. Dry and wet season flies caught from tsetse non-suppressing areas of South Omo zone

Parameter	**Trapping sites**	**No of traps deployed**	**No of flies caught**	**Tsetse caught**				**F/T/D**	**Biting flies**		
				Total no	*Gp*	M	F		Sto	Tab	Hae
Dry season Tsetse non-suppressing sites	Zegerma	4	39	17	17	4	13	2.13	15	5	2
	Kara Labuk	4	30	26	26	7	19	3.25	2	2	-
	Kuchuru	4	20	15	15	2	13	1.87	3	2	-
	Tirga	4	13	7	7	2	5	0.88	2	1	3
	Total	16	102	65	65	15	50	**2.03**	22	10	5
Wet season Tsetse non-suppressing sites	Zegerma	4	29	6	6	1	5	0.75	22	1	-
	Kara Labuk	4	32	9	9	2	7	1.13	16	2	5
	Kuchuru	4	10	3	3	-	3	0.38	4	1	2
	Tirga	4	18	0	0	-	-	0	14	4	-
	Total	16	89	18	18	3	15	**0.56**	56	8	7

Gp= *Glossina pallidipes*, M= Male, F= Female, Sto= *Stomoxys*, Tab= *Tabanus*, Hae= *Haematopota*

Among the non-suppressing districts, highest apparent density was recorded in Hammer district during both dry and wet season with apparent density of 2.68 F/T/D and 0.94 F/T/D, respectively (Table 18).It was 1.37 F/T/D and 0.18 F/T/D in Gnangatom district during dry and wet (rainy) season respectively. In dry season, the tsetse apparent density was increased by 3.63 (2.03/0.56) times that of wet season in tsetse non- suppressing area. Mechanical vectors of genus *Stomoxys*, *Tabanus* and *Haematopota* with their counted number indicated on (Table 17) were also recorded during entomological survey.

Table 18. Negative binomial regression model result showing incidence ratio (IR) of *Glossina pallidipes* in study area

Risk factor	**Category**	**IR**	**95% CI**		**P-value**
			LL	UL	
Altitude	> 1500 m.a.s.l	2.77	0.22	39.73	0.41
	1000-1500 m.a.s.l	2.85	0.33	26.80	0.32
	500-1000 m.a.s.l	1.27	0.36	5.43	0.71
	< 500 m.a.s.l	1*			
No of traps deployed	5 traps	1.15	0.25	4.21	0.83
	6 traps	NA	NA	NA	NA
	4 traps				
Seasons	Wet	0.21	0.097	0.47	0.0000*
	Dry				
Suppression status	Suppressing	0.50	0.087	2.87	0.41
	Non-suppressing				

IR = Incidence Ratio, 1* = Reference, CI = Confidence Interval, NA= Not Analyzed, m.a.s.l= meter above sea level

By taking four variable (altitude, number of traps deployed, season of vector survey and suppression status of the area), Poisson regression model was drawn to see the effect of above mentioned predictors on *Glossina* count which is dependent variable of the model. The output of Poisson regression model (Annex 5) indicated that suppression status of the area, number of traps deployed and the season of vector survey were significantly associated with *Glossina* count in the study area.

But due to lack of fit of the data on poison regression model because of over-dispersion seen on Poisson model diagnosis (Annex 5), the final model fit for this study *Glossina* count data was negative binomial regression model with the output displayed on table 18 above. By using negative binomial model fitted for the data, only one predictor variable i.e. season was the only variable significantly associated ($P < 0.05$) with *Glossina* count in the current study area. The result indicated that the incidence *G. pallidipes* during wet season was decreased by the factor of 0.21 when compared to its incidence in dry season by holding other variables constant.

4.4. Trypanocidal Drug Utilization Practice Survey Result

The findings of questionnaire survey disclosed that majority of the respondents were male and illiterate. Accordingly, 84.67% and 74.19% of the respondents were male and illiterate from tsetse suppression area respectively. Likewise, 86.7% and 88.3% were also male and illiterate from tsetse non-suppression areas, respectively.

About 63.7% of the respondents from tsetse-suppression area give priority to draft oxen in trypanosomosis treatment. But 58.3% of interviewee from non-tsetse suppression areas declared that they give priority to milking cow in trypanosomosis treatment (Figure 4). 63.70% and 81.7% of respondents respectively from tsetse suppression area and tsetse non-suppression areas obtain trypanocidal from private veterinary drug. Veterinarians and other animal health experts have very little role in treatment of animals with trypanocidal and other medication in the study area as 79.03% and 81.7% of respondents respectively from tsetse suppression area and tsetse non-suppression areas witnessed that they treat their sick animals by themselves (Figure 4).

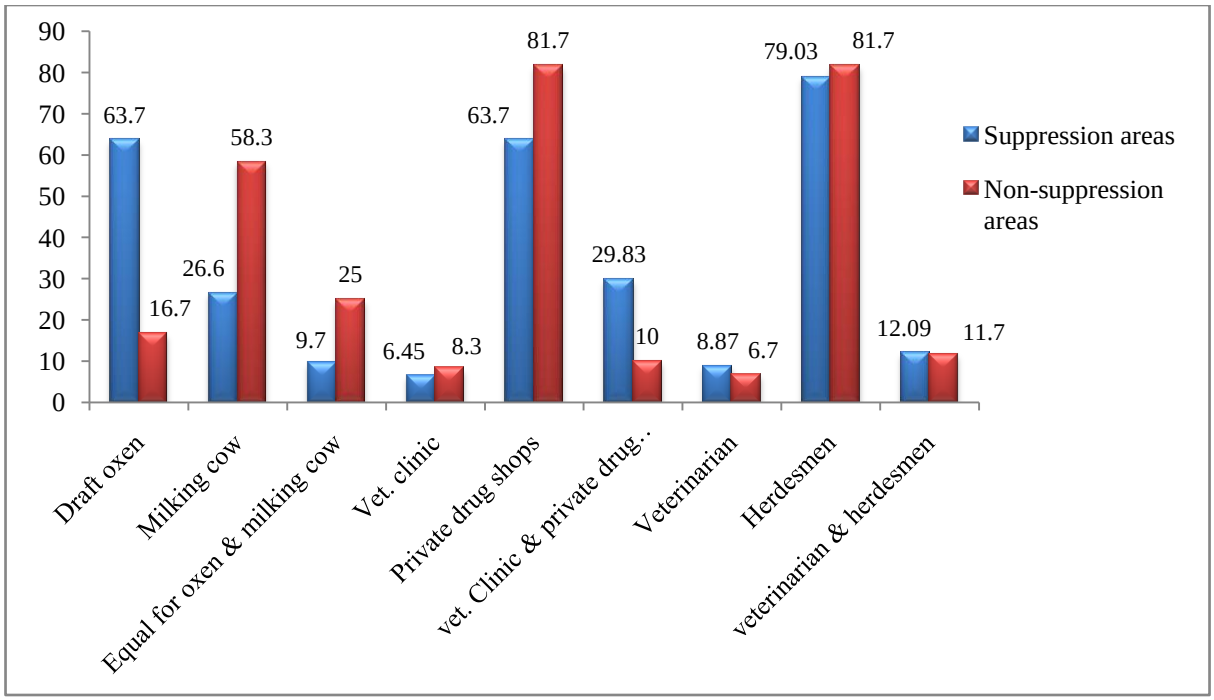


Figure 4. Respondent's priority in trypanosomosis treatment and source of trypanocidal drugs

Diminazine aceturate (DA) is the most preferred drug by herd owners of tsetse non-suppression areas as affirmed by 70% of respondents which is followed by both DA and ISM (16.7%). But in tsetse suppression area both DA and ISM (62.1%) occupy the first place in the choice of trypanocidal followed by DA (18.7%) and both DA and homidium (5.6%) (Figure 5).

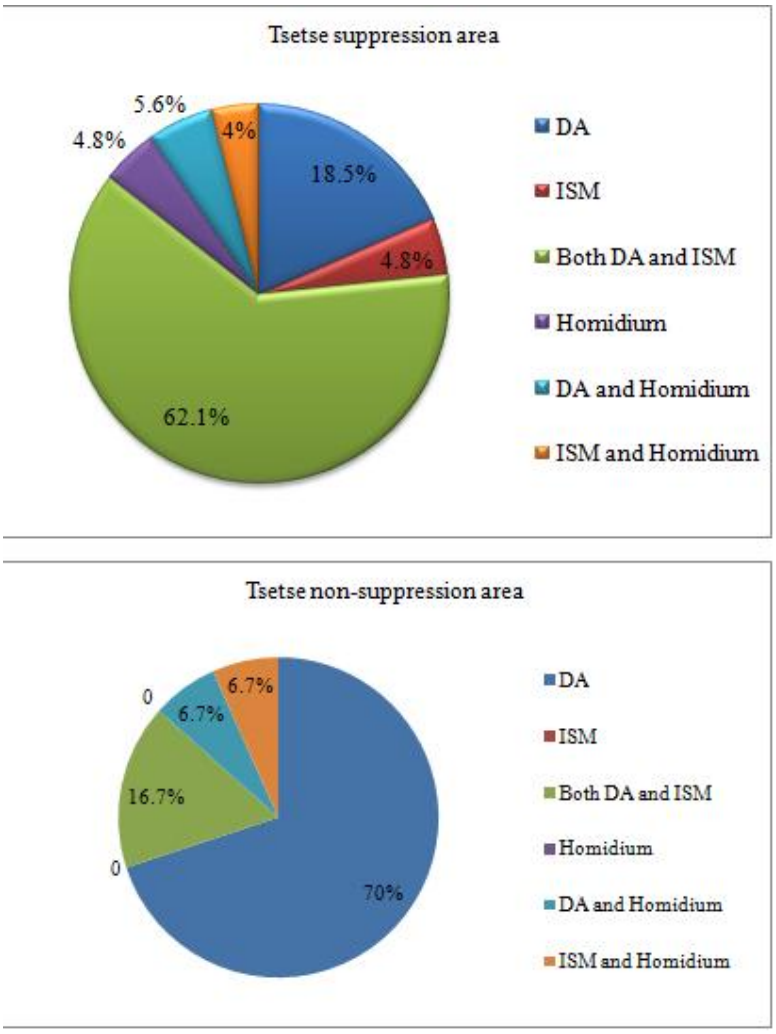


Figure 5. Trypanocidal drug preference by respondents

Respondents from both tsetse suppression area (81.5%) and non-suppression areas (73.3%) replied that their animals show frequent symptom/sign of trypanosome infection such as raising of hair, raising of hair and depression and raising of hair and drying of feaces (Table 5) during wet season as compared to dry season. With occurrence of disease symptom, the owners treat their animals with their own preferred trypanocidal. But after the treatment the animals continue showing the disease symptom which was accepted as the failure of the trypanocidal to cure the patient. This condition was witnessed by 83.1% and 86.7% of the respondents from tsetse suppression area and non-suppression areas respectively. This failureness of trypanocidal to cure the patient was most common when the origin of the drug is from private drug shops when compared with drugs from governmental veterinary clinic as the cattle owners' response indicated (Figure 6).

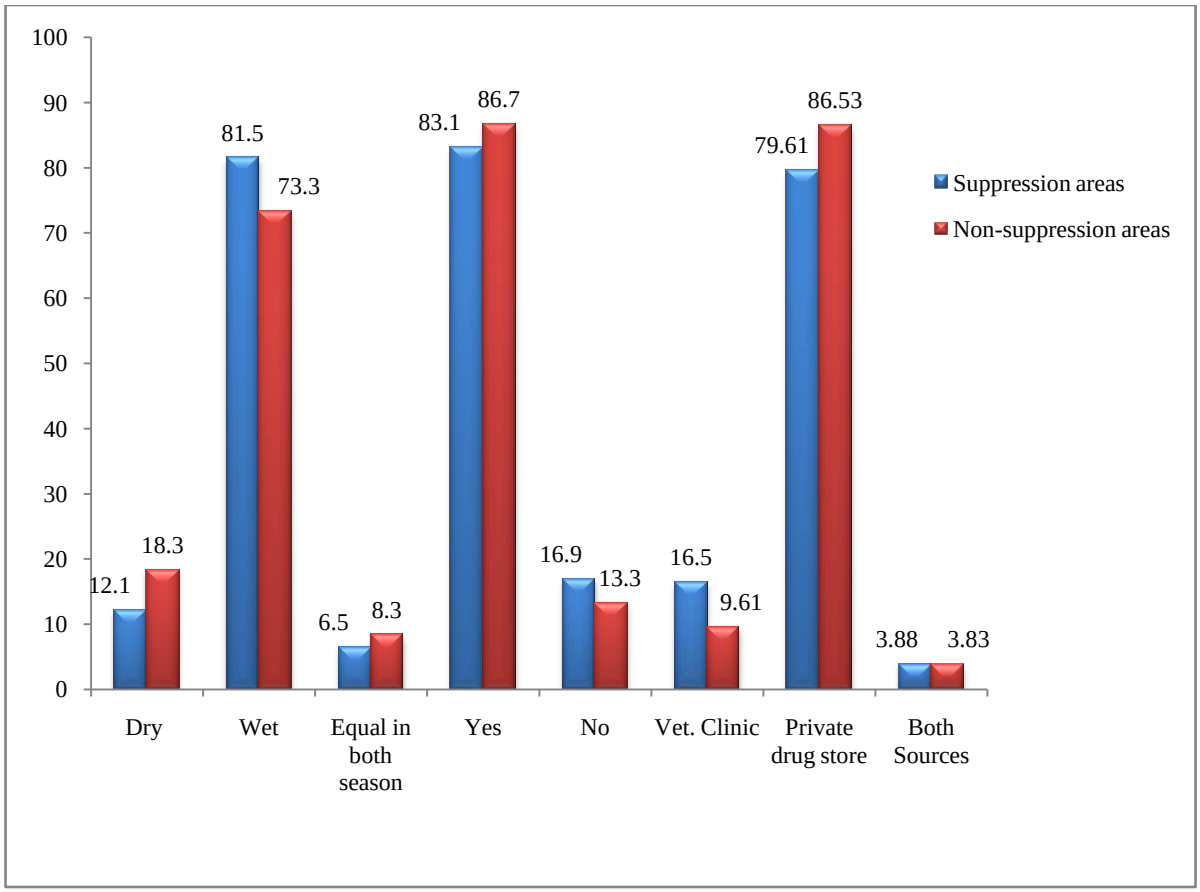


Figure 6. Season of frequent trypanosomosis occurrence, occurrence of treatment failure and source of more failing trypanocidal.

Diminazine aceturate is the most dominant trypanocidal in showing failures after treatment which was respectively declared by 54% and 70% of respondents from tsetse suppression areas and non-suppression areas districts and followed by both DA and ISM in both areas (Figure 7). The frequency of trypanocidal treatment per a year is higher in tsetse suppression area as half of the respondents replied that they treat their animals four times a year followed by three times a year (25.8%). But in tsetse non-suppression areas, trypanocidal medication of two times a year (60%) is maximal followed by three times a year (16.7%) as interviewee confirmed (Figure 7).

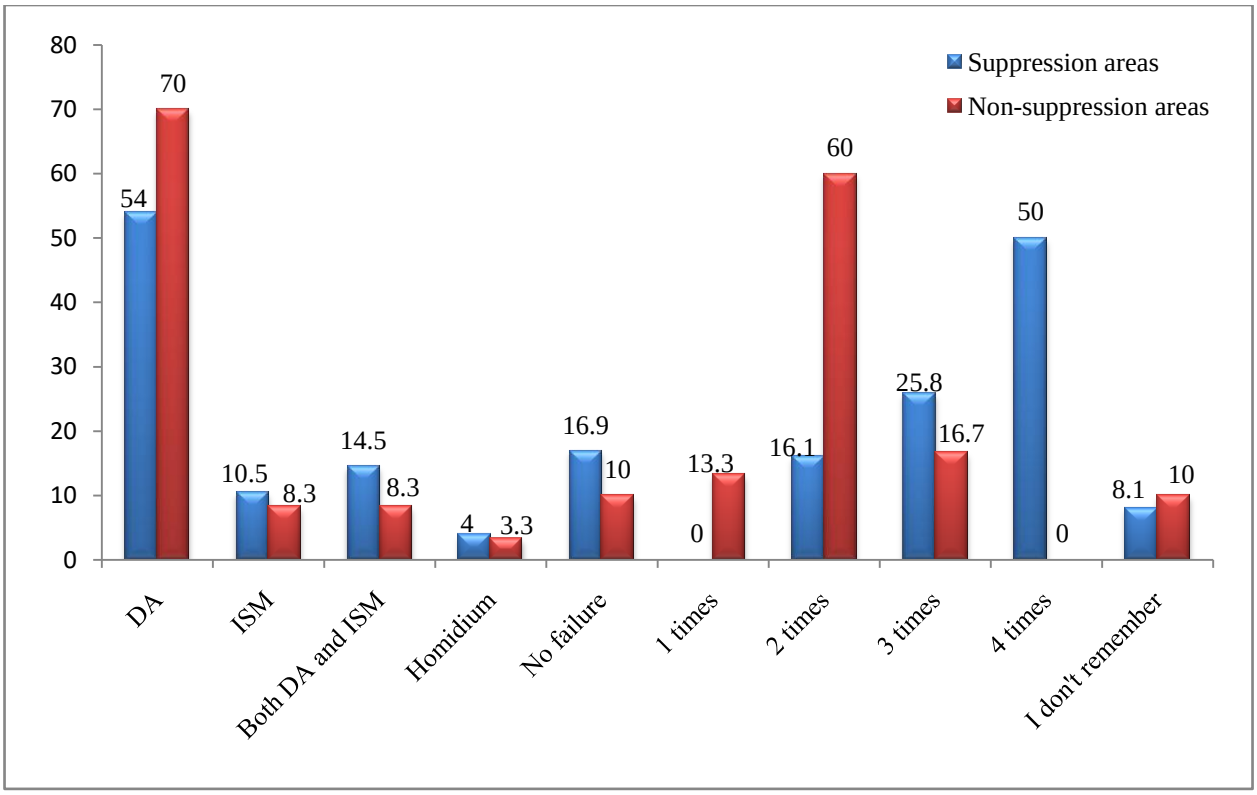


Figure 7. Failures of trypanocidal after treatment and frequency of treatment per year

Rising of hair (47.58% and 65% respectively in tsetse suppression area and non-suppression area) was the dominant symptom manifested by cattle owners as indicative of their animal sick by trypanosomosis which was followed by concurrent occurrence of rising of hair and depression in both study area. In tsetse suppression area, although the cattle owners invest more than 500 ETB to purchase trypanocidal in a year, about 39.51% ascertained that they repeatedly encountered death due to the disease in past five years. The cattle owners response indicated less average cost on trypanocidal purchase in tsetse non-suppression area which is ranged between 100-200 ETB per year as compared to >500 ETB per year in tsetse suppression area (Table 19). The respondents from tsetse non-suppression area also affirmed that they encountered less death due to trypanosomosis in past five years as witnessed by 73.3% of the respondents (Table 19).

Table 19. Interviewee response on cost for trypanocidal in a year, death occurrence due to trypanosomosis and prominent symptoms of the disease

Description of interview	Responses	Frequency		Percentage (%)	
		TSA	TNSA	TSA	TNSA
Cost for trypanocidal per one year	50-100 ETB	21	13	16.93	21.7
	100-200 ETB	20	20	16.12	33.3
	200-500 ETB	31	9	25.00	15.0
	> 500 ETB	32	9	25.80	15.0
	I don't know	20	9	16.12	15.0
	Total	**124**	**60**	**100.0**	**100.0**
Death due to Trypanosomosis in past 5 years	Yes	49	16	39.51	26.7
	No	75	44	60.48	73.3
	Total	**124**	**60**	**100.0**	**100.0**
Indicative signs/symptoms of trypanosomosis	Raising of hair and diarrhea	12	0	9.67	0.00
	Raising of hair	59	39	47.58	65.0
	Raising of hair and depression	45	13	36.29	21.7
	Raising of hair and drying of faces	8	8	6.45	13.3
	Total	**124**	**60**	**100.0**	**100.0**

Note: TSA= Tsetse Suppressing Area, TNSA= Tsetse Non- Suppressing Area, ETB= Ethiopian Birr

5. DISCUSSION

5.1. Parasitological Prevalence

The current trypanosome infection report indicated that the disease is still the main bottle neck for livestock production in the study zone. The overall prevalence of trypanosome infection in the current survey was found to be 11.05%. It is comparable with the previous reports of Ethiopia by Begna *et al. (*2011), Chanie *et al*. (2012) and Leta *et al*. (2016) who respectively revealed 14.2%, 13.8% and 13.3% from Humbo, Borena and Benshangul Gumuz Region. In contrary, reports by Abera *et al*. (2016), Ayele *et al*. (2012) and Belete (2017) respectively from Konta special district, Deramallo and Gnangatom district have shown higher infection rate in cattle. On the other hand, lower prevalence of trypanosome infection had been documented in Ethiopia by Alemayehu *et al*. (2012). Bekele *et al*. (2018), Biyazen *et al*. (2014) Gona *et al*. (2016), Leta *et al*. (2016) and Olani and Bekele (2016) respectively from Kefa zone (6.9%), Didessa district (5.47%), Dale-Wobera district (2.86%), three districts of Wolaita zone (6.6%), SNNPRS (7.8% - by meta-analysis) and Lalo-Kile district of Kallem Wollega (7.7%).

As indicated by parasitological prevalence report, the prevalence of bovine trypanosomosis in tsetse suppression areas and non-suppression areas was statistically not different within the same season although there was vector suppression activity conducting in tsetse suppression areas. This may be due to high challenge of the disease in tsetse suppression areas despite of suppression program. This can be further described in a way that either vector suppression activities were not sustainable in tsetse suppression areas or the vector or trypanosomosis challenge in tsetse non-suppression areas was not that much high so that the disease prevalence was found to be comparable to tsetse suppression areas. The current report also indicated that in both study sites, the disease prevalence was statistically significant among the seasons (dry versus wet) meaning that there was high prevalence report in dry season than wet season in both tsetse suppression areas and non-suppression areas. This implies that change in season was the main driving factor in alteration in incidence of trypanosomosis in both study sites.

In general higher and significant prevalence of trypanosomosis in both study site during dry season as compared to wet season was accredited to the fact that during dry season there was increased stress on animals due to lack of quality and quantity feed, more interaction of the host and vector around the park areas in where there was a number of reservoir wild animals for the disease causing parasite, animal become exhausted due to long distance movement in searching for feed and water, diminution of immunity due to other disease challenge which in turn open an easy route to trypanosome infection. Similar report was disclosed by Zekarias *et al*. (2014) who reported higher trypanosome infection prevalence in dry season (5.5%) than the wet season (3.5) along with increase in vector density in and around intensive suppression areas of STEP project site. However, reports of Dagnachew (2004) and (Tilahun *et al*. (2012) have shown a discrepancy in that they reported higher trypanosomosis prevalence in late rainy season than dry season respectively from Abbay Basin areas and South West Oromia region.

The dominant *T. congolense* followed by *T. vivax* report of the present study sites is in agreement with the earlier reports in Ethiopia by (Chanie *et al*. (2012), Tekle and Mekonen (2013) and (Biyazen *et al*. (2014), who reported that *T. congolense* as the prevailing species followed by *T. vivax.* The species proportion of the current study was slightly higher than the above authors report. An increased proportion of infection with *T. congolense* in the study area may be due to the major cyclical vectors of Savannah tsetse flies, *Glossina pallidipes* which are effective transmitters of *T. congolense* than *T. vivax* since the study area is located at tsetse belt of Ethiopia. Another reason also may be due to high number seroderms of *T. congolense* as compared to *T. vivax* and the development of better immune response to *T. vivax* infected animal. Different experimental drug resistant reports from various angles of Ethiopia (table 3) also indicated *T. congolense* as the leading species in developing resistant to currently available trypanocidal so that this may also be true in the case of current study areas with such trypanocidal drug treatment with unskilled herdsmen as indicated by respondents of this study sites. Disagreeing study results by Sinshaw (2004) from three Districts of Amhara region reported *T. vivax* as the dominant species causing the disease in the areas. Furthermore, Nimpaye *et al.* (2011) from Cameroon also added *T. vivax* as the major species of trypanosomosis by using PCR diagnostic tool.

There was no mixed infestation report in the area. This may be due the low sensitivity of the test (buffy coat method) in identifying species-specific prevalence so that animals with mixed infection

which developed chronic stage of the disease might be missed by the test. However, surprisingly Cox *et al*. (2010) have not found mixed infection of trypanosome species by using Whatman FTA matrix card test which has better sensitivity than buffy coat method although underestimation of the prevalence is also common in this method.

Overall dry season parasitological prevalence revealed that two risk factors (sex and grazing system) were significantly associated with bovine trypanosomosis. Higher and significant prevalence of the disease in female than male may be due to higher stress on female due milking and lactating of their young in addition to other common stressful condition of the dry season. In addition to this more care was given to male animals especially to draft oxen for their contribution in agricultural land preparation while the female animals were allowed to graze on communal range land very close to the wild life conservation area where the chance to be infected was very high.

The higher number of female animals checked for parasitological prevalence during the current study may also favor for higher prevalence report of the disease in female than male. Similarly, Belete (2017) supports the current finding who reported significantly higher infection on female (29.3%) than male (18.5%) from Gnangatom district of South Omo zone. Other authors; Ayele *et al*. (2012) and Begna *et al*. (2011) also reported higher disease prevalence on female than male but their report didn't declare significant variation. On the other hand, Dagnachew (2004) and Tilahun *et al.* (2012) reported equal infection of bovine trypanosomosis in male and female from Abbay Basin areas and South West Oromia region respectively. Significantly higher prevalence of trypanosomosis in tethered animal may be due to closeness of most of agricultural lands to the wild conservation area (tsetse habitat during dry season) where the animals (oxen) were tethered around agricultural land after traction this in turn expose them easily to the carrier vector from the park. The animal management system (no shelter at all) also increases the tethered animal's chance of infection by the disease in such high risk areas.

The overall wet season's equal and insignificant chance of infection across the whole studied risk factors may indicate that all animals across the study sites had equal chance of getting the disease regardless of sex, age, BCS, grazing system and suppression status of the area because there was almost similar distribution of vector in all areas during wet season due to the creation of suitable environment everywhere. Hence, all hosts have equal chance to be fed by the vector and became

infected. Decreased prevalence during this period may be due to decrease in stress (available water in residence areas, rehabilitation of pasture lands, withdrawal of the animals from the park areas, avoidance of long distance movement in searching for feed and water). The owner's habit of treating their animals at the start of rainy season by assuming the high occurrence of the disease in wet season which is associated with increased fly population was also another factor in decreasing the disease prevalence in wet season.

According to simple and multiple logistic regression analysis of tsetse suppression areas, there was significant difference in disease prevalence among sex groups, BCS, grazing system and season of the study period. But the strength of association between the analyzed category of risk factors (sex groups, BCS, grazing system and season) was different when the variables act as a single risk factor and in combined form. In general the statistically significant and higher prevalence of the disease in dry season than wet season (for season), in female animal than male animal (for sex) and in tethered animal than communal grazers (for grazing system) was discussed on above section. In the case of body condition score, the disease prevalence was higher in poor body conditioned animal than medium and good. Likewise, Gona *et al*. (2016), Belete (2017) and Ayele *et al*. (2012) also reported similar findings of higher trypanosomosis prevalence on poor animal than medium and good body conditioned animal. Poor body condition status of an infected animal was may be due to high immune-suppression nature of the disease so that the infected animal can be easily affected by concurrent infections and then become emaciated. It was also known that one of the clinical manifestations of trypanosomosis by itself was emaciation of the diseased animal. In addition to this infected animals were reluctant to feeding and water intake therefore their body condition status became lowered.

In the case of tsetse non-suppression areas, only sex of the study animal and season had significant effect on the prevalence of the disease with higher prevalence on female than male and on dry season than wet season by using both simple and multiple logistic regression analysis with similar strength of association in both analysis (OR = 0.51 in simple logistic regression, OR= 0.48 in multiple logistic regression, for sex and OR= 0.52 in simple logistic regression, OR= 0.41 in multiple logistic regression for season). The above discussion (about sex and seasonal difference in trypanosomosis prevalence) holds true for this also.

In both tsetse suppression areas and non-suppression areas there was no significant association between the occurrences of trypanosomosis and selected study districts. This may be due to similarity in conditions favoring for occurrence of the disease among the selected districts. For instance, in tsetse suppressing districts, the animals from both districts share the same grazing lands during dry and wet season which is around the wild life conservation areas thus their probability to be infected was the same in both districts. Age group was another risk factor without significant declaration and this may attributable to non-segregation of age groups by the pastoralists and agro-pastoralists of current study area because all age groups including one week young calf were allowed to move with their dam unless they were seriously weakened; which allow all age group equal chance to be infected. Findings of Dagnachew (2004), Ayele *et al*. (2012 and Tilahun *et al*. (2012) were consistent with the current finding. However, Alemayehu *et al*. (2012), Gona *et al*. (2016) and Belete (2017) reported significant difference in the prevalence of the disease among different age groups i.e. there was lower disease prevalence in young age groups than the older animals. There was also known disagreeing evidence in which suckling calves are not to suffer from serious attacks of trypanosomosis, possibly because of the influence of maternal antibodies in their systems Dwinger *et al*. (1992). Furthermore, the evidence of tsetse preference to feed on larger animal than the smaller one so that higher prevalence on older than the young is also inconsistent with the current report Vale and Torr (2005).

5.2. Hematological Findings

Packed cell volume (PCV) is one of the most fundamental quantitative measurements to estimate anaemic status of the tested animal. Since anaemia is one of the striking pathological manifestations of trypanosomosis which happen due to massive destruction of RBC by the trypanosome, by measuring PCV, the current study animals were classified as anaemic and non anaemic by setting cut-off value of PCV measurement for bovine species. Overall mean PCV record of current study indicated that there was statistically significant difference in mean PCV of parasitaemic (23.57%) and aparasitaemic (27.80%) animals. This might be due to erythrocyte destructive nature of trypanosome in infected animals. The overall mean PCV was also higher in animals diagnosed in wet season (28.45%) than those diagnosed in dry season (26.22%) regardless of infection status which may be attributable to more conducive condition of wet season in the areas which decreased

stressful condition which otherwise results in decrease in PCV reading. For example dehydration that occur during dry season due to lack of enough drinking water or fresh feed may result in low PCV measurement but during wet season the animal can get enough amount of drinking water as well as fresh grasses in near distance so that to be dehydrated and becoming anaemic is very low or null

Mean PCV of animals infected with *T. congolense* (23.59%) and those infected with *T. vivax* (23.26%) have statistically insignificant in current study. But it was known that T. congolense is the most virulent species in Sub-Saharan African countries causing serious pathological consequences, of which anaemia (low PCV) is the primary one. Similarly, Motloang *et al*. (2014) also added that the prevalence of highly virulent *T. congolense* Savannah type was very high in wildlife-livestock interface with resultant pathological sequel. However, Gona *et al.* (2016) disagree with present study report who found significant difference in PCV measurement between *T. congolense* infected and *T. vivax* infected animals in Wolaita zone by explaining more RBC invasion nature of *T. congolense* when compared with *T. vivax* in which the later can invade other tissues such as lymph node, eye and heart.

Comparison of anaemia with parasitaemia indicated that both in tsetse suppression areas and tsetse non-suppression areas, anaemic animals (PCV < 24) were more parasitaemic than non-anaemic animals (PCV > 24). The current study revealed that 26.3% of anaemic animals were infected (parasitaemic) as compared to 7.10% infected from non-anaemic animals from tsetse suppression areas. The same analysis for tsetse non-suppression areas also indicated that 25% of anaemic animals were parasitaemic and only 7.01% of those non-anaemic were infected by the parasite. In both cases there was statistically significant difference in infection rate of anaemic and non-anaemic animals. This study result indicated that most of anaemic animals were trypanosome positive and their lowered PCV was due to the infection although there are a number of factors that can cause anaemia. However, it is impossible to conclude all anaemic animals as parasitaemic because less sensitivity of PCV measurement used may lower the PCV scale and categorize the animal as anaemic but the animal was not parasitaemic. In the same manner, all parasitaemic animals may not be anaemic because there are hosts which are positive for the disease but able to control the pathological consequences such as anaemia, maintain their weight gain and perform well in such condition; for example trypanotolerant breeds of Africa (Mekuriaw and Kebede, 2015).

5.3. Entomological Survey

Entomological survey report of current study indicated that *G. pallidipes* was the solely cyclical vector species in the areas. Previous studies by Zekarias *et al*. (2014) from in and around intensive suppression areas of STEP project sites and Ayele *et al*. (2012) from Deramallo district also reported this species as the only cyclical vector. This may be due to the suitability of current study area for this species which in turn associated with habitat suitability range, ecological and climatic preferences. Likewise, Leta *et al*. (2015) report on spatial analysis of the distribution of tsetse flies in Ethiopia strength this assumption by elaborating that *G. pallidipes* had highly suitable areas in SNNPR with the required habitat, ecological and climatic preference. However, a number of reports from Ethiopia by Abebe and Wolde (2010); Tilahun *et al*. (2012); Desta *et al*. (2013) and Duguma *et al*. (2015) as well as from other African countries by Malele *et al*. (2007) from Tanzania and by Pagabeleguem *et al*. (2012) from Burkina-Faso have shown that the overlapping of two to three tsetse species was common.

Dry season entomological reports of 2.64 F/T/D and 2.03F/T/D, respectively for tsetse suppression areas and tsetse non-suppression areas of current study were slightly higher than the report of Zekarias *et al*., (2014) in intensive suppression areas of STEP project during dry season who reported apparent density of 1.6 F/T/D. Additionally low apparent density of *G. pallidipes* species up to 1.35 F/T/D were reported from the low land category of southern Rift valley of tsetse eradication and control program area by Jembere (2004) and from selected three districts of Wolaita zone by Gona *et al*. (2016). However, Zekarias *et al*. (2014) reported very high apparent density of *G. pallidipes* (280.54 F/T/D) in Nech-Sar national park around Arbaminch. Ayele *et al*. (2012) from Deramallo (19.14 F/T/D), Duguma *et al.* (2015) from South Western Ethiopia (7.45 F/T/D) also indicated higher cyclical vector density although the later reported apparent density of *G. pallidipes* with other Glossina species.

Higher apparent density of Glossina vector during dry season when compared with wet/rainy season in both current study sites may be associated with accumulation of flies around river and shady areas (especially around Mago national park) following favorable temperature and humidity created there during dry season. However, during wet season almost similar climatic condition was noticed everywhere and flies were sparsely dispersed here and there in the area. Yeshitila *et al*. (2006)

report from Sokoru district of Jimma zone was disagree with this justification who revealed relatively higher density of tsetse in late rainy season and null apparent density during dry season in Abelty area (Sokoru district). Similarly, Tilahun *et al*. (2012) also reported higher apparent density of Glossina species in late rainy season than dry season from Southwest Oromia. Zero apparent density report of Zekarias *et al*. (2014) during wet season in intensive suppression areas of STEP project site deviate from the wet season report of tsetse suppression areas and tsetse non-suppression areas of current study sites.

The high proportion of female Glossina species is most probably attributed to the fact that they live longer (mean female fly life span being eight weeks, but only four weeks in males); hence more females could be caught as described by Leak (1999) who also explained that females would represent 70 % to 80 % in unbiased sampling. Lower sex ratio compared to this study was disclosed by (Duguma *et al*., 2015) who reported 1 male to 1.37 females for *G. m. submorsitans*, 1 male to 1.32 females for *G. pallidipes* and 1 male to 1.22 females for *G. f. fuscipes*. Experimental study conducted by Desa *et al*. (2018) discovered 1 male to 4 females (1:4) was the best due to higher fecundity combined with lower mortality than the other ratios in artificially rearing colonies of *G. F. fuscipes* and *G. pallidipes*.

Season was the only driving factor affecting tsetse multiplication and their abundance in current study areas as indicated on tsetse count data analysis. Other factors such as altitudinal range of survey areas, numbers of traps deployed to catch the fly and suppression status of the area were not significantly associated with Glossina count. This may be partially due to the suitability of all available habitats in current study areas for *G. pallidipes* if the season is favorable for the fly's multiplication regardless of altitudinal range and suppression status of the areas. It may also partially due to too early analysis of suppression effect in the area. This implication was also explained by Mr. Aschenaki (personal communication, STEP project Arbaminch center director) who said that the suppression activity in South Omo zone is too young and the effect of suppression will be magnified in the future. Non-sustainability of vector suppression activities due to different constraints and poor community participation in suppression works conducting in the area may also further make its effect negligible and non-significant. Multivariable analysis made by Duguma *et al*. (2015) explained altitude was the only variable retained to affect tsetse apparent density in his study areas which was disagree with this study report.

Rapid reinvasion ability of Glossina species may also affect the suppression activity. In the case of South Omo zone, there are wild life conservation areas (especially Mago national park) which borders most of the areas where suppression activities undergoing. This park is suspected to be the major source of tsetse to re-invade the suppression areas since there were no natural or artificial barriers to obstacle the fly's movement. Similar thought was explained by Zekarias *et al*. (2014) who said that the presence of densely populated tsetse flies in the Nech-Sar National Park remains a great potential risk for reinvasion of cleared areas at the vicinity of the Park. Since the national park policy by itself does not allow applying suppression activity inside the park and it is also impossible to create artificial barrier (by traps and targets) around the whole park area, the future success of the suppression campaign will also be questionable. Likewise, Percoma *et al*. (2018) explained the importance of artificial barrier around tsetse suppression zones in Burkina-Faso and how the local community participation helped in integrated control campaign landed in the country. In the same way, Alemu *et al*. (2007), Vreysen *et al*. (2013), Duguma *et al*. (2015), Shaw *et al*. (2017) and Tesfaye *et al*. (2017) also described the lack of effective natural barrier and non-sustained control efforts allowed easily reinvasion of cleared zones with tsetse despite decades of implemented control attempts in Ghibe, Didessa and Southern Rift valley areas.

5.4. Trypanocidal Drug Utilization Practice

According to the questionnaire survey result of current study, trypanosomosis (locally known as "kusupho", meaning the disease caused by flies) is one of the major bottle-neck for livestock production in both tsetse-suppressing and tsetse non-suppressing areas of South Omo Zone. All the respondents (100%) from both study areas witnessed that their animals were encountered the disease for many decades. Similar reports about trypanosomosis was revealed from tsetse infested and non-tsetse infested areas of Northwest Ethiopia by Dagnachew *et al*. (2017), Abbay basin areas of Northwest Ethiopia by Dagnachew (2004), Tselemti District of Tigray region by W/yohannes *et al*. (2010), Western Ethiopia by Tewelde (2001) and also from South Western Ethiopia by Tekle *et al*. (2018).

From diseased animal's treatment point of view, the respondents had different approach in giving priority to their animals. Animal owners from tsetse suppression areas give priority to draft oxen in

treatment of trypanosomosis and reasoned that oxen is the most valuable class of animal in agricultural land preparations since they are practicing mixed farming system and the market fetch value of oxen is higher than other class of animals even after long term serving on traction. Seyoum *et al*. (2013) asserted similar condition of bovine trypanosomosis severity and more investment of owners to treat draft oxen than other class of animals in Baro-Akobo and Gojeb river basins in SNNPR, Southwestern Ethiopia. However, Van den Bossche *et al*. (2000) reported equal priority giving by the owners in trypanosomosis management to both draft oxen and milking cows which were taken as the most valuable animals in Eastern Zambia. On the contrary to tsetse suppression areas, respondents from tsetse non-suppression areas gave priority to milking cow in treatment of trypanosomosis and stated that milk is their primary base of livelihood since most of respondents from tsetse non-suppression areas were pastoralists who rear livestock for their livelihood.

Private drug shops were the major source of trypanocidal for the residents of South Omo Zone (for both tsetse suppression areas and tsetse non-suppression areas) followed by both sources (government vet clinic and private drug shop) and government veterinary clinic. Similarly, Dagnachew *et al*. (2017) report from tsetse infested areas of Northwest Ethiopia (Jabitehenan) was consistent with the current report about private drug shops as 48% of his interviewee responded this route as their primary drug source. But the same author was also reported government vet clinic as primary drug source for the cattle owners living in tsetse free areas of Bahir-Dar Zuria District, Northwest Ethiopia. Due to strict control made on unauthorized drug sellers (personal communication with each study district's livestock and fishery department head who is the member of unauthorized drug control committee) , the vet drug from this route is negligible in the study area. Community awareness creation on outcome of unauthorized drug sources and strong participation of authorized drug sellers in the control action playing the key role in control of unauthorized veterinary drug selling.

However, Tekle *et al*. (2018) reported unauthorized drug sources (56%) as the primary route followed by government vet clinic (25%) and private drug sellers (19%) for residents of Guraghe zone of SNNPRS and Jimma zone of Oromia regional state. Moreover, Manyazewal *et al*. (2014) stated that unauthorized drug sellers (smugglers) as the major source of vet drugs in Chewaka settlement station and Bikiltu Didessa peasant associations of South West Ethiopia. Dagnachew *et al.* (2017) also disclosed 34% and 10% of unauthorized drug sources contribution to cattle owners

of Jabitehenan (tsetse infested district) and Bahir-Dar Zuria District (tsetse free area) respectively in Northwest Ethiopia.

Assessment on person responsible for treatment of sick animals with trypanocidal drugs indicated that 79.03% and 81.7% of respondent's; respectively from tsetse suppression areas and tsetse non-suppression areas explained that they themselves were responsible for treatment of their sick animal. Only 8.87% and 6.7% of the respondents respectively from tsetse suppression areas and tsetse non-suppression areas send their sick animals to veterinary clinic or animal health post to be treated with veterinarian or other trained personnel. The cattle owners reasoned out that they were far away from animal health post and vet clinics so that the only chance to save their sick animal is treating by themselves. The respondents also explained that one animal health expert or community animal health worker (CAHW) was assigned for clusters of PAs (one cluster contain 3-4 PAs according to data obtained from SOZLFD (2018)) so that waiting for his/her service is very difficult to save their diseased animal. Survey reports from tsetse infested areas of North West Ethiopia by Tsegaye (2014), from South West Ethiopia by Tekle *et al*. (2018) and from Goro, Ameya and Kota districts of Southwest Oromia Tilahun *et al.* (2012) declared similar response on person responsible for trypanocidal treatment. On the other hand, Abebe (2018) report from Assosa and Bambasi districts of Assosa zone and Seyoum *et al*. (2013) from selected districts of Baro-Akobo and Gojeb River basins indicated that majority of the respondents send their ill animals to vet clinic or animal health posts for treatment.

Diminazine aceturate (DA), locally known as "kacho" was the major drug of choice for treatment of animals with trypanosomosis symptom in tsetse non-suppression areas by reasoning out that DA was cheaper than ISM ("singula") in the private drug stores. However, it was both DA and ISM in tsetse suppression areas according to current survey report. DA preference by cattle owners of tsetse non-suppression areas was agreed with reports of Van den Bossche *et al*. (2000) and Tewelde (2001) who stated that most farmers prefer to use DA than ISM. Assessment report of Tsegaye (2014) supported tsetse suppression areas drug preference of current study who stated that 68% of the respondents from tsetse infested areas of North West Ethiopia prefer ISM over DA. The same author also stated equal preference was given to both DA (40%) and ISM (40%) in Bahir-Dar Zuria district of North Western Ethiopia.

Trypanocidal treatment frequency of 4 times per year (50%) and two times per year (60%) was reported respectively from tsetse suppression areas and tsetse non-suppression areas of current study area. Comparable treatment frequencies were reported from tsetse free areas of North West Ethiopia (1-3 times per year) by Tsegaye (2014) and Metekel district of Benshangul Gumuz region (ever four months = three times per year) by Afewerk (1998). On the contrary, very higher treatment frequency reports were disclosed by Dagnachew (2004) in the lowland (3-4 times per month) and midland (1-2 times per month) areas of Abbay basin, Tewelde (2001) in Western Ethiopia (2.5-3.5 times per month), Tsegaye (2014) in tsetse infested areas of North West Ethiopia (> 10 times per year) and Seyoum *et al*. (2013) in Baro-Akobo and Gojeb River basins (5.7 times).

Higher treatment frequency in tsetse suppression areas compared to tsetse non-suppression areas in current study may indicate higher challenge of the disease although there were vector suppression activities in the tsetse suppression areas. It may also indicate non-sustainability and less participation of community on vector suppression activities. Close interaction of wild life conservation areas with tsetse suppression areas study districts may increase the chance of their animals to be infected by the disease since wild animals in the conserved areas may act as source of infection. This in turn increases the frequency of treatment in the area as compared to tsetse non-suppression areas. Easy access to trypanocidal drug sources may also play its part in increased frequency of treatment in tsetse suppression areas since most of the owners in tsetse suppression areas were close to main city (Jinka) where all sources (vet clinic and private drug vendor shops) of trypanocidal were concentrated.

High trypanosomosis occurrence during wet season according to respondents of current study was similar with the report of Abebe (2018). The respondents of this study associated high wet season occurrence of the disease with increased fly number in the area following rain since they associate the disease with fly population (“kusupho” in local language meaning disease caused by flies). They also explained that there was a tree called “gadaq” in local language whose flower can highly attract the flies (including tsetse) during wet season so that their animals were easily infected by the disease caused by flies (“kusupho”). On the contrary, equal occurrence of trypanosomosis in both dry and wet seasons was reported by Tewelde (2001) from Western Ethiopia, by Afewerk (1998) from Metekel district and by Ngare and Mwendia (2000) from Narok district of Kenya.

Treatment failure was witnessed by 83.1% and 86.7% of the interviewee from tsetse suppression areas and tsetse non-suppression areas respectively. Similarly, Tekle *et al*. (2018) survey report indicated that all of his respondents perceived the occurrence of treatment failure after medication. The current survey result also indicated that drugs from authorized private drug stores show more failure than those drugs from government vet clinic. However, reports of Tekle *et al.* (2018) pointed that treatments were more likely to be successful when the drugs are sourced from both government veterinary clinics and authorized private sources.

Diminazine aceturate (DA) is the most horrible trypanocidal drug that exhibit treatment failure in current study areas of South Omo Zone as approved by 54% and 70% participant respondent, respectively from tsetse suppression areas and tsetse non-suppression areas. Different reports from various angles of Ethiopia as well as other Sub-Saharan African countries revealed this situation through assessment as well as via experimental studies by using laboratory animals. Experimentally Moti *et al*. (2012) in Gibe valley, Chaka and Abebe (2003) in Gibe valley, Bedelle and Soddo, Mekonnen *et al.* (2018) in Tigray and Afar and Afewerk *et al*. (2000) in Metekel reported single as well as multiple trypanocidal drug treatment failure (resistant development). Low market price of DA when compared with ISM, its high preference by cattle owners of the study area (especially of tsetse non-suppression areas) and lack of dosage knowledge as well as administration by unskilled personnel may predispose this drug as the most prone for failure development.

Higher treatment cost per year in tsetse suppression areas as compared to tsetse non-suppression areas in this study may indicate high challenge of the disease to the residents of the area although there were vector control activities undertaken in the area. It may also indicate better awareness of cattle owners in prophylactic treatment of their non-sick animals in tsetse suppression areas which may increase annual cost for trypanocidal. Similar assumption was reported by Uilenberg (1997) who said that the number of treatment over a year reflects the magnitude of trypanosomosis challenge in an area. Mean annual treatment cost report for trypanosomosis from Gimbo and Guraferda of Baro-Akobo and Gojeb river basins in SNNPRS by Seyoum *et al*. (2013) was almost similar with the current treatment coast report from tsetse non-suppression areas but it is lower than annual treatment cost of respondents of tsetse suppression areas.

6. CONCLUSION AND RECOMMENDATIONS

The current study on bovine trypanosomosis in South Omo zone indicated that the disease was caused by the two common species of trypanosomes; *T. congolense* and *T. vivax* with alarming prevalence level in both tsetse suppression and tsetse non-suppression areas of the zone. Moreover, this study indicated that trypanosomosis is bottle neck for livestock production and productivity in the area. Despite tsetse suppression activities conducted in selected areas of the zone, its effect on cyclical vector density as well as on trypanosomosis prevalence was not significantly different from tsetse non-suppression areas. Non-sustainability of vector suppression activities, less community participation in suppression work and high risk of vector reinvasion from closely available wildlife conservation areas were the major obstacle for successful vector suppression crusade in the area although it was too early for complete evaluation of the campaign. High trypanocidal drug dependent control of the trypanosomosis by agro-pastoralists/pastoralists in conjunction with such frequent treatment per year by unskilled herdsmen may aggravate the development of trypanocidal drug resistance in the area even though its current status was also not disclosed. Therefore, based on the above remarks the following recommendations were forwarded:

- Integrated approach (vector control and chemotherapy) should be undertaken as control strategies of trypanosomosis in the area.
- Sustainable and participatory vector control activities should be employed in the area to tackle the vector.
- Awareness should be created among herdsmen about the risk of indiscriminate usage of trypanocidal drugs and to allow them to consult veterinary professionals to treat their sick animals.
- Drug resistance tests should be conducted in the area to know the status of currently available trypanocidal drugs in healing the disease.
- Smooth communication and collaboration should be made between the wildlife conservation area administration to conduct the vector suppression activities in and around the park to curve the vector reinvasion to suppression areas.

7. REFERENCES

Abd-Alla, A.M.M., Bergoin, M., Parker, A.G., Maniania, N.K., Vlak, J.M., Bourtzis, K., Boucias, D.G. and Aksoy, S. (2013): Improving Sterile Insect Technique (SIT) for tsetse flies through research on their symbionts and pathogens. *J Invertebr Pathol*. **112**: 1–20.

Abebe, B.H. (2018). Epidemiology of bovine Trypanosomosis in Selected Districts of Benshanhul Gumuz Region with Emphasis on Trypanosoma Congelense Trypanocidal Drug Resistance and Alternative Therapy in Mice. MVSc thesis submitted to AAU CVMA, Bishoftu, Ethiopia.

Abebe, R. and Wolde, A. (2010). A cross-sectional study of trypanosomosis and its vectors in donkeys and mules in Northwest Ethiopia. *Parasitol. Res*. **106**: 911–916.

Abera, A., Sibhat, B. and Tonamo, A. (2016). Full Length Research Paper A study on prevalence of bovine trypanosomosis in selected areas of Konta Special District. *African Journal of Agricultural Research.* **11**: 500–506.

Ademe, M. (1998). Field study on drug resistance trypanosome population of Bovine in Kindokoyisha, Southern Ethiopia, DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia. 35.

Afewerk, Y. (1998). Field investigation on the appearance of drug resistant population of trypanosomes in Metekel district, northwest Ethiopia. Addis Ababa University and Freiuniverstat Berlin, Faculty of Vetrinary Medicine, Ethiopia, MSc Thesis.

Afewerk, Y., Clausen, P.-H., Abebe, G., Tilahun, G. and Mehlitz, D. (2000): Multiple-drug resistant Trypanosoma congolense populations in village cattle of Metekel district, north-west Ethiopia. *Acta Trop.* **76**: 231–238.

Akoda, K., Van den Bossche, P., Marcotty, T., Kubi, C., Coosemans, M., De Deken, R. and Van Den Abbeele, J. (2009): Nutritional stress affects the tsetse fly's immune gene expression. *Med. Vet. Entomol*. ***23***: 195–201.

Aksoy, S. and Rio, R.V.M. (2005): Interactions among multiple genomes: Tsetse, its symbionts and trypanosomes. *Insect Biochem. Mol. Biol.* **35**: 691–698.

Alemayehu, B., Bogale, B., Fentahun, T. and Chanie, M. (2012): Bovine trypanosomosis : A threat to cattle production in Chena district , Southwest Ethiopia. *Open Journal of Animal Sciences.* **2**: 287–291.

Alemu, T., Kapitano, B., Mekonen, S., Aboset, G., Kiflom, M., Bancha, B., Woldeyes, G., Bekele, K. and Feldmann, U. (2007): Area- wide control of tsetse and trypanosomiasis; Ethiopian experience in the southern rift valley, in: Vreysen, M.J.., Hendrichs, A.S., J., R. and (Eds.), Springer, Dordrecht, the Netherlands, pp. 325–335.

Allen, C.L., Goulding, D. and Field, M.C. (2003): Clathrin-mediated endocytosis is essential in Trypanosoma brucei. *EMBO J.* **22**: 4991–5002.

Alsford, S., Eckert, S., Baker, N., Glover, L., Sanchez-Flores, A., Leung, K.F., Turner, D.J., Field, M.C., Berriman, M. and Horn, D. (2012): High-throughput decoding of antitrypanosomal drug efficacy and resistance. *Nature.* **482**: 232.

Alsford, S.A.M., Kelly, J.M., Baker, N. and Horn, D. (2013): Genetic dissection of drug resistance in trypanosomes. *parasitology*. **140** (12): 1478–1491.

Auty, H., Mundy, A., Fyumagwa, R.D., Picozzi, K., Welburn, S. and Hoare, R. (2008): Health management of horses under high challenge from trypanosomes: a case study from Serengeti, Tanzania. *Vet. Parasitol*. **154**: 233–241.

Ayele, T., Ephrem, D., Elias, K., Tamiru, B., Gizaw, D., Mebrahtu, G. and Mebrat, E. (2012): Prevalence of Bovine Trypanosomosis and its Vector Density in Daramallo District , South Western Ethiopia. *J. Vet. Adv.* **2**: 266–272.

Babokhov, P., Sanyaolu, A.O., Oyibo, W.A., Fagbenro-beyioku, A.F. and Iriemenam, N.C. (2013): A current analysis of chemotherapy strategies for the treatment of human African trypanosomiasis. *Pathogens and Global Health*. **107** (5): 242-252

Baral, T.N. (2010). Immunobiology of African Trypanosomes : Need of Alternative Interventions. *Journal of Biomedicine and Biotechnology*. **2010**: 1-24

Begna, F., Abebe, S. and Bekele, M. (2011): Bovine Trypanosomosis in Selected Villages of Humbo District , Southern Ethiopia. *Global Veterinaria*. **7**: 192–198.

Bekele, N., Kebede, A. and Mulatu, E. (2018): Journal of Veterinary Science & Prevalence of Bovine Trypanosomosis in Didessa District , Oromiya Region, Ethiopia. *J Vet Sci Technol.* **9**: 1–5.

Belete, D.A. (2017). A cross-sectional study on the occurrence of bovine trypanosomiasis and the risk factors in Gnangatom district, South Omo Zone. Glob. *J. Vet. Med. Res.* **5**: 148–152.

Biryomumaisho, S., Rwakishaya, E.-K., Melville, S.E., Cailleau, A. and Lubega, G.W. (2013): Livestock trypanosomosis in Uganda: parasite heterogeneity and anaemia status of naturally

infected cattle, goats and pigs. *Parasitol. Res*. **112**: 1443–1450.

Biyazen, H., Duguma, R. and Asaye, M. (2014): Trypanosomosis , Its Risk Factors , and Anaemia in Cattle Population of Dale Wabera District of Kellem Wollega Zone , Western Ethiopia. *J. Vet. Med. 1-6*

Bourn, B.D., Grant, I.A.N., Shaw, A., Torr, S., Protection, C.E., House, O., Street, S. and Me, K. (2005): Cheap and safe tsetse control for livestock production and mixed farming in Africa. *Aspects of Applied Biology*. **75**: 1–12.

Bouyer, J., Stachurski, F., Gouro, A.S. and Lancelot, R. (2009): Control of bovine trypanosomosis by restricted application of insecticides to cattle using footbaths. *Vet. Parasitol.* **161**: 187–193.

Büscher, P., Ngoyi, D.M., Kaboré, J., Lejon, V., Robays, J., Jamonneau, V., Bebronne, N., Van der Veken, W. and Biéler, S. (2009): Improved models of mini anion exchange centrifugation technique (mAECT) and modified single centrifugation (MSC) for sleeping sickness diagnosis and staging. *PLoS Negl. Trop. Dis.* **3**: e471.

Byamungu, M., Matembo, S., Benedict, K. and Mashenga, G. (2011): Storage time effect on blood diet for tsetse mass production in sterile insect technique. *J. Vector Borne Dis*. **48**: 93–95.

Cattand, P., Desjeux, P., Guzmán, M.G., Jannin, J., Kroeger, A., Medici, A., Musgrove, P., Nathan, M.B., Shaw, A. and Schofield, C.J. (1994): Chapter 23 Tropical Diseases Lacking Adequate Control Measures : Dengue , Leishmaniasis , and African Trypanosomiasis 451–466.

Cecchi, G., Mattioli, R.C., Slingenbergh, J. and De La Rocque, S. (2008): Land cover and tsetse fly distributions in sub -Saharan Africa. *Med. Vet. Entomol.* 1365-2915.2008.00747.x

Chaka, H. and Abebe, G. (2003): Drug resistant trypanosomes: a threat to cattle production in the southwest of Ethiopia . *Rev. Elev. Med. Vet. Pays Trop*. **56**.

Chanie, M., Arega, C. and Bogale, B. (2012): Hematopathology and Hematological Parametric Alterations in Indigenous Cattle Due to Trypanosomosis. *Global Veterinaria*. **9**: 546–551.

Chappuis, F., Loutan, L., Simarro, P., Lejon, V. and Büscher, P. (2005): Options for field diagnosis of human African trypanosomiasis. *Clin. Microbiol. Rev.* **18**: 133–146.

Chitanga, S., Marcotty, T., Namangala, B., Van den Bossche, P., Van Den Abbeele, J. and Delespaux, V. (2011): High prevalence of drug resistance in animal trypanosomes without a history of drug exposure. *PLoS Negl. Trop. Dis*. **5**: e1454.

Claes, F., Büscher, P., Touratier, L. and Goddeeris, B.M. (2005): Trypanosoma equiperdum: master of disguise or historical mistake? *Trends Parasitol.* **21**: 316–321.

Cossic, B.G.A., Adjahoutonon, B., Gloaguen, P., Dibanganga, G.L., Maganga, G., Leroy, P., Macleod, E.T. and Picozzi, K. (2017): Trypanosomiasis challenge estimation using the diminazene aceturate (Berenil) index in Zebu in Gabon. *Tropical Animal Health and Production*. **49** (3): 619–624.

Cox, A.P., Tosas, O., Tilley, A., Picozzi, K., Coleman, P., Hide, G. and Welburn, S.C. (2010): Constraints to estimating the prevalence of trypanosome infections in East African zebu cattle. *Parasites and Vectors*. **3**: 82.

Cross, G.A.M. (2001). African Trypanosomes in the 21st Century : What is Their Future in Science and in Health ?

CSA (2017). Agricultural sample survy; Report on Livestock and Livestock characterstics. Volume II, 1–194.

CSA (2013). Population Projection of Ethiopia for All Regions At Wereda Level from 2014 – 2017. Addis Ababa. FDRE-CSA.

CSA (2007). Population and Housing Census of Ethiopia: Administrative Report By Central Statistical Authority.

Dagnachew, S. (2004). Epidemiology of Bovine Trypanosomosis in the Abbay Basin Areas of Northwest Ethiopia, MVSc thesis submitted to AAU CVMA, Debre-Zeit, Ethiopia.

Dagnachew, S., Tsegaye, B., Awukew, A., Tilahun, M., Ashenafi, H., Rowan, T., Abebe, G., Barry, D.J., Terefe, G. and Goddeeris, B.M. (2017): Prevalence of bovine trypanosomosis and assessment of trypanocidal drug resistance in tsetse infested and non-tsetse infested areas of Northwest Ethiopia. *Parasite Epidemiol. Control.* **2:** 40–49.

De Deken, R. (2012). Arthropod Vectors: Tsetse flies Anatomy/Morphology, Distribution, life cycle and control. pp. 1–46.

Desa, G., Tsegaye, M., Lelisa, K., Argiles, R., Lema, B., Mekonnen, S., Briasco, M., Beyene, D., Parker, A.G. and Bouyer, J. (2018): Optimizing the Sex Ratio to Maximize the Yield of Sterile Males in Tsetse Mass- Rearing Colonies Optimizing the Sex Ratio to Maximize the Yield of Sterile Males in Tsetse Mass-Rearing Colonies. *Academic Journal of Entomology*. **11** (3): 59-65

Desquesnes, M., Holzmuller, P., Lai, D.-H., Dargantes, A., Lun, Z.-R. and Jittaplapong, S. (2013):

Trypanosoma evansi and Surra: A Review and Perspectives on Origin, History, Distribution, Taxonomy, Morphology, Hosts, and Pathogenic Effects. *Biomed Res. Int.* **2013**: 1-23

Desta, M., Menkir, S. and Kebede, A. (2013): The study on tsetse fly (Glossina species) and their role in the trypanosome infection rate in Birbir valley, Baro Akobo River system, western Ethiopia. *J. Vet. Med. Anim. Heal.* **5**: 186–194.

Dohoo, L., Martin, W. and Stryhn, H. (2003): Veterinary Epidemiologic Research. Charlottetown Prince Edward Island Canada, Canada.

Duguma, R., Tasew, S., Olani, A., Damena, D., Alemu, D., Mulatu, T., Alemayehu, Y., Yohannes, M., Bekana, M., Hoppenheit, A., Abatih, E., Habtewold, T., Delespaux, V. and Duchateau, L. (2015): Spatial distribution of Glossina sp . and Trypanosoma sp . in south-western Ethiopia. *Parasit and Vectors.* ***8:*** 1–10.

Dumesa, T. and Demessie, Y. (2015): Review on Tsetse Transmitted Bovine Trypanosomosis in Ethiopia **7**: 255–267.

Dwinger, R.H., Grieve, A.S., Snow, W.F., Rawlings, P., Jabang, B. and Williams, D. (1992): Maternal antibodies in N'Dama calves kept under natural trypanosomiasis risk in The Gambia. *Parasite Immunol.* **14**, 351-354.

Eisler, M.C., Dwinger, R.H., Majiwa, P.A.O., Picozzi, K., Maudlin, I., Holmes, P. and Miles, M. (2004): Diagnosis and epidemiology of African animal trypanosomiasis. *Trypanos.* **253**: 267.

Elhaig, M.M., Youssef, A.I. and El-Gayar, A.K. (2013): Molecular and parasitological detection of Trypanosoma evansi in camels in Ismailia, Egypt. *Vet. Parasitol.* **198**: 214–218.

Eyob, E. and Matios, L. (2013). Review on camel trypanosomosis (surra) due to Trypanosoma evansi: Epidemiology and host response. *J. Vet. Med. Anim. Heal.* **5**: 334–343.

FAO (2014). Testse and Trypanosomosis Information : Program Against African Trypanosomiasis, in: Dargie, J. (Ed.), . FAO, Rome, pp. 1–117.

FEWSNET (2012). Famine Early Warning Systems Network—Informing Climate Change Adaptation Series: A Climate Trend Analysis of Ethiopia.

Gedech, E.K. and Guangul, A.T. (2017): Tourism potential and constraints : considering the natural and cultural attractions of South Omo, Ethiopia. *AJHTL.* **6**: 1–23.

Getachew, A. (2005): Tyrypanosomosis in Ethiopia. *Ethiop. J. Biol. Sci.* **4**: 75–121.

Giordani, F., Morrison, L.J., Rowan, T.G., De Koning, H.P. and Barrett, M.P. (2016): The animal trypanosomiases and their chemotherapy: a review. *Parasitology.* **143**: 1862–1889.

Glare, T.R. and Callaghan, M.O. (1999): Environmental and health impacts of the insect juvenile hormone analogue , S-methoprene: report for the ministry of health, Biocontrol and Biodiversity, Grasslands Division, AgResearch PO Box 60, Lincoln.

Gona, Z., Teshale, A. and Tilahun, A. (2016): Study on prevalence of bovine trypanosomosis and density of its vectors in three selected districts of Wolaita Zone, Southern Ethiopia. *J. Vet. Med. Anim. Heal.* **8**: 128–135.

Gray, M.A., Kimarua, R.W., Peregrine, A.S. and Stevenson, P. (1993): Drug sensitivity screening in vitro of populations of Trypanosoma congolense originating from cattle and tsetse flies at Nguruman, Kenya. *Acta Trop.* **55**: 1–9.

Hagos, A.T. (2010). Equine trypanosomosis in Ethiopia: Epidemiology, characterization and control, PhD thesis, Faculty of Bio-science Engineering, Katholic University of Leuven, Belgium, PhD thesis number 915.

Hargrove, J.W. (2003). Tsetse eradication: sufficiency, necessity and desirability. Research report, DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh,. UK.

Hargrove, J.W. and Langley, P.A. (1993): A field Trial of pyriproxyfen-treated targets as an alternative methods for controlling tsetse (Diptera: Glossina). *Bull. Entomol. Res.* **83**: 361–368.

Holmes, P.H., Eisler, M.C. and Geerts, S. (2004): Current chemotherapy of animal trypanosomiasis.

Hoppenheit, A., Murugaiyan, J., Bauer, B., Clausen, P.-H. and Roesler, U. (2014): Analysis of Glossina palpalis gambiensis and Glossina tachinoides from two distant locations in Burkina Faso using MALDI TOF MS. *Parasitol. Res.* **113:** 723–726.

Hung, C.-H., Qiao, X., Lee, P.-T. and Lee, M.G.-S. (2004): Clathrin-Dependent Targeting of Receptors to the Flagellar Pocket of Procyclic-Form Trypanosoma brucei. *Eukaryot. Cell.* **3**: 1004–1014.

Joshua, R.A., Obwolo, M.J., Bwangamoi, O. and Mandebvu, E. (1995): Resistance to diminazine aceturate by Trypanosoma congolense from cattle in the Zambezi Valley of Zimbabwe. *Vet. Parasitol.* **60**: 1–6.

Kalu, A.I. (1995). Sensitivity of animal-derived Trypanozoon stocks from sleeping sickness endemic foci of Nigeria to trypanocides and human plasma. *Rev. d'élevage médecine vétérinaire des pays Trop.* **48**: 139–144.

Kebede, A., Ashenafi, H. and Daya, T. (2017): Review on Sterile Insect Technique (SIT) and its Potential towards Tsetse Eradication in Ethiopia. *Adv. Life Sci. Technol.* **37**: 24–44.

Leak, S.G., Peregrine, A.S., Mulatu, W., Rowlands, G.J. and D'Ieteren, G. (1996): Use of insecticideimpregnated targets for the control of tsetse flies (Glossina spp.) and trypanosomiasis occurring in cattle in an area of south-west Ethiopia with a high prevalence of drugresistant trypanosomes. *Trop. Med. Int. Heal.* **1**: 599–609.

Leak S.G.A. (1999). Tsetse Biology and Ecology: Their Role in the Epidemiology and Control of Trypanosomosis. Wallingford: CABI Publishing;

Leta, S., Alemayehu, G., Seyoum, Z. and Bezie, M. (2016): Prevalence of bovine trypanosomosis in Ethiopia : a meta-analysis. *Parasit. Vectors.* **139**.

Leta, S., Habtamu, Y., Alemayehu, G. and Shigute, B.A. (2015): Spatial analysis of the distribution of tsetse flies in Ethiopia using high resolution environmental datasets and Maxent modeling technique.

Magez, S., Brussel, V.U. and Caljon, G. (2010): Current status of vaccination against African trypanosomiasis. *Parasitology*. **137** (14): 2017-2027

Magez, S. and Radwanska, M. (2009). African trypanosomiasis and antibodies: implications for vaccination, therapy and diagnosis. *Future Microbiol*. **4:** 1075–1087.

Malele, I.., Kinung'hi, S.., Nyingilili, H.., Matemba, L.., Sahani, J.., Mlengeya, TDK. *et al.* (2007): Glossina dynamics in and around the sleeping sickness endemic Serengeti ecosystem of northwestern Tanzania. *J Vector Ecol.* **32**: 263–8.

Mamoudou, A., Delespaux, V., Chepnda, V., Hachimou, Z., Andrikaye, J.P., Zoli, A. and Geerts, S. (2000): Assessment of the occurrence of trypanocidal drug resistance in trypanosomes of naturally infected cattle in the Adamaoua region of Cameroon using the standard mouse test and molecular tools. *Acta Tropica*. **106** (2): 1–16.

Manyazewal, A.Z., Basu, A. and Yilkal, A. (2014): Socioeconomic Assessment of a Tsetse and Trypanosomosis Control Program in Southwest Ethiopia. J. Adv. Vet. Res. **4**: 126–134.

Masumu, J., Marcotty, T., Geerts, S., Vercruysse, J. and Van den Bossche, P. (2009): Cross-protection between Trypanosoma congolense strains of low and high virulence. *Vet.*

Parasitol. **163**: 127–131.

Masumu, J., Marcotty, T., Geysen, D., Geerts, S., Vercruysse, J., Dorny, P. and Van den Bossche, P. (2006): Comparison of the virulence of Trypanosoma congolense strains isolated from cattle in a trypanosomiasis endemic area of eastern Zambia. *Int. J. Parasitol.* **36**: 497–501.

Matovu, E., Iten, M., Enyaru, J., Schmid, C., Lubega, G., Brun, R. and Kaminsky, R. (1997): Susceptibility of Ugandan Trypanosoma brucei rhodesiense isolated from man and animal reservoirs to diminazene, isometamidium and melarsoprol. *Trop. Med. Int. Heal.* **2**: 13–18.

Matthews, K.R., Mcculloch, R., Morrison, L.J. and Matthews, K.R. (2015): The within-host dynamics of African trypanosome infections. *Phil. Trans. R. Soc.* B 370.

Mekonnen, G., Mohammed, E.F., Kidane, W., Nesibu, A., Id, H.B., Yohannes, H., Reet, N. and Van, Bu, P. (2018): Isometamidium chloride and homidium chloride fail to cure mice infected with Ethiopian Trypanosoma evansi type A and B. *Plos Neglected Tropica Disease*. **12** (9) 1–12.

Mekuriaw, G. and Kebede, A. (2015): A review on Indigenous Cattle Genetic Resources in Ethiopia : Adapitation , Status and Survival. *Online J. Anim. Feed Res.* **5**: 125–137.

Melaku, A. and Birasa, B. (2013): Drugs and Drug Resistance in African Animal Trypanosomosis : A Review. *Eur. J .Appl. Sci.* **5**: 84–91.

Moti, Y., Fikru, R., Van Den Abbeele, J., Büscher, P., Van den Bossche, P., Duchateau, L. and Delespaux, V. (2012): Ghibe river basin in Ethiopia: present situation of trypanocidal drug resistance in Trypanosoma congolense using tests in mice and PCR-RFLP. *Vet. Parasitol.* **189**: 197–203.

Motloang, M.Y., Masumu, J., Mans, B.J. and Latif, A.A. (2014): Virulence of Trypanosoma congolense strains isolated from cattle and African buffaloes (Syncerus caffer) in KwaZulu-Natal, South Africa. *Onderstepoort J. Vet.Res.* **81**.

Muktar, Y., Asmelash, M. and Mekonnen, N. (2016): Prevalence and associated risk factors of bovine trypanosomosis in Benatsemay district, SouthOmo zone, Ethiopia. *Livest. Res. Rural Dev.* **28**: 12.

Mulugeta, W., Wilkes, J., Mulatu, W., Majiwa, P.A.O., Masake, R. and Peregrine, A.S. (1997): Long-term occurrence of Trypanosoma congolense resistant to diminazene, isometamidium and homidium in cattle at Ghibe, Ethiopia. *Acta Trop.* **64:** 205–217.

Mungube, E.O., Vitouley, H.S., Allegye-cudjoe, E., Diall, O., Boucoum, Z., Diarra, B., Sanogo, Y.,

Randolph, T., Bauer, B. and Zessin, K. (2012): Detection of multiple drug-resistant Trypanosoma congolense populations in village cattle of South East Mali. *Parasites and Vectors*. **5**: 1–9.

Munhenga, G., Brooke, B.D., Chirwa, T.F., Hunt, R.H., Coetzee, M., Govender, D. and Koekemoer, L.L. (2011): Evaluating the potential of the sterile insect technique for malaria control : relative fitness and mating compatibility between laboratory colonized and a wild population of Anopheles arabiensis from the Kruger National Park , South Africa.

Namangala, B. (2011). Contribution of Innate Immune Responses towards Resistance to African Trypanosome Infections. *Scand. J. Immunol.* **75**: 5–15.

Ngare, P.M.. and Mwendia, C.M.T. (2000): Tsetse and trypanosomosis: An epidemiological survey in Osupuko and Mara division of Narok district. "Proc. *25th International Scientific Council for Trypanosomosis Research and Control (ISCTRC)* Mombassa, Kenya.

Nichlson, J.M. and Butherworth, M.H. (1986): A Guide to Body Condition Scoring of Zebu Cattle. ILCA; A monograph. Addis Ababa Ethiopia.

Nimpaye, H., Njiokou, F., Njine, T., Njitchouang, G.R., Cuny, G., Herder, S., Asonganyi, T. and Simo, G. (2011): Trypanosoma vivax, T. congolense "forest type" and T. simiae: prevalence in domestic animals of sleeping sickness foci of Cameroon. *Parasite.* **18**: 171–179.

NMSA (2005). National meteorological services agency agrometeorological bulletin: seasonal agro meteorological bulletin.

OIE (2017). OIE Reference Laboratory for Animal Trypanosomoses of African Origin. Compendium of Standard Diagnostic Protocols for Animal Trypanosomoses of African Origin. Office International des Epizooties (OIE). Montpellier, France.

Olani, A. and Bekele, D. (2016): Epidemiological Status and Vector Identification of Bovine Trypanosomosis in Lalo-Kile District of Kellem Wollega Zone , Western Ethiopia 3, 1045.

Pace, J.E. and Wakeman, D.L. (2003): Determining the age of cattle by their teeth. *J. Am. Vet. Med. Assoc.* **121**: 483.

Pagabeleguem, S., Sangare, M., Bengaly, Z., Akoudjin, M., Belem, A.M.G. and Bouyer, J. (2012): Climate , Cattle Rearing Systems and African Animal Trypanosomosis Risk in Burkina Faso 7.

Percoma, L., Sow, A., Pagabeleguem, S., Dicko, A.H., Serdebéogo, O., Ouédraogo, M., Rayaissé,

J., Bouyer, J., Belem, A.M.G. and Sidibé, I. (2018): Impact of an integrated control campaign on tsetse populations in Burkina Faso 1–13.

Peregrine, A.S. (1994). Chemotherapy and delivery systems: haemoparasites. *Vet. Parasitol.* **54**: 223–248.

Ponte-Sucre, A. (2016). An overview of Trypanosoma brucei infections: an intense host–parasite interaction. *Front. Microbiol.***7**: 2126.

Roditi, I. and Lehane, M.J. (2008): Interactions between trypanosomes and tsetse flies. *Curr. Opin. Microbiol.* **11**: 345–351.

Rowlands, G.J., Mulatu, W., Leak, S.G., Nagda, S.M. and D'Ieteren, G.D. (1999). Estimating the effects of tsetse control on livestock productivity-a case study in southwest Ethiopia. *Trop. Anim. Heal. Prod.* **31**: 279–294.

Saror, D.I., Ilemobade, A.A. and Nuru, S. (1979): The haematology of N'Dama and Zebu cattle experimentally infected with Trypanosoma vivax, in: International Scientific Council for Trypanosomiasis Research and Control. 16th Meeting, Yaounde, Cameroon. pp. 287–294.

Senait, G. and Asinake, B. (2016): Epidemiological Study of Bovine Trypanosomosis in Selective PAs of South Omo Zone , Ethiopia. *J. Biol. Agric. Healthc.* **6:** 108–114.

Seyoum, Z., Terefe, G. and Ashenafi, H. (2013): Farmers' perception of impacts of bovine trypanosomosis and tsetse fly in selected districts in Baro-Akobo and Gojeb river basins, Southwestern Ethiopia. *BMC Vet Res.* **9**: 214.

Shaw, A.P.M., Torr, S.J., Waiswa, C., Cecchi, G., Wint, G.R.W., Mattioli, R.C. and Robinson, T.P. (2013): Estimating the costs of tsetse control options : An example for Uganda. *Prev. Vet. Med.* **110**: 290–303.

Shereni, W., Anderson, N.E., Nyakupinda, L. and Cecchi, G. (2016): Spatial distribution and trypanosome infection of tsetse flies in the sleeping sickness focus of Zimbabwe in Hurungwe District. *Parasit. Vectors.* **9**: 605.

Shiferaw, S., Muktar, Y. and Belina, D. (2015): A review on trypanocidal drug resistance in Ethiopia. *Journal of Parasitology and Vector Biology*. **7**: 58–66.

Singh, V. and Singla, L.D. (2013): Trypanosomosis (surra) in livestock. *Vet. Parasitol.* Indian Perspect. Satish Ser. Publ. House, Delhi 277–302.

Sinshaw A. (2004). Prevalence of trypanosomosis of cattle in three district of Amhara Region, Ethiopia, Msc Thesis, FVM,AAU, Debre Zeit.

SOFED (2012). South Omo Zone Finance and Economic Development (SOFED) report (2004).

SOZLFD (2018). South Omo Zone Livestock and Fishery Department (SOZLFD), zonal livestock survey data.

Stein, J. (2011). Trypanotolerance and phenotypic characteristics of four Ethiopian cattle breeds. Doctoral thesis. ISBN 978-91-576-7564-4

STEP (2018). Southern Tsetse Eradication Program, Field data record on tsetse suppression activities in South Omo Zone conducted by STEP project Arbaminch center.

STEP (2012). Southern Tsetse Eradication Program, Field data record on tsetse suppression activities in South Omo Zone conducted by STEP project Arbaminch center.

Stevens, J.R., Brisse, S., Maudlin, I., Holmes, P.H. and Miles, M.A. (2004): The Trypanosomiases. Wallingford, Oxfordsh. OX10 8DE, UK CABI Publ.

Stijlemans, B., Caljon, G., Natesan, S.K.A., Saerens, D., Conrath, K., Perez-Morga, D., Skepper, J.N., Nikolaou, A., Brys, L. and Pays, E. (2011): High affinity nanobodies against the Trypanosome brucei VSG are potent trypanolytic agents that block endocytosis. *PLoS Pathog.* **7**: e1002072.

Tabel, H., Wei, G. and Shi, M. (2008: T cells and immunopathogenesis of experimental African trypanosomiasis. *Immunol. Rev.* **225**: 128–139.

Taylor, K. and Authié, E.M.-L. (2004): Pathogenesis of Animal Trypanosomiasis. *Trypanos*. 331.

Taylor, M.A., Coop, R.L. and Wall, R.L. (2007): Parasites of dogs and cats. *Vet. Parasitol.* **3**: 429–430.

Tekle, T., Terefe, G., Cherenet, T., Ashenafi, H., Akoda, K.G., Abbeele, J. and Van Den, Gari, G. (2018): Aberrant use and poor quality of trypanocides : a risk for drug resistance in south western Ethiopia. *BMC Veterinary Research.* **14** (4): 1–8.

Tekle, Y. and Mekonen, S. (2013): Prevalence of Bovine Trypanosomosis in Tsetse Controlled and Uncontrolled Areas of Eastern Wollega , Ethiopia. *J. Sci. Innov. Res.* **2**: 61–75.

Tewelde, N., Abebe, G., Eisler, M., McDermott, J., Greiner, M., Afework, Y., Kyule, M., Münstermann, S., Zessin, K.-H. and Clausen, P.-H. (2004): Application of field methods to assess isometamidium resistance of trypanosomes in cattle in western Ethiopia. *Acta Trop.* **90**: 163–170.

Tewelde, T. (2001). Study on the occurrence of drug resistant trypanosomes in cattle in the farming in tsetse control areas (FITCA) project in western Ethiopia. Addis Ababa University,

Faculty of Veterinary Medicine, Ethiopia, MSc Thesis.

Tihon, E., Imamura, H., Broeck, F. Van Den, Vermeiren, L., Dujardin, J. and Abbeele, J. Van Den (2017): Drugs and Drug Resistance Genomic analysis of Isometamidium Chloride resistance in Trypanosoma congolense. *IJP Drugs Drug Resist.* **7**: 350–361.

Tilahun, A.D.., Asfaw, Yilkal, A. and Yacob, H.T. (2012): Bovine trypanosomosis in three districts of Southwest Oromia, Ethiopia. *Ethiop. Vet. J.* **16**: 23–39.

Torr, S.J., Maudlin, I. and Vale, G.A. (2007): Less is more: restricted application of insecticide to cattle to improve the cost and efficacy of tsetse control. *Med Vet Entomol.* **21**: 53–64.

Tsegaye, B. (2014). In vivo Drug Sensitivity Tests of Trypanosoma vivax Isolates from Selected Tsetse Infested and Tsetse free areas of North West, Ethiopia, A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Tropical Veterinary Parasitology.

Tsegaye, B., Dagnachew, S. and Terefe, G. (2015): Review on Drug Resistant Animal Trypanosomes in Africa and Overseas. *African J. Basic & Appl. Sci.* **7**: 73–83.

Uilenberg, G. (2011). Theileria sergenti. *Vet. Parasitol*. **175**: 386.

Uilenberg, G. and Boyt, W.P. (1998): A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis. FAO, Rome, Italy.

Vale, G.A. and Torr, S.J. (2005): User-friendly models of costs and efficacy of tsetse control: application to sterilising and insecticidal techniques. *Med. Vet. Entomol.* **19**, 293–305.

Vale, G.A., Hargrove, J.W., Chamisa, A., Grant, I.F. and Torr, S.J. (2015): Pyrethroid Treatment of Cattle for Tsetse Control : Reducing Its Impact on Dung Fauna. *Plos Neglected Tropical Diseases*. **9** (3): 1–11.

Van den Bossche, P., Doran, M. and Connor, R.J. (2000): An analysis of trypanocidal drug use in the Eastern Province of Zambia. *Acta Trop.* **75**: 247–258.

Van den Bossche, P., Shumba, W. and Makhambera, P. (2000): The distribution and epidemiology of bovine trypanosomosis in Malawi. *Vet. Parasitol.* **88**: 163–176.

Vitouley, S.H. (2014). Trypanocidal drugs and the problem of drug resistance in West Africa.

Vreysen, M.J.B., Saleh, K.M., Ali, M.Y., Abdulla, A.M., Zhu, Z.R., Juma, K.G., Dyck, V.A., Msangi, A.R., Mkonyi, P.A. and Feldmann, H.U. (2000): Glossina austeni (Diptera: Glossinidae) eradicated on the Island of Unguja, Zanzibar, using the sterile insect

technique. *J. Econ. Entomol.* **93**: 123–135.

W/yohannes, D., Etsay, K. and Getachew, A. (2010): Study on the assessment of drug resistance on Trypanosoma vivax in Tselemti district , Tigray, Ethiopia. *Ethiop. Vet. J.* **14**: 15–30.

Wilkinson, S.R. and Kelly, J.M. (2009): Trypanocidal drugs : mechanisms , resistance and new targets. *Expert Rev. Mol. Med.* **11**: e31.

Wiser, M.F. (2011). Protozoa and Human Disease. Garland Science Taylor and Francis Group.

Yeshitila, A., Getachew, A., Hagos, A. and Basu, A. (2006): Prevalence Of Bovine Trypanosomosis In Sokoru District, Jimma Zone, Oromiya Region, Southwest Of Ethiopia. *Bull. Anim. Heal. Prod. Africa.* **54**: 242–258.

Yimer, M.M., Bula, D.G., Tesama, T.K., Tadesse, K.A. and Abera, B.H. (2015): Prevalence of salivary gland hypertrophy syndrome in laboratory colonies and wild flies of Glossina pallidipes in Ethiopia. *Onderstepoort J. Vet. Res.* **81**: 1–6.

Zekarias, T., Kapitano, B., Mekonnen, S. and Zeleke, G. (2014): The Dynamics of TseTse Fly in and Around Intensive Suppression Area of Southern Tsetse Eradication Project Site , Ethiopia. Ethiop. *J. Agric. Sci.* **24**: 59–67.

8. ANNEXES

Annex 1. Questionnaire survey format for trypanocidal drug utilization practice

Name of interviewee_____, Age_____ Sex_____ Educational level _____

District_____PA _____Village _____

1. What is the grazing system of your animals?
   (1) Free grazing on communal land (2) Tethering (3) Zero grazing (4) Free grazing & Tethering
2. Have your animals encountered trypanosomosis
   (1) Yes (2) No
3. If yes for Q2 which animal group does trypanosomosis most affect?

(1) Cattle (2) goat (3) Sheep (4) Equine

4. Which category of animals do you give priority in trypanocidal drug treatment
   (1) Oxen (2) Milking cows (3) Calves
5. Which drug is your choice for treatment/ the most popular?
   (1) ISM only (4) Homidium
   (2) DIM only (5) ISMM and Homidium
   (3) Both ISMM and DIM (6) DIM and Homidium
6. Source of the trypanocidal drugs?
   (1) Government veterinary clinics (3) Privately run drug outlets
   (2) Private practitioners (4) All of the above
7. Who administers trypanocidal drug to your animals?
   (1) Government veterinarians (2) Farmers/herdsmen (3) All of the above
8. Which type of water used to dilute the drug?
   (1) Distilled water (2) boiled water meant for home use
9. How many times do you treat your animals in a year
   (1) 1 times (2) 2 times (3) 3 times (4) 4 times
10. In which season your animal show sign of trypanosomosis frequently?

(1) Dry season (2) Wet season

11. Have you encountered any treatment failure after treating your animal?

(1) Yes (2) No

12. If yes for Q11, drugs from which source encountered more treatment failure?

(1) Government veterinary clinics (3) All of the above
(2) Private practitioner's clinic/pharmacy (4) No failure

13. Which drug encounters more treatment failure?

(1) DIM (2) ISMM (3) Homidium (4) Both DIM and ISMM (5) No failure

14. Which signs your animals shown could be indicative of trypanosomosis infection?

(1) Raising of hair and diarrhea (4) Raising of hair and depression
(2) Raising of hair (5) Raising of hair and loss of tail tip
(3) Raising of hair, diarrhea and depression (6) Raising of hair, coughing & eating of soil

15. Do you know how the disease (trypanosomosis) is transmitted?

(1) By Flies (2) by Ticks (3) by contact (4) I don't know

16. Do you differentiate tsetse flies from other haematophagous flies?

(1) Yes (2) No

17. How many do trypanosomosis treatment costs you in a year?

(1) 50-100 ETB (2) 100-200 ETB (3) 200-500 ETB (4) >500 ETB

18. Do you encounter any death of your animal do to trypanosomosis in past five years?

(1) Yes (2) No

Thank You!

Name of interviewer ……..

Date…… Signature……

Annex 2. Data collection format during blood sample collection and lab analysis

District _____ PA _____

S. no	**Owner's data**			**Animal data**					**Season of collection**	**Specimen collected**	**Lab technique conducted**	**Lab result**					**Remark**
												Tryps Species					
	Name	Age	Sex	Breed	Age	Sex	BCS	Grazing system				Tc	Tv	Tb	other	PCV	

Annex 3. Data collection format for tsetse fly survey and identification

District	PA name	Vegetation type	No of traps deployed/PA	Total tsetse caught	Sex of tsetse		Species of tsetse caught/number				Other flies caught			No of days traps stayed
					M	F	*G.p*	*G.m*	*G.f*	*G.t*	*Sto*	*Tab*	*Hae*	

Note: M= Male, F= Female, G.p= *Glossina pallidipes*, G.m= *G. morsitans*, G.f= *G. fuscipes*, G.t= *G. tachinoides*, Sto= *Stomoxys*, Tab= *Tabanus*, Hae= *Haematopota*

Name of veterinarian _____ Date _____ Signature _____

Annex 4. Geo-reference data of selected study sites (PAs) from each study districts

Study districts	Selected study PAs	Latitude	Longitude	Altitude (m.a.s.l.)
Debub Ari	Kure	05^0 47’ 338’’,	036^0 29’ 442’’	1319
	Tembel	05^0 67’.464’’	036^0 53’.542’’	1541
	Chelegod	05^0 55’.337’’	036^0 12’.564’’	1277
Benatsemay	Goldiya	05^0 37’.345’’	036^0 34’.365’’	1198
	Diziaman	05^0 29’.142’’	036^0 34’.842’’	1266
	Enchete	05^0 24’.666’’	036^0 57’.371’’	545
Hammer	Kara Labuk	05^0 19’.186’’	036^0 15’.548’’	444
	Zegerma	05^0 09’.609’’	036^0 51’.157’’	547
Gnangatom	Kuchuru	05^0 25’.580”	036^0 13’.027”	389
	Tirga	05^0 42’.279”	035^0 44’.759”	455

Annex 5. Poisson regression model output for Glossina count data (by R- software)

```
Tsetse2<-glm(`Tsetse count`~factor(`suppression
status`)+factor(Altitude)+factor(`Number of
traps`)+factor(Season),family = "poisson",data = tsetse_poison)
>Summary (Tsetse2)
```

Risk factor	Category	IR	95% CI		P-value
			LL	UL	
Altitude	> 1500 m.a.s.l	1.22	0.45	3.36	0.69
	1000-1500 m.a.s.l	2.30	0.94	5.79	0.069
	500-1000 m.a.s.l	1.15	0.69	1.83	0.56
	< 500 m.a.s.l	1*			
No of traps deployed	5 traps	2.35	1.50	3.86	0.00034**
	6 traps	NA	NA	NA	NA
	4 traps	1*			
Season	Wet	0.19	0.13	0.26	0.0000***
	Dry	1*			
Suppression status	Suppressing	0.43	0.19	0.88	0.027*
	Non-suppressing	1*			

```
pchisq(Tsetse2$deviance, df=Tsetse2$df.residual, lower.tail =
FALSE)
```

P-value = 3.741176e-25. *This p-value (*3.741176e-25*) is significant, indicating the lack of fit of the data in poison regression model.*

Checking over-disperssion

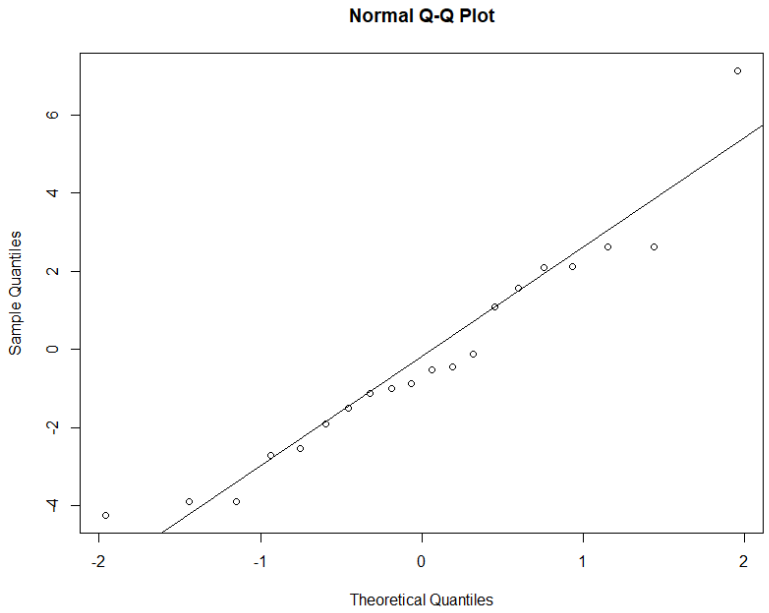
```
dispersion<-dispersiontest(Tsetse2,trafo=1)
>dispersion
```

```
Overdispersion test

data:  Tsetse2
z = 1.957, p-value = 0.02518
Alternative hypothesis: true alpha is greater than 0
Sample estimates:
Alpha
6.531259
```

Interpretation: *evidence of over dispersion (c is estimated to be* 6.53*) which speaks quite strong against the assumption of equidispersion (i.e. c=0)*

```
res<- residuals(Tsetse2, type="deviance")
>qqnorm(res, plot.it = TRUE)
>qqline(res)
```



Normal Q-Q plot (sample quantiles vs theoretical quantiles) indicating non-normal distribution of errors when the tsetse count data was analyzed by using poison regression model

Annex 6. A Guide to Body Condition Scoring of Zebu Cattle

Score	Condition	Features
1	**L -**	Marked emaciation (animal condemned at ante mortem examination)
2	**L**	Transverse process project prominently, neural spines appear sharply
3	**L+**	Individual dorsal spines are pointed to the touch: hips, pins, tail-head and ribs are prominent
4	**M -**	Ribs, hips and pins clearly visible. Muscle mass between hooks and pins slightly concave. Slightly more flesh above the transverse process than in L+.
5	**M**	Ribs usually visible, little fat cover, dorsal spines barely visible
6	**M +**	Animal smooth and well covered: dorsal spines cannot be seen, but are easily felt
7	**F -**	Animal smooth and well covered, but fat deposits are not marked. Dorsal spine can be felt with firm pressure, but felt rounded rather than sharp
8	**F**	Fat cover in critical areas can be easily seen and felt; transverse processes cannot be seen or felt
9	**F +**	Heavy deposits of fat clearly visible on tail-head, brisket and cod; dorsal spines, ribs and pins fully covered and cannot be felt even with firm pressure

Source: (Nichlson and Butherworth, 1986)

Annex 7. Picture gallery of field and laboratory works

Traditional housing (crash-housing) (left) and community bore hole (‘chirosh’) (right) for animals (from Hammer district)

Glossina species collected from field and their identification to species and genera level


Parasite identification and PCV recording at Jinka regional veterinary laboratory

Disease ranking with Hammer pastoralists (Kara Labuk PA)

Ventral views of male tsetse fly with hypopygium (left) and Hatchet cell (under stereo microscope) for tsetse fly identification (right)

Questionnaire survey with agro-pastoralists/pastoralists