HAWASSA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE
FACULTY OF VETERINARY MEDICINE

SEASONAL PREVALENCE OF BOVINE TRYPANOSOMOSIS AND TSETSE FLY DENSITY IN ZALA DISTRICT, GOFA ZONE, SOUTHERN ETHIOPIA

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BY

TONE TOKA TOKOSSA

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I dedicate this thesis to my father Toka Tokossa, my beloved wife Hewan Diyakon and my brothers for nursing me with affection, their unconditional love and their dedicated partnership in the success of my life.

DECLARATION

I, the under signed, declare that the information presented here in my thesis is my original work, has not been presented for a degree in any other university and that all sources of materials used for the thesis have been duly acknowledged.

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LIST OF ABBREVIATIONS

CSA	Central Statistics Authority
FAO	Food and Agricultural Organization
GDP	Gross Domestic Product
GPS	Geographic positioning system
ILRAD	International Laboratory for Research Institute
MOARD	Ministry of Agriculture and Rural Development
NTTICC	National Tsetse and Trypanosomosis Investigation and Control Center
OIE	Office International Des Epizooties
STEP	Southern Rift Valley Tsetse Eradication Project
WHO	World Health Organization
ZWADO	Zala Woreda Agricultural Development Office

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ABSTRACT

Bovine trypanosomosis is a parasitic disease causing serious economic losses in livestock productivity and agricultural development. However, the seasonal information of bovine trypanosomosis and tsetse fly density is very limited in many parts of the country including the southern rift valley of the country, particularly in Gofa Zone. Therefore, the objectives of this cross-sectional study were to estimate seasonal prevalence of bovine trypanosomosis and to assess tsetse fly apparent density in Zala district. For the parasitological study, a total of 560 animals (280 in each season) were examined for trypanosomosis by using buffy coat technique. For the entomological survey, 80 NGU traps odour-baited with acetone were deployed in watering and grazing points in which the animals and vector are believed to have frequent contact. An overall prevalence of trypanosomosis was 6.96%, of which 4.28% and 9.64% accounted for the dry and wet season, respectively. The prevalence of bovine trypanosomosis was significantly higher during the wet season (OR = 2.38; $p < 0.05$), in black and black spotted coat colour (OR = 4.61, $p < 0.05$) and poor body conditioned (OR = 4.55; $p < 0.05$) animals. Two species of trypanosomes, *Trypanosome congolense*, 87.18% and *Trypanosome vivax*, 12.82% were circulating in the area both in dry and wet season. The mean PCV value in infected animals (20.89 ± 4.04) was significantly lower than in non-infected animals (22.9 ± 6.32 %). The entomological study revealed the presence of only one Glossina species, known as *G. pallidipes* and two species of other biting flies, *Stomoxys* and *Tabanus* were distributed in the study area. From all traps deployed in both wet and dry season of the study district, a total of 564 tsetse and other biting flies were caught of which, 56.9% belongs to *G. pallidipes* and 43.1% were other biting flies. Overall apparent densities of *G. pallidipes* and biting flies in the study area were 1.38 and 1 flies/trap/day, respectively. Relatively, higher Glossina/trap/day caught in the wet season, (1.78 flies/trap/day) than in the dry season (1 flies/trap/day). Therefore, bovine trypanosomosis is one of the major impediments to livestock development and a potential threat to health and productivity of cattle in Zala district. Hence, the tsetse and trypanosomosis control scheme in tsetse infested areas of the district should be strengthened in coordinated manner to minimize the burden of the disease.

Key words: *Bovine; Ethiopia; Season; Southern Ethiopia; Trypanosomosis; Zala district*

1. INTRODUCTION

African animal trypanosomiasis is one of the major animal health problems posing a significant effect on the settlement and socioeconomic development over large tsetse belt regions of the continent. It is a serious disease in domestic livestock that causes a significant negative impact in food production and economic growth in many parts of the world, particularly in Sub-Saharan Africa (Cecchi *et al*., 2008). In Sub-Saharan Africa, including East Africa, the vector of a disease is distributed over 10 million km^2 of potential grazing lands in 37 countries, exposing the lives of around 55 million people and 160 million cattle to the risk of a disease (Stijlemans *et al*., 2018).

The overall economic loss (both direct and indirect) is estimated to be about 500 billion dollars a year in terms of mortality, production, abortion, reduced fertility, and ability to work as traction animals. Furthermore, the disease is responsible for an annual loss of millions of dollars in livestock health and production as a result of the cost related to treatment, prevention, and vector control efforts (Assefa and Abebe, 2001).

Ethiopia has huge livestock population in Africa and the livestock sector plays a significant role in the national economy and livelihood of farmers and pastoralists (CSA, 2018). The sub-sector contributes about 16.5% of the national gross domestic product (GDP) and 35.6% of the agricultural GDP. Despite this huge livestock number, productivity is too low and even below the average for most Eastern and Sub-Saharan African countries, due to a number of complex and interrelated factors, such as inadequate feed and nutrition, widespread diseases, poor genetic potential of local breeds and inefficiency of livestock development services (Leta and Mesele, 2014). Among these, trypanosomosis is one of the major animal health constraints to livestock production and agricultural development (Abebe *et al*., 2017).

Trypanosomosis is a chronic hemoprotozoan disease of domestic animals and humans caused by different species of unicellular eukaryotic parasite of the genus *Trypanosome* (Abebe *et al*., 2017). With an exception of *Trypanosoma equiperdum* of equines, which causes a venereal disease, all have arthropod vectors in which the transmission is either cyclically by tsetse flies of the *Glossina species* or non-cyclical by many other insects (Urquhart *et al*., 1992, Gebisa *et*

al., 2020). Cattle affected with trypanosomosis can show major clinical manifestations of a disease, such as intermittent fever, anemia, anorexia, dullness, apathy, watery ocular discharge, reproductive disorder, and superficial lymph node enlargement. The animals progressively become emaciated and cachectic, and finally die if untreated (Constable *et al*., 2017).

There are six pathogenic species of trypanosomes which are recorded in Ethiopia, namely *T. vivax*, *T. congolense*, *T. brucei*, *T. evansi*, *T. equiperdum* and *T. rhodesiense*. But the most important trypanosomes in the country are *T. vivax* and *T. congelense*. Both species affect a great number of cattle which are the most important species of the domestic animals in Ethiopia (Abebe, 2005). The prevalence varies from locality to locality depending on agro-climatic conditions, seasons and as part of activities which are intended to control the impact of the disease (Tadesse and Tsegaye, 2010).

Trypanosome transmitted by tsetse fly continues to be a major constraint in livestock production. The disease greatly affects social, economic and agricultural development of communities in tsetse infested areas (Radostits *et al.*, 2007). The resistance of trypanosome to existing anti-trypanosomal drugs, increasing vectors' resistance to insecticides, absence of effective vaccines and adverse effects of existing anti-trypanosomal drugs are challenges in controlling the disease. People have been using both plant and animal species for treatment and control of trypanosomosis and as tsetse fly repellent in Ethiopia (Abdeta *et al.*, 2020).

Moreover, trypanosomosis is a very serious disease of cattle, which causes great socioeconomic losses in the country in general and study area in particular. Its socioeconomic impact is reflected on direct losses due to mortality, morbidity and reduction in milk and meat production, abortion and stillbirth and also costs associated with combat of the disease are direct losses. Consequently, studying the prevalence and magnitude of the vector is inevitably important to develop appropriate control measures (Abebe, 2005).

Over the past few decades, many efforts have been made to control tsetse and trypanosomosis in Ethiopia through coordinated action of the government, non-governmental organizations and local community. The control interventions commonly used in Ethiopia include; insecticidal pour-on, insecticide-impregnated traps and targets and use of different trypanocidal drugs (Abebe *et al.,* 2017, Duguma *et al.,* 2015). However, information related to

temporal and spatial dynamics of tsetse and trypanosomes remain very limited and may be a reason that control strategies are less effective and fail in endemic areas (Eyasu *et al*., 2021, Nnko *et al*., 2017).

Hence, the epidemiological knowledge on seasonal prevalence of bovine trypanosomosis and distribution of the tsetse fly are paramount in formulating appropriate strategies for the control of these problems (Van Den Bossche and De Deken, 2002). Also in Ethiopia, a few studies were conducted regarding the seasonal dynamics on the prevalence of the disease and apparent density in the tsetse fly, while systemic studies have not yet been carried out on seasonal prevalence and disease associated risk factors in the current study area. Therefore, this study was conducted with the aim to estimate seasonal prevalence of bovine trypanosomosis, to assess potential risk factors associated with trypanosomal infection and tsetse fly apparent density in Zala district, Gofa zone, Southern Ethiopia.

1.2. Statement of the problem

In Ethiopia, extensive and longtime tsetse trypanosomosis control operations have been running for several years, trypanosomosis is still remaining as a major constraint of livestock production in tsetse and trypanosomosis infected areas of the country in general and the study area in particular. According to the systematic review performed by Meyer *et al.*, 2016 on past and ongoing tsetse and animal trypanosomosis control operations in five African countries, including Ethiopia with a long history of their considerable effort to control of trypanosomosis and its vector; tsetse fly indicated that there is lack of evaluations. So, these indicated that there is limited scientific information available about the seasonal prevalence of trypanosomosis and its tsetse fly distribution and also lack of evaluation of the control programs undertaken. So, the seasonal disease prevalence and its tsetse fly distribution information are important to device appropriate control measures.

In Gofa Zone, bovine trypanosomosis is one of the most important livestock diseases, which poses a serious threat to the lives and livelihood of entire communities and constitutes the greatest disease constraint to livestock production.

Apart from the problem most studies in the Southern Nation Nationalities and peoples region conducted in dry seasons or wet seasons using snapshot cross-sectional studies, information about both dry and wet seasons were very limited, especially in the study area of Zala district.

In the district, there is no compilied data and reliable information on the prevalence of bovine trypanosomosis and associated risk factors. Consequently, the present study was conducted to observe the problem both in dry and wet seasons in selected Kebele' s of Zala district.

1.3. Objectives of the Study

1.3.1. General objective

- To estimate the seasonal prevalence of bovine trypanosomosis and tsetse fly distribution in selected areas of Zala district, Gofa zone, Southern Ethiopia.

1.3.2. Specific objectives

To achieve the above general objectives, the study included the following specific objectives:-

- To estimate the seasonal prevalence of bovine trypanosome species prevailing in the study area.
- To assess seasonal dynamics of vector distribution in study area.
- To assess potential risk factors associated with trypanosome infection.

1.4. Significance of the study

The significance of the study is to improve the knowledge and basic understanding on challenges and opportunities associated with the seasonal prevalence of bovine trypanosomosis, fly distribution and associated risk factors and also aid to develop appropriate strategy to control and prevention of bovine trypanosomosis and provide information for further study. Besides, this study generated data that would be added to the existing information in the country.

2. LITERATURE REVIEW

2.1. The trypanosomes

African animal trypanosomosis or Nagana is a complex chronic, debilitating and often fatal diseases of animals caused by different species of flagellated unicellular parasites belonging to the genus *Trypanosome* and found in the blood and other tissues fluids of vertebrates including livestock and wild animals (Singh and Singla, 2012). It is mainly transmitted cyclically by the genus Glossina (Tsetse flies), but also transmitted mechanically by several biting flies like Tabanids, Stomoxys, Haematopota and Chrysops. The disease can affect various species of mammals but, from an economic point of view, tsetse-transmitted trypanosomosis, is particularly important in cattle (Desquesnes and Davila, 2002).

Trypanosomes are single celled flagellated protozoan parasites that live and multiply extracellularly in blood and tissue fluids of their mammalian hosts and transmitted by the bite of vector flies. The name Trypanosome is derived from Greek word *trypano* (borer) and *some* (body) because of their cork-screw like motion. The trypanosome consists of a single cell varying in size from 8 to 50 μm. The different trypanosome species differ in morphological characteristics as described by in appearance, shape and size between the various species allowing specific identification (Maudlin *et al*., 2004).

2.2. Taxonomy and classification of trypanosomes

Trypanosomes are unicellular protozoan parasites of the phylum Sarcomastigophora, order Kinetoplastida, due to the presence of a kinetoplast at the base of the flagellum (Eloy and Lucheis, 2009), family Trypanosomatidae and genus *Trypanosome*. Genus *Trypanosome* presents flagella and an organelle recognized by its kinetoplast (Eloy and Lucheis, 2009). On the basis of the site of development in the insect vector, the genus *Trypanosome* is divided into two sections: stercoraria and salivarian. In the stercoraria section the metacyclic trypanosomes develop in the hindgut of the vector and are thus transmitted to the mammalian host via faeces. In contrast, salivarian trypanosomes develop in the anterior portion of the fly's digestive tract

in the salivary glands (*T. brucei*) or in the midgut, *T. congolense* and in the proboscis (*T. vivax*) and are transmitted via the saliva (Peacock *et al*., 2012).

2.3. Etiology of trypanosomosis

Trypanosomosis is an important protozoan disease caused by the genus *trypanosome* transmitted through bites by different species of *Glossina* and mechanically by a number of biting flies such as *Tabanus* and *Stomoxys* species. Bovine trypanosomosis is a parasitic infection caused by an extracellular hemoparasites known as trypanosomes. They swim in body fluids by flagellum, boring their way between cells. They generally, possess a kinetoplast and undergo cyclical development in an arthropod vector. Their biological adaptations, morphology and pathogenicity are fascinating and are being extensively studied (Magona *et al*., 2003). Three main pathogenic species of trypanosomes are recorded in Ethiopia. These are: *T. congolense, T. vivax* and *T. brucei*. *T. vivax* and *T. congolense* are the main pathogens of cattle (Desquesnes and Davila, 2002).

2.4. Morphological characteristics

The different trypanosome species differ in morphological characteristics as described by (Maudlin *et al*., 2004). *Trypanosome congolense* is smaller in size, usually without free-flagellum, but has marginally located medium sized kinetoplast. It is divided into four subtypes with different distributions and pathogenicity: savannah type, forest type, Tsavo type and Kilifi type. *Trypanosome congolense* savannah type is the most pathogenic of the four and is capable of causing severe anemia and even death of infected cattle (Bengaly *et al*., 2002). Other *T. congolense* types cause mild disease that in certain instances does self-cure.

Trypanosome vivax is a monomorphic parasite with distinct free flagellum, larger and terminal kinetoplast. In East Africa, there are two types of *T. vivax* isolates: the hemorrhagic *T. vivax* that causes an acute hemorrhagic syndrome and the mild strain (Magona, *et al.*, 2008). Cattle infected with the hemorrhagic *T. vivax* produce auto-antibodies to red blood cells, a phenomenon that is not observed in the non-hemorrhagic *T. vivax* (Bett *et al*., 2004).

Parasites in *Trypanosome brucei* group show pleomorphic with slender, intermediate or stumpy forms. They have small sub-terminal kinetoplast, undulating membrane with conspicuous posterior end taper to a point except in stumpy forms. During the course of the infection, there is a change in the trypanosome population from the long thin forms, through the intermediate to the short stumpy and this altered appearance is accompanied by a change in the type of respiration, as the trypanosome prepares for its period within the tsetse fly. The short stumpy forms are adapted to living and developing in the tsetse, while long thin forms are the true mature blood forms which die in the gut of the insect. Similar metabolic changes also occur in other trypanosome species, but there are no such obvious morphological changes associated with them as in *T. brucei* (Maudlin *et al*., 2004).

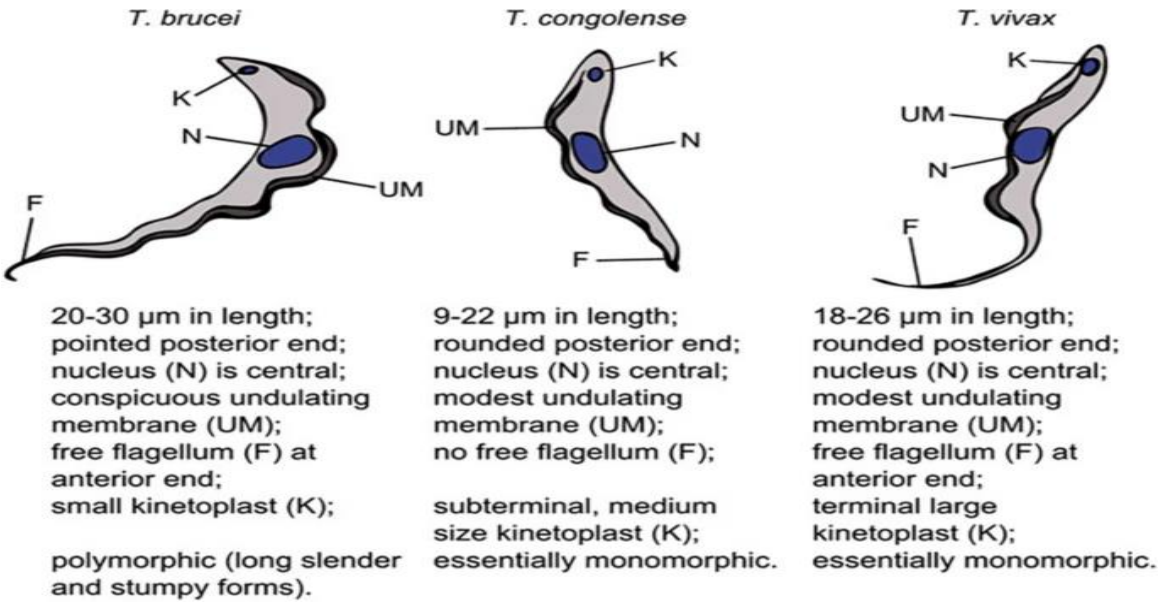

Figure 1: Morphology of trypanosome species (Source: - OIE, 1982)

2.5. Life cycle of trypanosomes

Most tsetse-transmission is cyclical and begins when blood from a trypanosome infected animal is ingested by the tsetse fly. The trypanosome loses its surface coat, multiplies in the fly, then reacquires a surface coat and becomes infective. *Trypanosoma brucei* species migrate from the gut to the proventriculus to the pharynx and eventually to the salivary glands; the cycle for *T. congolense* stops at the hypopharynx and the salivary glands are not invaded; the entire cycle for T.vivax occurs in the proboscis. The animal infective form in the tsetse

salivary gland is referred to as the metacyclic form. The life cycle in the tsetse may be as short as 1week with *T. vivax* or extend to a few weeks for *T. brucei* species (OIE, 1982).

The African trypanosomes have four major life cycle stages. The procyclic form (PF), epimastigote form (EMF) and metacyclic form (MCF) all develop in tsetse while the blood stream form (BSF) is found in the mammalian host (Peacock *et al*., 2012). Tsetse flies ingest infective blood stream trypomastigotes when they feed on infected hosts and may remain infected, acting as a continual source of infection. Tsetse ingests trypomastigote that found in the blood and lymph while feeding on an infected host. The trypomastigotes lose their glycoprotein surface coat and become elongated multiplying in the midgut before migrating forward to the salivary glands and proboscis. They then transform into epimastigote forms which multiply and then transform again into small typical metacyclic trypomastigotes which are the infective stages and are introduced into the vertebrate host during feeding. These metacyclic have been shown to have a small repertoire of Variable Surface Glycoprotein (VSG) genes and they multiply at the inoculation site for a few days before invading blood stream and lymphatics (Pays *et al.*, 2001). This life cycle has variation in reference with the *trypanosome* species involved. Development of *T. congolense* occurs in the midgut and proboscis while development of *T. brucei* takes place in the mid gut and salivary glands (Peacock *et al*., 2012).

Trypanosome vivax completes cyclical development exclusively within the mouth parts of tsetse flies (Peacock *et al*., 2012). The blood trypomastigote forms are taken up by tsetse along with its blood meal and undergo stages of complex biological development inside the insect host before becoming infective. Both male and female flies are capable of transmitting trypanosomes. *Trypanosome vivax* usually multiplies rapidly in blood and is evenly dispersed throughout the cardiovascular system, whereas *T. congolense* tends to aggregate in small blood vessels and capillaries of the heart, brain and skeletal muscle from where a small proportion of parasites enter the blood circulation. *Trypanosome brucei* localize in tissues aside blood vessels (Langousis and Hill, 2014). The parasites are then transmitted to a subsequent host at the next blood meal (Brun *et al*., 2010).

2.6. Mode of transmission

Most trypanosomes must develop for one to a few weeks in tsetse flies (*Glossina spp*), which act as biological vectors before transmitted to susceptible hosts. The tsetse fly becomes infected with trypanosomes when feeding on an infected animal. When an infected tsetse fly bites an animal, the parasites are transmitted to susceptible host in the saliva (CFSPH, 2009). Trypanosome species that commonly infect cattle in Ethiopia such as *T. congolense, T vivax* and *T. brucei* are transmitted to cattle biologically via the bite of infected tsetse flies. Other studies made in different parts of Ethiopia revealed that, in addition to *Glossina spp*, other biting flies such as *tabanids*, *haemtopota* and *stomoxy* are responsible for mechanical transmission of trypanosomes to susceptible animals (Worku *et al*., 2017).

2.7. Pathogenesis

The pathogenesis of African animal trypanosomosis depends on several factors, including parasite-related aspects (species and virulence), host (species, breed, age and nutritional status and physical condition), vector (species, density and infection rate and host preference) and the environment (the availability of food and water and the season (Van den Bossche and Delespaux, 2011). During a blood meal on a mammalian host, an infected tsetse fly injects metacyclic trypanosomes in to the skin tissues (Hunt, 2010). Following inoculation, trypanosomes then continue to proliferate by binary fission for a few days, leading to a local inflammatory response called a chancre. The size of chancre is determined by the animal immune status, the virulence of the infecting trypanosome species and the inoculation dose (Nwoha, 2013). The chancre‟ disappears 3 to 15 days post-infection and trypanosomes enter to the lymph nodes and the blood stream and transform in to blood stream trypomastigotes, which are carried to other tissues. The chancre not only forms a site for the establishment of the infection but also is a focus for multiplication and persistence of trypanosomes before their dissemination in to blood stream (Elnasri, 2005).

2.8. Clinical signs

Bovine trypanosomosis causes severe anemia, edema, immunosuppression and various neurological disorders, which may eventually produce the death of the affected animals (Gonzatti *et al*., 2014). The basic clinical syndrome appears after an incubation period of 8-20 days. There is fever, which is likely to be intermittent and to last for a long period. Affected animals are dull, anorexic and apathetic have a watery ocular discharge and lose condition. Superficial lymph nodes become visibly swollen, mucous membranes are pale, diarrhea occasionally occurs and some animals have edema of the throat and underline. Estrus cycles become irregular, pregnant animals may abort and semen quality progressively deteriorates. The animal becomes very emaciated and cachectic and dies within 2-4 months or longer. Thin, rough-coated, anemic, lethargic cattle with generalized lymph node enlargement are said to have 'fly struck' appearance. Furthermore, intercurrent bacterial, viral, or other parasitic infections may mask or complicate the basic clinical syndrome (Cannor *et al*., 2004).

2.9. Diagnosis

Diagnosis of trypanosomosis in tsetse, humans or domestic livestock is a basic requirement for epidemiological studies as well as for planning and implementing chemotherapy and for monitoring vector control operations. Accurate diagnosis of trypanosome infection in livestock is required for a proper appreciation of the disease in any geographical locality. The general clinical picture is as follows but there are many variations determined by the level of tsetse fly challenge, the species and strain of the trypanosome and the breed and management of the host (Radostitis *et al.*, 2007). Definitive diagnosis of the disease is ultimately dependent on the detection of the trypanosome in blood samples from infected animals (Abebe, 2005).

2.9.1. Clinical diagnosis

The clinical manifestation of bovine trypanosomosis is influenced by the host as well as the trypanosomes species and "strain" (Bezie *et al*., 2014). Infection of cattle by one or more of the three African animal trypanosomes results in subacute, acute, or chronic disease. However,

under natural challenge, disease manifestation may be more complex (Taylor and Authié, 2004).

2.9.2. Parasitological diagnosis

Wet blood film: These are made by placing a droplet of blood (about 2 µl) on a clean microscope slide and covering with a cover slip (22 × 22 mm). The blood is examined microscopically at 40x total magnification with condenser aperture, phase-contrast or interference contrast. Approximately 50–100 fields are examined. Trypanosomes can be recognized by their movement among the red blood cells (RBCs). The method is simple, inexpensive and gives immediate results (OIE, 2021). Final confirmation of the species is made by the examination of the stained preparation. The diagnostic sensitivity of the method is generally low, but depends on the examiner's experience and the level of parasitaemia. Sensitivity can be improved significantly by lysing the RBCs before examination using a haemolytic agent such as sodium dodecyl sulfate (Ndao *et al*., 1995).

Thick blood films: These are made by placing a drop of blood (5–10 µl) on a clean microscope slide and spreading it over an area of approximately 2 cm in diameter, using the corner of another slide. The thickness of the resultant film should be such that when dry, the figures on a wristwatch dial can just be read through it. The film is dried thoroughly by rapidly waving in the air and without fixation is de-haemoglobinised by immersion in distilled water for a few seconds and dried before staining. A dry smear should be kept dry and protected from dust, heat, flies and other insects (OIE, 2021). It is stained for 30 minutes with 4% diluted Giemsa stain in phosphate buffered saline, pH 7.2. Therefore, it is important to start with the manufacturer's directions and to vary staining time and stain concentration to obtain the optimal result. The stained smear is then washed with buffered water and examined at 500 to 1000x total magnification (OIE, 2021).

Thin blood smear: These are made as in the case of blood smears to detect on the blood parasites like trypanosomes. They are fixed by methanol and stained with Giemsa stain, or with one of the more recent test stains such as Diff-Quik, field's stain, which have the advantage of acting much faster than Giemsa. They are read using oil immersion objectives,

for identification of trypanosomes. Hence, what is most important thing of using such a method is that specific diagnosis of trypanosomes is possible. Nevertheless, the sensitivity is extremely low and the main use of thin smear is in fact the specific identification of trypanosomes found in wet or thick (Geysen *et al*., 2003).

2.9.3. Parasite concentration techniques

In this procedure, heparinized capillary tubes are three quarters filled with the suspected blood sample containing an anticoagulant. The dry ends of the capillary tubes are sealed with cristaseal and centrifuged at 12, 000 rpm for 5 minutes. After centrifugation, the buffy coat/plasma junction is located between the plasma and the red blood cells and contains white blood cells as well as the parasites. The capillary tubes are then mounted on a woo chamber and can then be directly viewed at low magnification for mobile parasites (Chappuis *et al*., 2005). The analytical sensitivity of BCT depends on the species of trypanosome as has been demonstrated by Paris (Salas *et al*., 2003), with the smallest numbers detectable per milliliter of blood being 2.5x102, 5x102 and 5x103, for *T. congolense, T. vivax* and *T. brucei*, respectively.

2.10. Epidemiology of trypanosomosis

The epidemiology of African trypanosomosis is determined mainly by the ecology of the tsetse fly which is found only in tropical Africa (Radostits *et al*., 2007). Tsetse flies (*genus Glossina*) are restricted to Africa from about latitude 15° N to 29° S. Ethiopia is situated at the East end of the African tsetse belt and in Ethiopia, tsetse flies are confined to south western and north western regions between longitude 33° and 38 °E and latitude 5° and 12°N of an area covers 220,000 km². The three main species that inhabit relatively distinct environments are: *G. morsitans* usually found in savanna country, *G. palpalis* prefers areas around rivers and lakes and *G. fusca* lives in high forest areas. All three species transmit trypanosomes and all feed on various mammals. The riverine species (*G. palpalis, G. tachinoides, and G. fuscipes* are important as vectors of bovine (Radostits *et al*., 2007).

2.11. Geographical distribution

Bovine trypanosomosis (Nagana) is found in the low lands of Ethiopia, especially in the "tsetse belt". For example, rift valley, Omo, Borena, Metekel Zone of Benshangul Gumuz region. The most important trypanosomes affecting cattle in Ethiopia are: - *Trypanosoma congolense, T. vivax* and *T. brcei* (Mekuria and Gadissa, 2015).

The general distribution of tsetse flies is determined principally by climate and influenced by altitude, vegetation and presence of suitable host animals. Out of the nine regions of Ethiopia, five (Amhara, Beninshangul Gumuz, Gambella, Oromia and Southern Nation Nationalities and peoples region) are infested with more than one species of tsetse flies. To date five species of *Glossina* (*Glossina morsitans submorsitans, G. Pallidipes, G. tachinoides, G. f. fuscipes and G. longipennis)* have been recorded from Ethiopia (Keno, 2005).

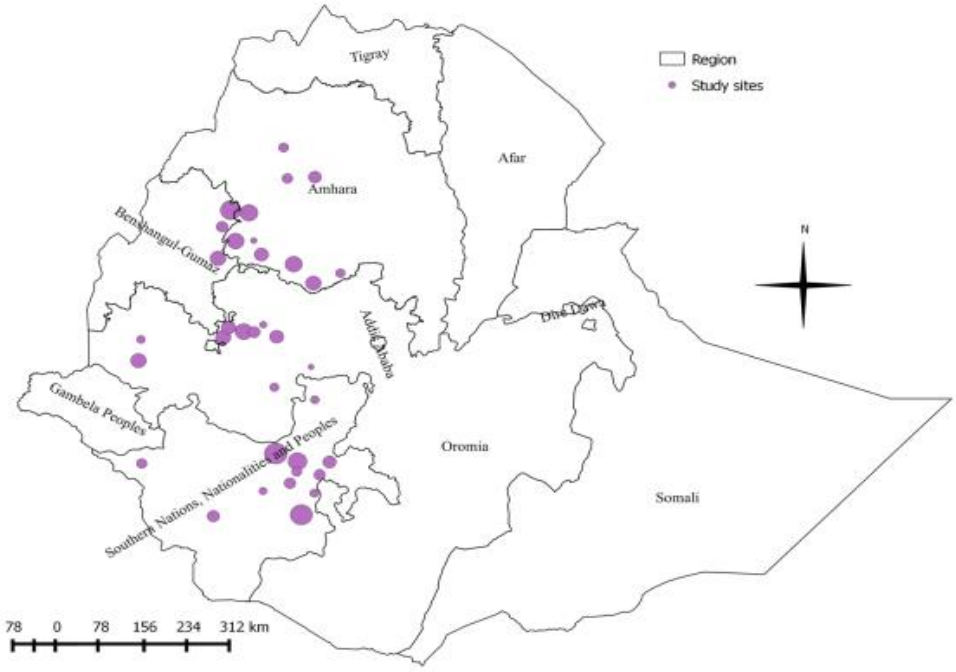

Figure 2: Observed spatial distribution of bovine trypanosomosis in Ethiopia (Leta *et al*., 2016)

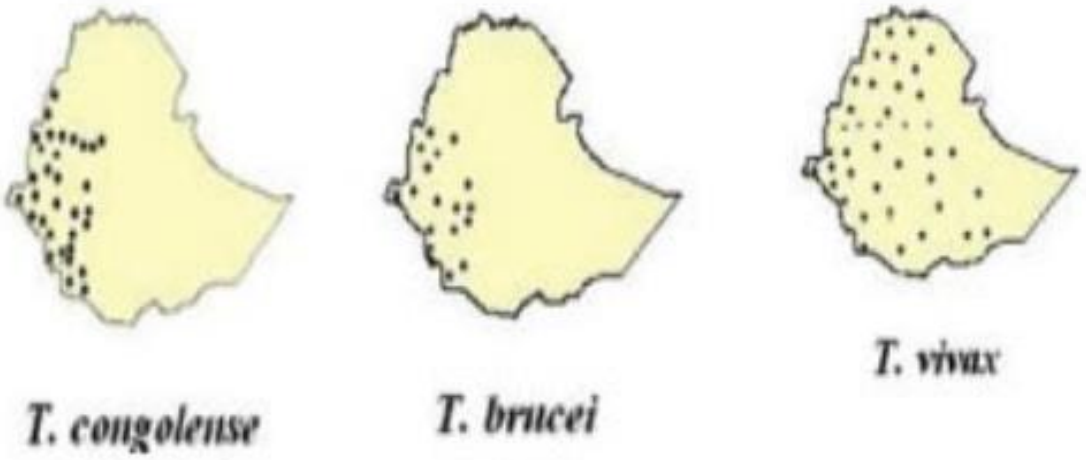

Figure 3: Distribution of pathogenic trypanosomes in Ethiopia (Uilenberg, 1998)

2.12. Risk Factors affecting bovine trypanosomosis

2.12.1. Host related factors

Trypanosomes can infect all domesticated animals; clinical cases have been described in cattle, sheep, goats, camels, horses, donkeys and other species of animals. In parts of Africa including Ethiopia, cattle are the main species affected due to the feeding preferences of tsetse flies (CFSPH, 2009). However, the effect of infection varies with the host in that in most wild animals, such as warthogs, bushbucks, kudus or buffalos, trypanosomes become established but do not produce the disease. This is because these animals and the parasites have evolved for many years resulting in a balanced host/parasite relationship. In domestic animals the relationship with the parasite has not fully developed leading to development of the disease (Namangala, 2011). The level of trypano-tolerance varies; depend on both genetic and environmental in origin. The indigenous zebu cattle are trypano-susceptible and West African Bos Taurus breeds are trypano-tolerant, i.e. they can survive and be productive without treatment under trypanosomosis risk. Exotic imported ruminants (improved dairy cattle) are more severely affected than local genotypes (Taylor and Authie, 2004). In Ethiopian, four cattle breeds namely Abigar, Gurage, Horror and sheko have been thought to be relatively trypano-tolerant when compared to the indigenous zebu cattle (Desta *et al*., 2011).

2.12.2. Environmental factors

Trypanosomosis maintains large area of Africa (so called “fly belts”) and it is suppose that wildlife have contributed a lot in the maintenance of the diseases in a relatively defined ecosystem (Reichard, 2002). The environment allows for the interaction between the *Glossina species*, vertebrate hosts and the trypanosomes in order for trypanosomosis to be occurred. In West Africa, tsetse habitats have been sub-divided along distinct North-South climatic gradients, with predominantly riverine tsetse species in the North and a mixture in the South (Hendrickx *et al*., 2004). In the North, arid conditions prevent fly spread and riparian vegetation constitutes suitable niches for the localized, well-demarcated pockets of tsetse populations. Outside these favorable micro-climates, tsetse hardly survives and it would appear that no links exist between pockets, except occasionally and in spatially limited neighboring areas during the rainy seasons. In the intermediary band, climatic conditions and vegetation become slowly more suitable. Distinct fly pockets tend to merge and tsetse distribution patterns become more linear along main streams. Tsetse population still remain concentrated in pockets during dry season, but spreading (Bouyer *et al*., 2009) during the rainy season over large parts of the river systems, including important tributaries and savannah buffers. In the humid South, there are no climatic limitations to fly distribution and flies are present along river systems and even around humid woodlands and forests.

Due to increasing human population and as a result the opening up of more land for crops, the morsitans group is disappearing in most places of Africa (Djiteye et al., 1997). Riparian tsetse species on the other hand are more versatile and can co-exist with human development. They are opportunistic feeders; where agricultural intensity is low; they feed on wild reptiles and rarely carry pathogenic trypanosomes (De la Rocque *et al*., 2001).

2.12. 3. Pathogen factors

In cattle, *T. vivax* generally produces a higher level of parasitemias than other species since its life cycle in the tsetse is also shorter. Parasite virulence is also an important factor influencing the epidemiology of African animal trypanosomosis. The pathogenicity appears to vary depending on which type or strain of *Trypanosome* is involved (Bengaly *et al.*, 2002). Within

T. congolense, different types exist (savannah, forest, kilifi and Tsavo) that have a different pathogenicity. *Trypanosome congolense* Savannah type is the most pathogenic and is responsible for acute infection and death of diseased animal. However, *T. congolense* forest and Kilifi types cause mild infections (Bengaly *et al*., 2002). Apart from *T. congolense*, other members of sub genus *Nannomonas* causing AAT include: - *T. simiae* (affecting domestic suids) and *T. Godfrey*i (Van Den Bossche *et al*., 2011).

2.13. Economic impacts of bovine trypanosomosis

In domestic animals, trypanosomosis is a disease with a great economic impact, affecting not only the wellbeing of the livestock population, but also efficient food production in crop-livestock production systems (Shaw *et al*., 2014). African animal trypanosomosis puts 50 million cattle at risk and leads to the death of three million animals every year, inflicting a direct annual loss of US$ 1.0-1.2 billion in cattle production (Cecchi *et al*., 2014).

In Ethiopia, a study conducted on socioeconomic impacts of trypanosomosis on cattle in Girja district of southern Oromia by Chanie *et al*., 2013 indicated that the total household expenditure on trypanocidal drugs was increasing from time to time. The finding of Chanie *et al*., 2013 also indicated that trypanosomosis has direct impact on livestock productivity by reducing 23% meat and milk off take, 5% increase in calving rate, 13.5% mortality and livestock management especially the number of livestock kept by farmers, the breed and species composition of the herd, 12% loss of draught power, 3% abortion and 28% cost of trypanocidal drugs and insecticides in the district.

Trypanosomosis directly affects the milk and meat productivity of animals, reduces birth rates, increases abortion as well as mortality rates and all of these reduce the herd size and herd composition. The indirect impact of the disease mostly lies on crop production through the availability and cost of animals that provide traction power (Swallow, 2000). The overall negative impact extends to the access and availability of cultivable areas, changes in land use and exploitation of natural resources, restrain of opportunities for diversification and intensification of agricultural activity.

2.14. Control of bovine trypanosomosis

There has been a long history of tsetse and trypanosomosis control in Africa, but today the problem is still far from being solved and there is no control method that can fully eradicate African animal trypanosomosis and the incidence of both animal and human trypanosomosis remained high with occasional endemic outbreak (Achukwi and Musongong, 2009).

Despite, extraordinary research efforts directed at the development of vaccines against trypanosomes, no vaccine has so far has been developed in the near future (Magez *et al*., 2010). Hence, control of animal trypanosomosis relies primarily on control of the vector, farming of trypano-tolerant breeds and use of trypanocidal drugs (Holmes, 2013). Prevention of successful establishment and/or maturation of trypanosomes within the tsetse fly have been proposed as possible future control method (Aksoy *et al*., 2003).

2.15. The tsetse vector

Tsetse fly (Diptera, Glossinidae) is large biting fly that inhabit about 10 million km^2 of area in 37 sub-Saharan Africa countries and are distributed discontinuously throughout their range, and each taxon is restricted to a relatively specific habitat (Gooding and Krafsur, 2005).

Depending on the environmental type, there are three main subgroups of Tsetse flies: Palpalis (riverine), Morsitans (savannah) and Fusca (forest-dwelling) (Wamwiri and Changasi, 2016). Approximately one-third of Africa's total landmass is infested by these flies (Leta *et al*., 2016). Vector distribution mainly confined to the Southern and Western regions between longitude of 33°and 38°E and latitude of 5°and 12°N (Kotye, 2006). Among 31 species of tsetse flies, five species: - *Glossina pallidipes, G. morsitans, G. fuscipes, G. tachinoides and G. longipennis* are known in different regions of Ethiopia (Amhara, Benishangul Gumuz, Gambella, Oromia and Southern Ethiopia (Bangu and Eyob, 2017).

Morsitans group: Are also called the savannah flies due to their preference to this environment and the most important vectors as the African savannah is a vast area and the flies come into contact with man, livestock and wild game animals. In Ethiopia, this group is

distributed in Didessa valley near the village of Wonago and Lado on the eastern side of Lake Abaya, Shambo, on the Mugher River, on the Dabous River (Wollega), on the Baro and Gilo Rivers (Gambella district), Illubabor associated with Akobo river, in the Savannah near Turmi and near Mizan Teferi (Shumago and Tekalign ,2016). All species belonging to this group are restricted to savannah wood lands and their distribution and abundance is tied with wild animal's distribution. During dry season, they are concentrated near the source of water courses and spread out in wooden savanna during the rainy season. The species under these groups are; *G. morsitans* and *G. pallidipes* identified from Ethiopia. *G. pallidipes* is highland species being present in some coastal areas and rift volley (Hordofa and Haile, 2017).

Palpalis group: The distribution of the palpalis group species is likewise associated with lowland rain forest (Vreysen *et al*., 2012), specific vegetation like riparian forests that line the hydrographical network or plantations of certain crops and extended along river systems in the humid savanna. They are also called riverine tsetse fly groups and can tolerate a wide range of climatic conditions (Vreysen *et al*., 2012). There are two species of palpalis group in Ethiopia; *G. tachinoides* and *G. fuscipes*. *G. fuscipes* is found in Maze, Gorgora, Bazo and Cuccia Rivers (Gamo Gofa), on the Ketto tributary and at Degeno of the Birbir (Wellega), on the tributary of the Gojeb (Kaffa) and near the bridge on the Omo River and Addis to Jimma high way (Hordofa and Haile, 2017).

Fusca Group: They are forest tsetse flies and are densely colonized where vegetation are found (Hordofa and Haile, 2017). In transition zones between true forest and wooden lands, they prefer dense shade and riverine thickets. These mainly forest-dwelling groups consequently have little epidemiological significance. In Ethiopia, the distribution of this vector is along the Walmal River (Bale), at tributary of Wabe Shebele in (Daghato River) in the Ogaden and near Lake Abaya, Gamo goffa and Keffa. Under this group, there are two species of tsetse flies i.e. *G. brevipalpis* and *G. longipennis*. *G. brevipalpis* is found only at the lower part of the Omo River (Hordofa and Haile, 2017). Both males and females are blood eaters and therefore, both sexes play a role as potential vector for trypanosome. In addition to that, once a tsetse fly has been infected, they remain infected throughout its life (Krasfur, 2010).

2.16. Status of tsetse fly and trypanosomosis in Ethiopia

Unfortunately, the development and intensification of livestock productivity in Ethiopia is hampered among others by cross-border epizootic diseases such as African animal trypanosomosis. Out of the nine regions of Ethiopia (Oromia, Benishangul Gumuz, Amhara, Gambella and SNNPR) are infested with more than one species of tsetse flies (Abebe, 2005).

In tsetse infested areas 14 million of cattle, equivalent number of small ruminants and more than 7.5 million equines and 1.2 million of camels are at risk of contracting trypanosomosis. Trypanosomosis also prevents full use of land and the introduction of highly productive exotic dairy animals and draught oxen to low land areas (Taye *et al*., 2012).

The prevalence of trypanosomosis in tsetse infested areas range from 11.85-37% (Fikru *et al.*, 2012). According to Geja *et al*, 2012, the fly density, trypanosomosis prevalence and mortality due to trypanosomosis have been significantly reduced and the government of Ethiopia has conducted a massive settlement program in early 2000s to the tsetse and trypanosomosis belt.

3. MATERIALS AND METHODS

3.1. Description of the study area

Zala district is found in Gofa zone in South Nation Nationality and Peoples regional state of Ethiopia. The area is characterized by two major climatic condition, which includes dry (December to February) and wet (June to September). The district has 36 Kebeles and from which three Kebeles were purposively selected namely: Deboch-Bena, Melagayle-Tosa and Wagesho for this study. The district is 510 Kms away from south of Addis Ababa, 284 km away West from Hawassa and 240 km away South-west from Arba-Minch. Zala district is bordered in the North by Kucha district, in the South by UbaDebretsehay district, in East by Kemba and Daramalo district and in the West by Demba Gofa District **(**Figure 3**)**.

The study Kebeles were covered by different types of vegetation (the vegetation is dominantly occupied wood grass land (WGL) and has an altitude of 1336m, 1310m and 1115m above sea level of Deboch-Bena, Melagayle-Tsosa and Wagesho, respectively. The districts mean annual temperature and humidity ranges from18°C–32°C and 10%–90%, respectively. The area receives an annual minimum rainfall of 800 and a maximum of 1000 ml. Zala district is characterized by bimodal rainfall pattern, the short rain falls between March and April and the long rainy season between June and September. Agriculture is the main livelihood in the area in which cattle and goats kept as the major livestock which are highly important for the livelihood of the local population. Livestock existing in the district is estimated to be 203,095 cattle; 4,015 sheep; 48,233 goats; 314,587 poultry; 213 mules and 13,171 donkeys (ZWADO, 2023).

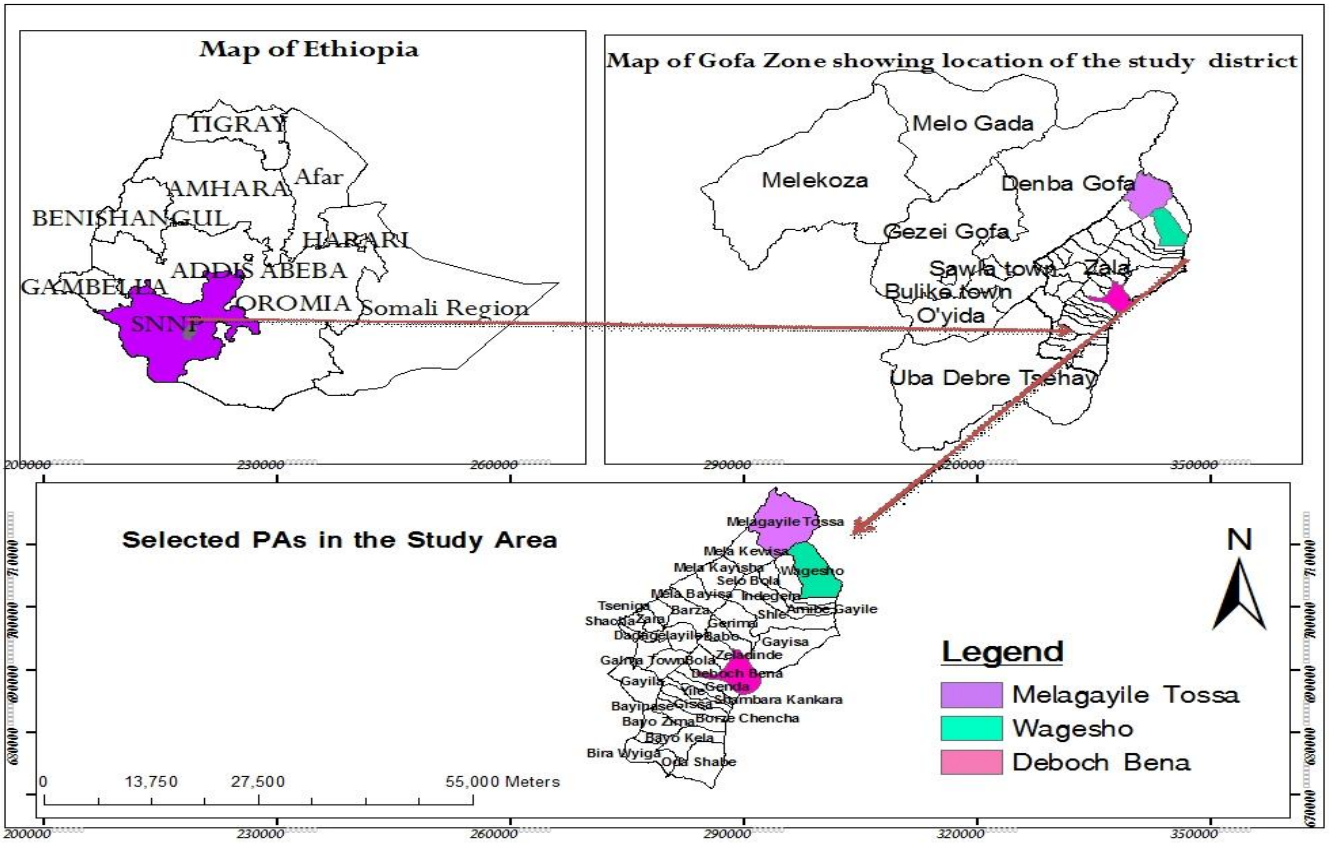

Figure 4: Map of the study area from different administrative hierarchies created by researcher using Arc GIS Version: 10.1.

3.2. Study animals

The study animals were local zebu cattle (*Bos indicus*), which were kept under an extensive management system, together with other livestock species in the three Kebeles of Zala district. They herded together during the day time and returned to their individual owner's house stead each evening. Both male and female cattle above one year of age were included in the study. During sampling, all parameters including; season, age, sex, body condition and colour of the animals were recorded. The age of study animals were estimated by means of their dentition as described by (Radostits and Gray, 2007) and conventionally classified into three age categories: less than 3 years, between 4 and 6 years and greater than 7 years of age. For convenience of the study, the colour of studied animals grouped as (black and black spot),

(red) and (gray, white and white spot). The body condition of the animals was also grouped as ‘good, medium and poor’ based on criteria described by (Nicholson and Butterworth, 1986).

3.3. Study design

Repeated cross-sectional study design was conducted to estimate the seasonal prevalence of bovine trypanosomosis and apparent density of *G. pallidipes* from December 2022 to September 2023 in Zala district, Gofa zone, Southern Ethiopia. This repeated cross-sectional study provides information about the seasonal prevalence of bovine trypanosomosis and tsetse fly distribution in selected areas of Zala district.

3.4. Sample size determination

The total number of animals required for the study were computed by using the expected prevalence of 10.17% as per the previous report in Rift valleys of Gamo zone in Southern, Ethiopia (Seyoum *et al*., 2022) which has similar ecology with the present study district with 95% confidence interval and absolute precision of 5%. Sample size was calculated by using the formula described by Thrusfield, (2018).

$$n=\frac{1.96^2 x Pexp\ (1-Pexp)}{d^2}$$

Where; n= required sample size

Pexp= expected prevalence

d^2= desired absolute precision

Z = 1.96 for 95% confidence interval.

Hence, the total sample computed was 140 cattle per season, which were 280 cattle for the two seasons. But to increase the precision, the sample size was increased by 100% for each season. Therefore, a total of 560 animals (280 in each season) were sampled for parasitological study.

3.5. Sampling techniques

Three study Kebeles were selected purposively based on the complaint by animal owners about trypanosomosis and history of tsetse fly infestation. Kebele refers to the lowest administrative division of a district in Ethiopia but greater than a village. The study animals were sampled by using systematic random sampling technique and sample size was allocated proportionally based on the cattle population that exists in selected Kebeles. Households were selected from the list obtained in the Kebele and within in the selected household's temporary id number given to the animals based on age and colour and first animal selected randomly from interval and continued until required sample size achieved. All parameters like: season, sex, age, body condition score and coat colour were recorded for each individual animal during sample collection. The age of study animals were estimated by means of their dentition as described by (Radostits and Gray, 2007) and conventionally classified into three age categories: less than 3 years, between 4 and 6 years and greater than 7 years of age. According to the body condition of the animals, they were grouped as poor, medium and good based on the appearance of ribs and dorsal spines applied for zebu cattle (Nicholson and Butterworth, 1986).

3.5.1. Study population of cattle and sample taken

The total cattle populations in the selected three Kebeles were 16,254 (ZWADO, 2023). Thus, sample sizes of 560 were considered for the study district. The cattle population of selected Kebeles, Melagayle-Tosa, Wagesho and Deboch-Bena were 6,224 (38.3%), 5,800 (35.7%) and 4,230 (26%), respectively. Therefore, the total sample size was allocated based on the cattle population proportion existed in the three selected Kebeles. Accordingly, the sample size allocated for Melagayle-Tosa, Wagesho and Deboch-Bena was 214, 200 and 146 cattle, respectively. The same procedure and sample size (280) were used in both seasons; 560 cattle were shown in (Table 2).

Table 1: Study population of cattle and coordinate points

Kebele	Study Population	Cattle Sampled	Altitude (By using GPS)	Longitude	Latitude
Melagayle-Tosa	6,224	214	1310	37.14009	6.44374
Wagesho	5,800	200	1115	37.17142	6.38833
Deboch-Bena	4,230	146	1336	37.09597	6.2306

3.5.2. Inclusion and exclusion criteria during sampling

The inclusion criteria required were animals whose age were more than one year which were not treated against trypanosomosis before at least one month prior to sample collection and managed under extensive production system. However, exotic breeds of animals and those animals managed under intensive production system were excluded from this study because the number of exotic breeds were low to get enough information in the study area and animals managed under intensive production system were less exposed to the vectors since they were kept close to the homestead where tsetse fly has been controlled.

3.6. Study Methodology

3.6.1. Identification of trypanosome parasite by using buffy coat technique

To estimate seasonal prevalence of bovine trypanosomosis and to assess risk factors associated with trypanosomosis, a cross-sectional study was conducted two times during the study period, in the dry and wet season. The superficial ear vein of the study animal was punctured and blood samples were collected by using heparinized capillary tubes. Then one end of the capillary tubes sealed with Crista seal. The capillary tubes were transferred to a hematocrit centrifuge and centrifuged immediately for 5 minutes at 1,200 revolutions per minute. After the centrifugation, packed cell volume (PCV) was measured by using hematocrit reader for the

determination of the presence or absence of anemia. Animals with PCV ≤ 24% were designated as anemic and those with PCV>24% were considered as non- anemic (Radostits *et al*., 1994). Then after, capillary tubes were cut by using a diamond pen at about 1 mm below the buffy coat to include the uppermost layers of the red blood cells and 3 mm above to include the plasma. The content of the capillary tubes were expressed onto a clean slide; the content was then carefully mixed and covered with a 22x22 mm cover slip. Finally, the slide was examined under 40x objective and 10x eye piece for movement of parasite and the trypanosome species were identified based on their movement pattern during the buffy coat examination as described by (Murray *et al*., 1977). The confirmation of trypanosome species by morphological characteristics was done after staining the blood smear with Giemsa and examination with oil immersion microscopy with 100x power of magnification (Luckins, 1992, Murray *et al*., 1977).

3.6.2. Entomological survey

In the present study, an entomological study was conducted to identify the tsetse fly species prevailing in the area and to estimate the seasonal apparent density of tsetse flies and other biting flies in the study district. This study was conducted in two seasons: dry season (December 2022 - February 2023) and wet season (June - September 2023). For the entomological survey, 80 NGU traps baited with acetone were used. The traps were deployed at approximate intervals of 200-250m for 72 hours in watering and grazing points in which the animals and vector are believed to have frequent contact (Pollock, 1982). The poles of each trap were greased to prevent fly predators, mainly ants. The coordinates of trap deployment sites were recorded by using GPS. Trap deployment sites were selected to represent all vegetation type or habitat that can be related to fly multiplication, behavior, feeding and other related aspects. After 72 hours of deployment, tsetse and other biting flies that trapped in the cages were counted and identified according to their morphological characteristics such as size, color, wing venation structure and proboscis at the genus and species level (Pollock, 1982, Wall and Shearer, 1997). Sexes of tsetse flies were identified by observing the posterior end of the ventral aspects of the abdomen. Male flies were identified by their enlarged hypophgeum in the posterior ventral end of the abdomen; but in female tsetse flies, this

structure is absent. An apparent density of the tsetse fly was calculated using the formula: fly catch/trap/day.

3.7. Data management and analysis

Data collected from parasitological and entomological survey were entered to Microsoft excel spread sheet 2010 and then filtered and coded before analysis. STATA version 14.2 software was used for the data analysis. The prevalence of trypanosomosis was calculated by dividing the number of infected individuals to the total number of examined animals and then multiplied by 100 (Thrusfield, 2018). The difference in the prevalence of trypanosomosis (dependent variable) between the study areas: season, sex, age, body condition and cattle of different coat colour (independent variables) were analyzed by using logistic regression analysis and the association of trypanosomosis with anemia (as determined by low mean PCV %) was evaluated by Student's t-test. PCV value cut off of 24% was used to determine the presence of anemia in the study animals. The test result was considered as significant when the calculated p-value was less than 0.05. Those risk factors with $p< 0.25$ in the initial univariable logistic analysis were checked for collinearity and those variables with gamma value between -0.6 and +0.6 were further subjected to multivariable Logistic regression analysis. Odd ratio (OR) was used to associate the statistical strength of trypanosomosis positivity with different potential risk factors. In all the analysis, confidence level and desired absolute precision were held at 95% and 5%, respectively. The apparent density (AD) of the tsetse fly population and biting flies were calculated by dividing the number of flies caught by the number of traps deployed and number of days of deployment and expressed as fly catch /trap/ day.

3.8. Ethical Consideration

The animal study was reviewed and approved by Animal Research Ethics Review Committee of Southern Ethiopia Tsetse and Trypanosomiasis Control and Eradication Institute. Written informed consent was obtained from the owners for the participation of their animals in this study. Animal owners were convinced that the study was not harm their cattle and proper caution was followed while sample collection and restraining procedures were taking place.

4. RESULTS

4.1. Prevalence of trypanosomosis

Out of the total 560 cattle examined (i.e., 280 in the dry and 280 in the wet seasons), 39 animals were found positive for trypanosome infection with an overall prevalence of disease at 6.96% (39/560) (Table 3).

Table 2: Prevalence of bovine trypanosomosis in selected Kebeles in Zala district

Kebeles	Number examined	Positive	Prevalence (%)	95% CI
Wagesho	200	13	6.5 %	4 -10.9
Melagayle-Tosa	214	18	8.4 %	5- 13
Deboch-Bena	146	8	5.47 %	2.7- 10.6
Total	560	39	6.96 %	5.1- 9

CI = Confidence interval

4.1.1. Proportion of trypanosome species identified

Two species of trypanosomes, *T. congolense* and *T. vivax* were identified in both dry and wet season of the study district. In both seasons of the study area, *T. congolense* was the predominant species which accounted for 87.2% (34/39) of the overall infection and *T. vivax* for 12.8% (5/39) (Table 4).

Table 3: Prevalence of trypanosome species identified in the dry and wet season (n = 39)

Season	Number Positive	*Tryp. Congo* (%)	*Tryp. Vivax* (%)	Proportion of Trypanosoma species (%)
Wet	27	25 (64.1%)	2 (5.1%)	27 (69.2%)
Dry	12	9 (23.1%)	3 (7.7%)	12 (30.77%)
Total	39	34 (87.2%)	5 (12.8%)	

Tryp. Congo = Trypanosoma congolense, CI = Confidence interval

4.1.2. Univariable and multivariable logistic regression analysis of risk factors

The analysis revealed that a significantly higher prevalence ($p< 0.05$) was recorded in wet season (9.64%) than dry season (4.28%). Also, there was a significant difference in the prevalence of bovine trypanosomosis among the different body condition score of animals ($p < 0.05$), which was higher in poor body condition score of cattle (11.5%) than in good (2.7%) and medium (4.19 %). The differences in the seasonal prevalence among various coat colours were also statistically significant ($p < 0.05$). Out of 560 samples collected both in dry and wet seasons, 8.33% and 6.25% samples were positive for bovine trypanosomosis in male and female, respectively. The prevalence of bovine trypanosomosis with the age categories was, 7.36%, 3.7% and 13.28 % in age of ≤3; 4-6 and ≥7, respectively (Table 5).

The multivariable logistic regression analysis was done after checking for collinearity; all variables with $p < 0.25$ in the univariable analysis (season, coat colour and body condition scores) were subjected to multivariable logistic regression analysis. The final multivariable logistic regression model for potential risk factors revealed that season, coat colour and body condition score had a significant association with trypanosome prevalence and hence, potential factors ($p < 0.05$). The Hosmer–Lemeshow goodness-of-fit test suggested that the model fits the data ($\chi 2 = 8.49$; $p = 0.29$) (Table 6).

Table 4: Univariable logistic regression analysis of risk factors

Variable	Category	No.of animal Examined	No.of infected Animal (%)	OR	95% CI	p-value
Season	Dry	280	12 (4.28%)	-	-	Ref
	Wet	280	27 (9.64%)	2.38	1.18- 4.80	0.015
Sex	Male	192	16 (8.33%)	-	-	-
	Female	368	23 (6.25%)	0.73	0.37 - 1.42	0.359
Age	≤3	163	12 (7.36%)	-	-	Ref
	4-6	269	10 (3.7%)	0.48	0.20 -1.15	0.101
	≥7	128	17 (13.28%)	1.92	0.88 - 4.19	0.099
BCS	Good	72	2 (2.77%)	-	-	Ref
	Medium	262	11 (4.19%)	1.53	0.33 - 7.08	0.584
	Poor	226	26 (11.5%)	4.55	1.05-19.66	0.042
C.colour	W+ G and Ws	183	10 (5.46%)	-	-	Ref
	Red	320	17 (5.3%)	0.97	0.43 - 2.16	0.942
	B and Bs	57	12 (21.05%)	4.61	-	0.001

BCS = Body condition score, OR= Odds ratio, W= White, G= Gray, Ws=White spot, B=Black, Bs= Black spot, CI: Confidence interval

Table 5: Multivariable logistic regression analysis of risk factors

Variable	Category	No.of animals Examined	No.of infected Animal (%)	OR	95% CI	p-value
Season	Dry	280	12 (4.28%)	-	-	Ref
	Wet	280	27 (9.64%)	2.24	1.08 - 4.62	0.029
BCS	Good	72	2 (2.77%)	-	-	Ref
	Medium	262	11 (4.19%)	1.82	0.38 - 8.69	0.451
	Poor	226	26 (11.5%)	5.94	1.31 - 26.87	0.020
C. colour	W+ G and Ws	183	10 (5.46%)	-	-	Ref
	Red	320	17 (5.3%)	0.95	0.42 - 2.17	0.916
	B and Bs	57	12 (21.05%)	5.32	2.07 - 13.66	0.001

W=White, G=Gray, WS= white spot, B= Black, Bs = Black spot, BCS = Body condition score, OR= Odds ratio, CI: Confidence interval

4.2. Hematological findings

The hematological examination revealed an overall mean PCV (±SD) 22.77% (±6.21) (95% CI = 22.26-23.29). The mean PCV of infected and non-infected animals were (20.89 ± 4.04) and (22.9 ± 6.32 %). Moreover, the dry and wet season mean PCV of studied animals were, (22.07 ± 6.33) and (23.48 ± 6.02), respectively (Table 7).

Table 6: Analysis of the association of trypanosome infection with mean PCV (%) of cattle

Variable	Category	Animal Examined	Mean PCV (%)	Std. dev.	95% CI	t- value	p-value
Infection status	Non infected	521	22.9 %	6.32	22.37- 23.46		
	Infected	39	20.89 %	4.04	19.58 -22.20	1.96	0.025
Season	Dry	280	22.07 %	6.33	21.32 - 22.8		
	Wet	280	23.48 %	6.02	22.77- 24.19	2.69	0.99
	Overall	560	22.77 %	6.21	22.26-23.29		

Std. dev. = Standard deviation, CI: Confidence interval, PCV= Packed cell volume

4.3. Entomological results

4.3.1. Seasonal proportion of fly count

A total of 80 NGU traps were deployed approximately 100-250m apart and left in position for three days each for dry and wet season. From all traps deployed both in wet and dry season, a total of 564 tsetse fly and other biting flies were caught in 72 hrs. Out of these, 56.9% (321/564) belongs to tsetse flies and the remaining 43.1% (243/564) were biting flies. *G. pallidipes* was identified as the only tsetse fly species in the study area. The proportion of biting flies that commonly encountered during the study period were *Tabanus species*, 37.9% (92/243) and *Stomoxys species* 62.1% (151/243). A total of 65.8% (371/564) of the flies were caught during the wet season and 34.2% (193/564) of the flies were caught during the dry season (Table 8).

Table 7: Proportion of fly caught in the wet and dry season (72 hours)

Season	Tsetse flies no. (%)				Biting flies no. (%)			Total fly
	Male	Female	Unkn sex	Total	Tabanus	Stomoxys	Total	
Wet	79(36.9)	126(58.9)	9(4.2)	214	63(40.2)	94(59.9)	157	371
Dry	41(38.3)	63(58.8)	3(2.8)	107	29(33.7)	57(66.3)	86	193
Total	120(37.4)	189(58.9)	12(3.7)	321	92(37.9)	151(62.1)	243	564

No = Number

4.3.2. Seasonal apparent density

The overall apparent densities of *G. pallidipes* and biting flies in the study area were 1.38 f/t/d and 1.01 f/t/d, respectively. The apparent density of *G. pallidipes* and biting flies was higher in the wet season than in the dry season (Table 9). Based on the study kebeles, in wet season, the highest tsetse fly density were observed in Wagesho and the lowest recorded in Melagayle-Tosa, which was 2.45 f/t/d and 0.93 f/t/d, respectively. In dry study season, the highest tsetse fly density were observed in Melagayle-Tosa and the lowest recorded in Deboch-Bena, which was 1.2 f/t/d and 0.37 f/t/d, respectively. Similarly, in wet season, the highest other biting fly

density was observed in Melagayle-Tosa and the lowest recorded in Wagesho, which was 1.47 f/t/d and 1.25 f/t/d, respectively and also in dry season, the highest other biting fly density was observed in Melagayle-Tosa and the lowest recorded in Deboch-Bena, which was 0.93 f/t/d and 0.3 f/t/d, respectively, of the study area (Table 9).

Table 8: Seasonal apparent density of *Glossina pallidipes* and biting flies

Season	Kebeles	No. of traps used	*G.pallidipes*					Biting flies	
			Male	Female	Un sex	Total	f/t/d	Count	f/t/d
Wet	Wagesho	20	62	78	7	147	2.45	75	1.25
	Deboch- Bena	10	11	27	1	39	1.3	38	1.27
	Melagayle-Tosa	10	6	21	1	28	0.93	44	1.47
	Total	40	79	126	9	214	1.78	157	1.3
Dry	Wagesho	20	27	43	2	72	1.2	49	0.8
	Deboch-Bena	10	3	8	0	11	0.37	9	0.3
	Melagayle-Tosa	10	11	24	1	36	1.2	28	0.93
	Total	40	41	75	3	119	1	86	0.7
	Overall	80	120	201	12	333	1.38	243	1.01

Un = Unknown, f/t/d =Fly per trap per day

5. DISCUSSION

In the present study, an attempt was made to estimate the seasonal prevalence of bovine trypanosomosis and the tsetse fly distribution. The finding of this study indicated that an overall prevalence of cattle trypanosomosis was 6.96%. This finding was in general agreement with previous studies by (Efrem *et al*., 2013 in Lalo-Kile district of Kelem Wollega Zone, Alemayehu *et al*., 2012 in Chena district of South-West Ethiopia and Dano *et al*., 2014) in Guto Gida Woreda of East Wollega Zone, who reported an overall prevalence of 6.86%, 6.90% and 7.81%, respectively.

The present result was higher than the reports of (Eyasu *et al*., 2021, Girma *et al*., 2014 and Anjulo *et al*., 2019), who reported an overall prevalence of 4.98%, 1.56% and 1.75%, respectively, from along the escarpment of Omo River, Loma district in Southern Ethiopia and Arba-Minch area. In contrast, the current finding was lower than the previous findings reported from different parts of Ethiopia (Seyoum *et al*., 2022, Bitew *et al*., 2011, Muktar *et al*., 2016 and Tesfaheywet and Abraham, 2012). They reported a prevalence of 10.17%, 21%, 29.5% and 27.5%, from Arba-Minch Zuria district, Jabi Tehenan district in western Ethiopia, Metekel district and Arbaminch district, respectively. The variation between the reports might be due to the difference in management system, animal susceptibility, fly control operations, the development of drug resistance and increasing of tsetse challenge due to higher vector density in the study area (Degneh *et al*., 2017, Amante and Tesgera, 2020).

Two species of trypanosome, namely *T. congolense* and *T. vivax*, were noted to cause bovine trypanosomosis in the present study areas. The majority of the infection (87.2%) was caused by *T. congolense*. The predominance of *T. congolense* in the current study is in a general agreement with various reports from different parts of south and south-western Ethiopia (Tadesse and Tsegaye, 2010, Ayele *et al*., 2012, Teka *et al*., 2012, Berhe *et al*., 2015, Sheferaw *et al*., 2016, Abebe *et al*., 2017, Bezabih and Bisho, 2017, Tadele and Ayichew, 2017). The relatively higher predominance of *T. congolense* infection in cattle suggests the increased contact of cattle with the savanna tsetse flies, particularly *G. pallidipes*, which are more efficient transmitters of *T. congolense* than *T. vivax* in East Africa (Abebe and Jobre,

1996). It may be also due to the high number of serodems of *T. congolense* compared with *T. vivax* and the development of better immune response to *T. vivax* by the infected animal in the study area (Ephrem and Geja, 2019).

The multivariable logistic regression analysis for potential risk factors showed significantly higher prevalence of trypanosomosis in the wet season (OR = 2.24, $p < 0.05$), black and black spot coated colour (OR = 5.32, $p < 0.05$) and poor body conditioned animals (OR = 5.94, $p < 0.05$). This result was in close agreement with the previous studies reported by (Eyasu *et al*., 2021, Seyoum *et al*., 2022, Dagnachew *et al*., 2005 and Degneh, *et al*., 2017). In the current study, seasonal prevalence of trypanosomosis was significantly higher in the wet season (9.64%) than in the dry season (4.28%). This result is in a general agreement with the previous study (Seyoum *et al*., 2022 and Eyasu *et al*., 2021) reported in Rift Valleys of Gamo Zone, and escarpment of Omo River, Loma district in Southern Ethiopia, respectively. This may support the statement that season is a well-known limiting factor for cattle trypanosomosis (Nnko *et al*., 2017). This might be due to an absolute increase in the number of tsetse and biting flies in the wet season due to favorable environmental factors: such as enough moisture, vegetation growth and suitable habitat, which in turn, lead to an increase in trypanosome challenge to cattle resulting into the observed difference during the two study seasons (Brightwell *et al*., 1987).

Bovine trypanosomosis in the present study was found to be influenced by the coat colour of cattle. There was significant difference in prevalence among three hair coat colored animals at the present study area. As shown by multivariable logistic regression analysis, trypanosomosis was significantly higher in cattle with black and black spot coat colour ($P < 0.05$) than in white, white spot, gray and red colored animals. The odd ratio of trypanosomosis in cattle with black and black spotted coat colour was 5.32 times higher than that of other colored animals. Black coat colour animals were more affected by trypanosomosis than the other colour considered in this study. This finding was in agreement with the observation of Abebe *et al*., 2017 in Omo-Ghibe tsetse belt, Sheferaw *et al*., 2016 in selected parts of the southern rift valley and Girma *et al*., 2014 around Arba-Minch town. The present finding also accords with the assumption that tsetse flies are more attracted to dark colour like the hides of cow and this

may increase the chance of black coat coloured animals to be bitten by the flies and facilitate the transmission of trypanosome (Leak, 1999).

Body condition of the studied cattle was another factor that has showed strong association with trypanosomosis prevalence. It is well established fact that trypanosomosis is a wasting disease that results in a progressive loss of body condition (Radostits *et al*., 2007), although poor body condition could result from other factors, such as concurrent nutritional and parasitic diseases, bovine trypanosomosis is also a devastating and wasting disease, which results in a progressive loss of body condition (Steverding, 2008). Besides, trypanosomosis was observed to be higher in animals with poor body condition when compared with those in good or medium body conditions. It meant that trypanosomosis causes emaciation of animals (Teka *et al*., 2012, Abebe *et al*., 2017). There was significant difference in prevalence among body condition scores of animals at the present study district. The present finding revealed that the occurrence of infection was 11.5% in animal with poor body condition which was higher than 4.19% and 2.77%, in animals with medium and good body condition, respectively. This finding is in line with the previous reports from Ethiopia (Ayele *et al*., 2012, Lelisa *et al*., 2014 and Abebe *et al*., 2017), who found the highest prevalence of trypanosomosis in poor body conditioned animals. This might be attributed to reduced resistance of those animals having poor body condition or related to the progressive weight loss arising from debilitating nature of the disease itself (Radostits *et al*., 2007).

The hematological findings revealed that, overall mean PCV value of all studied animals was 22.77%. The mean PCV of the infected animals (20.89 ± 4.04) was significantly ($p < 0.05$) lower than that of the non-infected ones (22.9 ± 6.32 %). The lower mean PCV value in the infected animals has been reported by authors from different corners of the country (Seyoum *et al*., 2022, Desta, *et al*., 2013, Amante and Tesgera, 2020, Yalew and Fantahun, 2017, Zeryehun and Abraham, 2012 and Anjulo *et al*., 2019). Variation between infected and non-infected animal mean PCV value indicates that trypanosomosis might be involved in the reducing of the PCV values in the infected animals (Murray *et al*., 1977). However, trypanosomosis is typically suspected to reduce the PCV. It is worthy to note that other factors like blood-sucking gastrointestinal parasites and nutritional deficiencies also cause the lowering of PCV (Eyasu *et al*., 2021, Van den Bossche and Rowlands, 2001).

In this study, entomological findings revealed the presence of only one *Glossina* species, *G. pallidipes* and other biting flies including *Stomoxys* and *Tabanus*. A total of 321 (56.9%) *G. pallidipes* and 243 (43.1%) other biting flies were caught during the study period. These species of *Glossina* and other biting flies have also been reported in the Southern parts of the country (Eyasu *et al*., 2021 and Seyoum *et al*., 2022). The overall apparent densities of *G. pallidipes* and biting flies in the study area were 1.38 f/t/d and 1.01 f/t/d, respectively. This finding was lower than that of Teka *et al*., 2012, Fentahun and Tekeba, (2013) and Lelisa *et al*., 2014, who reported 14.97, 13.01 and 11.9 f/t/d, respectively from western part of Ethiopia. The relative low level of tsetse population in the present study may be due to the control intervention under taken in Zala district by National Tsetse and Trypanosomosis Investigation and Control Center (NTTICC) and expansion of farm lands leading to the destruction of tsetse habitat and elimination of their wild hosts (Van den Bossche *et al*., 2010).

The seasonal apparent density of tsetse flies was higher in wet season than dry season in Zala district. An overall apparent density of *G. pallidipes* in the wet and dry season was 1.78 and 1 f/t/d, respectively, which was similar to the results obtained (Eyasu *et al*., 2021, Dagnachew *et al*., 2005 and Degneh *et al*., 2017) in different parts of the country. This could suggest an absolute increase in the number of tsetse and biting flies due to favorable environmental conditions (Bright well *et al*., 1987). In streak to this finding, Lulu *et al*., 2019 who reported a higher apparent density of *G. pallidipes* in Dale Sadi District, West Oromia which was 5.34 fly/trap/day whereas Girma *et al*., 2014, who reported a lower apparent density of *G. pallidipes* which was 0.194 fly/trap/day in and around Arba-Minch, Gamo Gofa Zone. The observed difference can be explained in view of the possible development of optimum vegetation, temperature and humidity favorable for tsetse fly breeding and survival in the wet season (Cherenet *et al*., 2006).

6. CONCLUSION AND RECOMMENDATIONS

A parasitological study revealed the overall prevalence of 6.96% in the study area with seasonal prevalence of 9.64% and 4.28% in the wet and dry season, respectively. The most prevalent pathogenic trypanosome species in the study area were; *T. congolense* and *T. vivax*, with significantly higher prevalence of *T. congolense*. The prevalence of trypanosomosis was significantly higher in wet season, black and black spotted coat colour and poor body conditioned animals. The entomological study revealed the presence of only one *Glossina* species, known as *G. pallidipes* and two species of other biting flies, *Stomoxys* and *Tabanus* were distributed in the study area. The overall apparent densities of *G. pallidipes* and other biting flies in the study area were 1.38 f/t/d and 1.01 f/t/d, respectively. A relatively higher *Glossina* was caught in the wet season than dry season. Moreover, this study showed a direct relationship between seasonal trypanosomosis prevalence and *G. pallidipes* apparent density. Therefore, based on the above conclusion, the following recommendations were forwarded:

- The need for strengthening the vector and parasite control interventions in the area.
- Government and non-government organization should conduct community based integrated tsetse fly control strategy in order to bring sustainable solution for livestock producing community.
- Extend and strengthen the national tsetse and trypanosomosis control scheme in tsetse infested areas combines with insecticide treated targets and cattle pour-on has to be implemented in the study area to minimize the burden of the disease.
- Further studies on the tsetse flies feeding pattern and blood meal analysis are required due to the awareness of tsetse fly feeding performance in the area is very important to draw rational decisions to guide control of trypanosomosis and their vectors.

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8. APPENDICES

Appendix 1: Appendix I: Packed Cell Volume (PCV) Determination

- ✓ Blood samples were collected by puncturing the ear vein with a lancet.
- ✓ The blood sample was collected directly in to a capillary tube, which was treated with heparin sealed one end with ‘’cristaseal’’.
- ✓ The capillary tubes were placed in a micro haematocrite centrifuge with the sealed and outermost.
- ✓ After screwing the rotary cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12,000rpm for five minutes.
- ✓ Then the capillary tubes were placed in haematocrite reader and expressed the reading as percentage of packed red cells to the total volume of whole blood.
- ✓ Finally, an animal with PCV ≤ 24% were considered as anemic.

(Source: Murray *et al*., 1977)

Appendix 2: Buffy Coat Technique

- ✓ Blood samples were collected by puncturing the ear vein with a lancet.
- ✓ The blood sample was collected directly in to a capillary tube, which was treated with heparin sealed one end with ‘’cristaseal’’.
- ✓ The capillary tubes were placed in a micro haematocrite centrifuge with the sealed and outermost.
- ✓ After screwing the rotary cover and closing the centrifuge lid,, the specimens were allowed to centrifuge at 12,000rpm for five minutes.
- ✓ The haematocrite tubes were cut by diamond tipped pencil few millimeters below the junction of the buffy coat/plasma level and the erythrocyte, the contents homogenized on to a clean slide covering with a 22x22mmcover slip.
- ✓ Then the slides were examined under a microscope using x40 objective and x10 eyepiece for movement of parasite.

(Source: Murray *et al*., 1977)

Appendix 3: Body condition scoring

BCS	1	Thin	Bone structure of shoulder, ribs, back and pins are sharp to the touch and easily visible. No evidence of fat deposits.
BCS	2	Thin	No evidence of fat deposition and there is muscle loss especially in the hindquarters. The spines processes feel sharp to the touch and are easily seen.
BCS	3	Thin	Very little fat cover over the loin, back and fore ribs. The backbone is still highly visible. Processes of the spine can be identified individually by touch and may still be visible. Spaces between the processes are less pronounced.
BCS	4	Borderline	Fore- ribs are slightly noticeable and the 12^{th} and 13^{th} ribs are still very noticeable to the eye. The transverse spinouse process can be identified only by palpation and feel rounded rather than sharp. Slight muscle loss in hind quarter.
BCS	5	Moderate	The 12^{th} and 13^{th} ribs are not visible to the eye unless the animal has been shrunk. The transverse spinouse process can only be felt with firm pressure.
BCS	6	Moderate	Ribs are fully covered and are not noticeable to the eye. Hindquarters are plump and full. Noticeable springiness over the fore-ribs and on each side of the tail-head. Firm pressure is now required to feel the transverse process.
BCS	7	Fleshy	Ends of the spines process can only be felt with very firm pressure. Spaces between processes can barely be distinguished. Abundant fat cover on either side of the tail-head with evident patchiness. Fat in the brisket.
BCS	8	Fleshy	Animal takes on a smooth, blocky appearance. Bone structure disappears from sight. Fat cover is thick and spongy and patchiness is likely. Brisket is full.
BCS	9	Fleshy	Bone structure is not seen or easily felt. The tail-head is buried in fat. The animal mobility may actually be impaired by excessive fat. Square appearance.

Body condition scores 1to 4 are classified as ‘’poor’’

Body condition scores 5 to 6 are classified as ‘’medium’’

Body condition scores 7 to 9 are classified as ‘’good’’

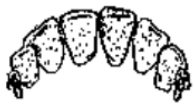
(Source: - (Nicholson and Butterworth, 1986)

Appendix 4: Guideline to determining the age of cattle by the teeth

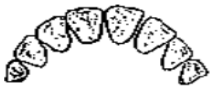
At birth to 1 month: Two or more of the temporary incisor teeth present. Within first month, entire 8 temporary incisors appear

2 years: As a long-yearling, the central pair of temporary incisor teeth or pinchers is replaced by the permanent pinchers. At 2 years, the central permanent incisors attain full development.

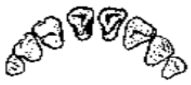
2-1/2 years: Permanent first intermediates, one on each side of the pinchers, are cut. Usually these are fully developed at 3 years.



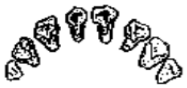
3-1/2 years: The second intermediates or laterals are cut. They are on a level with the first intermediates and begin to wear at 4 years.



4-1/2 years: The corner teeth are replaced. At 5 years the animal usually has the full complement of incisors with the corners fully developed.



5 to 6 years: The permanent pinchers are leveled, both pairs of intermediates are partially leveled, and the corner incisors show wear.



7 to 10 years: At 7 or 8 years the pinchers show noticeable wear; at 8 or 9 years the middle pairs show noticeable wear; and at 10 years, the corner teeth show noticeable wear.

12 years: After the animal passed the 6th year, the arch gradually loses its rounded contour and becomes nearly straight by the 12th year. In the meantime, the teeth gradually become triangular in shape, distinctly separated, and show progressive wearing to stubs. These conditions become more marked with increasing age.

(Source: Radostits, O. and Gray, C. (2007)

Appendix 5: Parasitological data collection sheet

Regin_____Zone_____Woreda_____Kebele_____Long_____Lat_____Alt_____

Season_____Total cattle for sampling_____

No.	Owner name	Animal name	Sex	Age	Colour	Bcs	PCV	Buffy coat result		
								T.cong	T.viv	T.brucei
1										
2										

Appendix 6: Entomological data collection sheet

Region____Zone____Woreda____Kebele____Season____Odur used_____Trap id_____

Lon	Lat	Alt	Veg	St Dat	St Tim	End Dat	End Tim	Tsetse flies					Biting flies	
								Spp	No. Ma	No. Fe	No. Un	Tot	Tab	Stom
1														
2														

Note: stDate (start date) = date of trap deployment; No.Usex = No. of unknown sex

StTime (start time) = time of trap deployment No.M = Number of male fly

EndDate (End date) = date of fly harvest No.F = Number of female fly

EndTime (End time) = Time of fly collection Veg =Vegetation

Alt= Altitude Lon =Longitude

- Dates should be in dd/mm/yy/format and it is in Gregorian Calendar
- Times should be 12hr medium format (from 6 am- 12 am in the morning and 12pm-6pm afternoon)

Appendix 7 : Some images captured during study period

Figure 5: NGU trap depletion, species identification and counting of the tsetse fly

Figure 6: Instrumental adjustment and laboratory procedures

Figure 7: Field blood examination for parasitological study

9. BIOGRAPHICAL SKETCH

The author, Tone Toka Tokossa, was born in 1981 E.C from his father Toka Tokossa and his mother Shameti Doncha in Gayla Kebele, Gofa Zone, Zala Woreda in Southern Nations Nationalities and People's Region. He attended his elementary education (1-6) at Gayla elementary school from 1991- 1996. He pursued his junior secondary education (7-8) at Gelta primary school from 1997-1998. He attended his secondary high school education (9-10) at Galma secondary school from 1999-2000. He attended his preparatory education at Sawla secondary and preparatory school from 2001-2002. He then joined **University of Gonder** in 2003 and was awarded **DVM degree** in **Veterinary Medicine** in 2008. After his graduation, he served in Zala Woreda Livestock and Fishery Development office as animal health coordinator and department head for five years. In September 2014, he joined the School of Graduate Studies at **Hawassa University** for the **Master of Science study in Veterinary Epidemiology**.